USE OF DIVERSITY ARRAY TECHNOLOGY (DArT) TO IDENTIFY QTLs FOR PHYSIOLOGICAL TRAITS IN MUNGBEAN (*VIGNA RADIATA*) AND SOYBEAN (*GLYCINE MAX*)

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ABSTRACT

This thesis reports the outcomes of research evaluating the application of Diversity Array Technology (DArT) to two important tropical legume crops, mungbean (*Vigna radiata* (L.) Wilczek) and soybean (*Glycine max* (L). Merrill.). Mungbean is comparatively under-studied, and is therefore likely to benefit from research to improve adaptation and productivity. Soybean has received more breeding attention than mungbean, but is still under-researched when compared with modern cereals like rice, wheat and maize. Drought remains an important constraint to soybean productivity in rainfed areas. DArT is a novel molecular marker technology that has been successfully applied in genetic studies in several plant species, but had not been previously applied to either of the crops of interest.

A DArT marker library/ array for mungbean was created using two mungbean cultivars, Berken and Kiloga, and two wild accessions *V. radiata.* ssp. *sublobata*, ACC 1 and ACC 87, and one or more accessions of five other *Vigna* species, viz. *V. lanceolata*, *V. mungo*, *V. mungo* var. *sylvestris*, *V. trilobata* and *V. vexillata*. A DArT library/ array for soybean was developed using two oilseed type cultivars, CPI 26671 and Valder, a landrace G2120, a wild soybean (*G. soja*) accession and one or more accessions of two wild *Glycine* species, *G. falcata* and *G. tomentella*. For each crop, two genomic complexity reduction methods, utilizing *Pst*I/*Taq*I and *Pst*I/*BstN*I restriction digests, were selected for DNA clonal library development and for the isolation in each case of 7,680 DArT clones from genomic representations of pooled DNA samples. While the *Pst*I/*BstN*I method produced more polymorphic clones than *Pst*I/*Taq*I for the soybean library, there was no significant difference between the two methods for the mungbean library. In the initial library evaluation, there were nearly 1,500 polymorphic clones identified for soybean. Polymorphism frequencies in mungbean were around twice those in soybean, reflecting greater diversity in the mungbean germplasm samples. The DArT marker transferability from soybean to mungbean (13.6%) was nearly five times higher than that from mungbean to soybean (3.1%). The percentage of DArT marker transferability between mungbean and several other *Vigna* species ranged from 3.4 to 20.2%. The genetic similarities among 11 diverse *Vigna* spp. samples, evaluated using the DArT mungbean library, were consistent with published information on these taxa.

These mungbean and soybean arrays were then used to evaluate the application of DArT markers for construction of genetic linkage maps and identifying putative qualitative and quantitative trait loci (QTLs). In mungbean, four F_5 recombinant inbred line (RIL) populations were derived from crosses between cultivars Berken and Kiloga and wild accessions ACC 1 and ACC 87. The F_5 RIL populations were evaluated for 54 qualitative and quantitative traits using plants grown in large pots on outdoor benches. There were large differences between the cultivated and wild parents and individual lines for all traits. Broad sense heritability estimates were moderate to high in most cases, with significant phenotypic correlations between many traits. A large number of polymorphic DArT

markers were selected for the four RIL populations (1062 – 2013). The four mungbean linkage maps contained 672 to 981 DArT markers with segregation distortion levels higher in the ACC 87 (44.1 – 47.8%) than in the ACC 1 crosses (33.7 – 42.4%). Maps consisted of 15 – 19 linkage groups (LGs) and spanned lengths of 629.7 to 883.5 cM with average inter-marker distances of 0.9 – 1.2 cM. Various putative QTLs (77 – 122 QTLs) were identified for the vast majority of the 54 evaluated traits. In addition, the level of congruence across populations was reasonably strong.

In soybean, one F_7 and two F_6 RIL populations derived from crosses between CPI 26671, Valder and G2120 were used in phenotypic evaluation and QTL mapping with DArT markers for physiological drought stress response traits. The RILs were grown in deep cylindrical pots in the glasshouse, and exposed to severe water deficit followed by re-watering. Traits recorded included relative water content (RWC), epidermal conductance (g_e), and recovery in growth following re-watering. The drought stress responses in the parental plants and RIL populations were broadly consistent with prior studies: As plant available water (PAW) in the soil declined, both RWC and g_e declined, with the relation between RWC and g_e exponential rather than linear as in previous studies. Analysis of variance showed significant differences at both population and genotypic levels for all key traits. However, there were large environmental effects on most traits, which resulted in high coefficients of variation and low estimates of broad sense heritability. The three individual linkage maps contained 196 – 409 DArT markers and 15 – 22 LGs with the aggregate length ranging from 409.4 to 516.7 cM. An integrated soybean map was constructed consisting of 759 DArT markers, 27 LGs and an expanded length of 762.2 cM. Total numbers of putative QTLs identified in the CPI 26671 x G2120 (CG) and VG (Valder x G2120) populations were 106 and 34 respectively. In each of the population, 10 LGs harboured QTLs associated with RWC, g_e and recovery ability, of which five similar LGs contributed to drought tolerance. A BLAST (Basic Local Alignment Search Tool) search for sequences of 19 selected DArT markers linked to QTLs conditioning drought response traits indicated that 18 DArT markers were unique and aligned to 12 soybean chromosomes. Comparison of these DArT markers with other markers associated with drought-related QTLs in previous studies confirmed that five of them overlapped whereas the remaining 13 had not been previously identified. However, except for chromosome 15, the chromosomes with which the DArT QTLs in the CG and VG populations were associated were ones that had been shown to harbour drought-related QTLs in previous studies.

This study is the first showing DArT development in mungbean and soybean and its application for manipulating QTLs associated with wild physiological traits in mungbean and drought tolerance in soybean. DArT was successfully developed for both species, with more polymorphisms evident in the mungbean than in the soybean arrays. The study demonstrated that DArT provides high quality markers which can be used for diversity analyses, the construction of high-density genetic linkage maps and for QTL analysis. Meanwhile the marker transferability between arrays enabled plausible discrimination of genetic relationships between related taxa.

In both mungbean and soybean, the large numbers of DArT markers that were generated contributed to relatively tight resolution in the genetic maps, enhancing the power for QTL detection. Potentially useful QTLs/ markers were identified for many traits in mungbean, including some potentially useful ones such as resistance to powdery mildew and thrips, late flowering, hardseededness and perenniality, and in soybean, for RWC, g_e and recovery after drought stress. In mungbean, further research is needed to identify an appropriate approach for the construction of an integrated map from the four RIL populations used in this study. In both species, follow-up research is required to verify the QTLs detected in this study before they can be used for marker-assisted selection in mungbean and soybean breeding programs. Nonetheless, the QTL analyses based on DArT markers in this study have been shown to be useful in the genetic dissection of both qualitative and quantitative traits in both species, and it is apparent that DArT markers will offer advantages for a range of molecular breeding and genomics applications.

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ABBREVIATIONS

H^2_b	Broad sense heritability
1xB	ACC 1 x Berken
1xK	ACC 1 x Kiloga
87xB	ACC 87 x Berken
87xK	ACC 87 x Kiloga
AFLPs	Amplified fragment length polymorphisms
ANOVA	Analysis of variance
BC	Backcross
BILs	Backcross inbred lines
BL	Branch length
BLAST	Basic Local Alignment Search Tool
blastn	Nucleotide basic local alignment search tool
bp	Base pair
CAPs	Cleaved amplified polymorphic regions
cDNAs	complementary DNA
CG	CPI 26671 x G2120
CIM	Composite Interval Mapping
cM	Centi-Morgan
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CTAB	Cetyl trimethylammonium bromide
CV	CPI 26678 x Valder
cv.	Cultivar
DArT	Diversity Array Technology
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
DFlo	Duration of flowering
dGTP	Deoxyguanosine triphosphate
DH	Double haploid
DM	Standing dry biomass
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DPc	Dry pod colour
DPM	Dry pod mass
DRTs	Domestication-related traits
DSI	Drought susceptibility index
DTT	Dithiothreitol
dTTP	Deoxythymidine triphosphate
E	Environment
EDTA	Ethylenediaminetetraacetic acid
ESTs	Expressed sequence tags
EST-SSRs	Expressed sequence tags - Simple sequence repeats
ETS-SSRs	Expressed tagged sites - Simple sequence repeats
FLc	Flower colour
Flo	Time to flowering
FloS	Flower size

FSt	Infloresence structure
G	Genotype
G x E	Genotype by Environment interaction
g_e	Epidermal conductance
g_{e70}	Epidermal conductance at 70% relative water content
GH	Growth habit
GS	Genome selection
HI	Harvest index
HLc	Hilum colour
HPc	Hypocotyl pigment
HS	Hardseededness
ICIM	Inclusive Composite Interval Mapping
ICIM – ADD	ICIM with additive effects
ICIM QTLs	QTLs detected by Inclusive Composite Interval Mapping method
IL	Internode length
IM	Interval Mapping
ISSR	Inter-simple sequence repeat amplification
LA	Leaf area
LGs	Linkage groups
LL	Leaflet length
LLb	Leaflet lobing
LOD	Logarithm of odds
LoS	Number of leaves on stem
LP glasshouse	Long Pocket glasshouse
LPc	Leaf petiole colour
LR	Leaflet ratio
LRc	Leaf rachis colour
Lus	Lustre
LW	Leaflet width
MAS	Marker-assisted selection
Mat	Growth duration
Mbp	Megabase pair
MIM	Multiple Interval Mapping
n.a	Not applicable
N1P	Node of 1st pod
NCBI	National Central for Biotechnology Information
NEB	New England Biolabs
NILs	Near isogenic lines
nLA	New leaf area
nLL	Number of new leaflets
nNS	Number of new stem nodes
NoB	Number of nodes on branches
NoS	Number of nodes on stem
NS1	Number of stem nodes before re-watering
NS2	Number of stem nodes after re-watering
OA	Osmotic adjustment
Ost	Overall visual seed traits
PAW	Plant available water
PC	Pod clusters

PCR	Polymerase chain reaction
PCR-SSCP	Single-strand conformation polymorphism
PD	Pod dehiscence
PdL	Peduncle length
PeL	Petiole length
PG	Time for pod growth
PHc	Plant hair colour
PHd	Plant hair density
PIC	Polymorphism information content
PIN	Largest *P*-value for entering variables in the stepwise regression of phenotype on marker variables
PL	Pod length
PM	Powdery mildew
Pops	Population
Pops/Gen	Genotypes within populations
Pos	QTL position on LG
PP	Number of pods per peduncle
PVC	Polyvinyl chloride
PVE	Phenotypic variation explanation
PVP-40	Polyvinylpyrrolidone 40
PW	Pod width
QBP glasshouse	Queensland Bioscience Precinct glasshouse
QTLs	Quantitative trait loci
R_RWC	Relative response relative water content
RAPDs	Random amplified polymorphic DNA
Rc	Recovery score
RE	Restriction enzyme
RFE	Recursive feature elimination
RFLPs	Restriction fragment length polymorphisms
RILs	Recombinant inbred lines
rLA	Remnant live leaf area
rLL	Number of remnant live leaflets
RT	Room temperature
RUE	Radiation use efficiency
RWC	Relative water content
RWCc	Critical relative water content
SAHN	Sequential, agglomerative, hierarchic, non-overlapping clustering
SCAR	Sequence characterised amplified region
SCN	Soybean cyst nematode
SCr	Seed coat ridging
SDS	Sodium Dodecyl Sulfate
SIM	Simple Interval Mapping
SL	Stem length
SM	Seed mottling
SML	Statistical machine learning
SML QTLs	QTLs detected by Statistical Machine Learning method
SNPs	Single nucleotide polymorphisms
SP	Number of seeds per pod
SS	Seed size

SSC	Saline sodium citrate
SSR	Simple sequence repeats
STc	Stem colour
STd	Stem diameter
STMs	Sequence-tagged microsatellite sites
STS	Sequence tagged site
SY	Seed yield
TC	Total pod clusters
TE	Tris-EDTA buffer
Thr	Thrips
TL	Surface texture layer
TLc	Texture layer colour
TrisHCl	Trisaminomethane hydrochloride
TuB	Perenniality
TW	Twining
UPGMA	Unweighted pair-group method using the arithmetic average
VG	Valder x G2120
w/v	Weight/ volume

CHAPTER 1. INTRODUCTION

The legume family is the third largest family of flowering plants after the Compositae and the Orchidaceae (Smartt 1990). It comprises more than 730 genera with 19,000 species distributed world wide (Lewis *et al*. 2005). Economically, legumes are second only to cereal crops (family Poaceae) and contribute 27% to the world's crop production (Graham and Vance 2003).

The legumes are divided into three families: Mimosaceae, Caesalpiniaceae and Fabaceae. Fabaceae contains nearly all the economically important crop legumes, including mungbean (*Vigna radiata* (L). Wilczek.) and soybean (*Glycine max* (L.) Merr.) which belong to the tribe Phaseoleae. Like most of the Phaseoleae, they are part of the Phaseoloid clade (one of the two clades in the Fabaceae) which includes the tropical or warm season legumes (Polhill 1994; Choi *et al.* 2004).

Both mungbean and soybean were domesticated from wild species in the Asian region. Mungbean, also known as green gram, was domesticated in Asia from the Indian subcontinent to the Far East (Smartt 1990). The genetic diversity of cultivated mungbean, as evaluated by microsatellite analysis, supports the view that mungbean was domesticated in South Asia (Sangiri *et al*. 2007). The putative progenitor of cultivated mungbean is the wild form, *V. radiata* ssp. *sublobata*, which is widely distributed from western Africa to northern and eastern Australia and Papua New Guinea (Lawn and Cottrell 1988; Tomooka *et al*. 2002).

Soybean was domesticated from the wild soybean, *Glycine soja* Sieb. et Zucc. in the cool, humid north-east of China (Hymowitz 1970), and was later disseminated to other Asian countries such as Korea, Japan, Indonesia, the Philippines, Vietnam, Thailand, and India. By the 16^{th} and 17^{th} centuries, soybean was introduced to Europe and North America (Singh and Hymowitz 1999). Since crop improvement aimed at soybean as a grain crop started in the 1920s in well- irrigated areas of United States, soybean has become a large commercial grain crop and has been bred for mechanized agriculture (Hymowitz 1988).

The legume family in general, and mungbean and soybean in particular, play a critical role in sustainable agriculture. Legume species benefit the environment because of their ability to sequester C while enhancing soil quality and tilth, which contributes to offsetting increases in atmospheric CO_2 levels (Graham and Vance 2003). Their capacity for symbiotic nitrogen fixation with compatible rhizobia (Graham and Vance 2003) provides essential and 'free' nitrogen for use by the host plants or by associated or subsequent crops. Worldwide, 40 to 60 million metric tons of nitrogen are annually fixed by agriculturally important legumes (Smil 1999). In addition, the inclusion of legumes in a cereal cropping rotation increases plant-available nitrate-N in the soil for the cereals, which in turn

increases their yield by 50 – 80%. For example, paddy rice or wheat in rotation with mungbean had yields increased by 0.5 – 1.1 t/ha (Ahmad *et al*. 2001).

Both cultivated soybean and mungbean have become increasingly important agricultural commodities because of their multitude of uses. Soybean supplies edible vegetable oil and high quality proteins for human consumption and for animal feed. Soybean contributes 57% to total oilseed production which makes it the world's number one oilseed crop in international trade markets, followed by other oil crops such as rapeseed, groundnut, sunflower and coconut (Singh and Hymowitz 1999; Pathan and Sleper 2008). Soybean seeds can be used for industrial, food and pharmaceutical products (Singh and Singh 1992; Lu 2004). There are also many specialist soybean culinary foods (e.g. tofu or bean curd, edamame or green beans, natto or fermented beans and soy sauce). Its most recent industrial application is the production of biodiesel (Pradhan *et al*. 2011; Yusuf *et al*. 2011). Mungbean is a rich source of protein and of other useful dietary needs such as vitamin A, iron, zinc and folate (Shanmugasundaram 2007). Its familiar form is crunchy bean sprouts which are widely used in oriental cuisine, salads and healthfoods (Lawn and Cottrell 1988; Manavalan *et al*. 2009; Ratnaparkhe *et al*. 2011). Other diverse products produced from mungbean include fried foods and desserts (Singh and Singh 1992).

As with legume crops generally, the genetic improvement of soybean and mungbean lags behind cereals (Borlaug 1973; Summerfield and Lawn 1987; Wojciechowski *et al.* 2004). One of the reasons is the limited genetic variation or extremely narrow genetic base of the germplasm used in breeding, resulting in difficulty in identifying unique traits for crop development. The pedigrees of the most popular mungbean lines grown worldwide were based on only a few dozen parental sources (Yang 1996; Yimram *et al*. 2009). Likewise, soybean breeding has been limited to hybridization within the different maturity groups (e.g. Delannay *et al*. 1983; Gizlice *et al*. 1993, 1994; Jong *et al*. 2004; Wang *et al*. 2008). While soybean is a large commercial crop that has been bred for mechanized agriculture, mungbean essentially is still a village crop and so fewer studies on its genetic diversity have been undertaken.

In order to widen the germplasm base, related wild species can sometimes be employed in crop improvement. Wild species provide a potentially important source of novel traits such as pest and disease resistance, unique photothermal responses, seed traits and abiotic stress tolerance. For instance, wild mungbean accessions have hardseededness and high seed protein content, disease resistance and saline, calcareous or cracking clay soil tolerance, all of which are potentially useful for mungbean variety improvement (Lawn and Cottrell 1988; Lawn and Rebetzke 2006). Likewise, the wild germplasm of soybean including the wild annual *G. soja*, and at least 26 perennial species of the subgenus *Glycine* may provide a source of useful traits including disease and pest resistance and herbicide tolerance (Singh and Hymowitz 1999; Chung and Singh 2008).

Abiotic stresses such as drought, high salinity and high temperature are increasingly serious threats to the stable production of crops worldwide. Of these, drought is the main climatic factor limiting soybean yields. For instance, drought stress occurring at the reproductive stage of soybean can cause yield reductions of 46 – 71% (Samarah *et al*. 2006). Water deficit occurring between initial flowering and seed fill reduced branch growth, branch seed number and branch seed yield, contributing to total seed yield reduction (Frederick *et al.* 2001). Soybean seed quality was also reduced (Vollmann *et al*. 2000).

Breeding for drought tolerance requires insight into the physiological traits affecting responses to water deficit. Genetic variation has been found in soybean germplasm for several physiological traits such as osmotic adjustment, epidermal conductance and critical relative water content (James 2004). These three traits have been shown to affect leaf area maintenance during stress, and ultimately the capacity for droughted plants to recover when the stress is relieved (Lawn and Likoswe 2008). However, physiological traits that have been linked to drought resistance are complex and difficult to measure and are subject to large environmental effects on their expression. This makes them difficult to manipulate in a breeding program.

It is well-known that many physiological traits are not qualitatively (i.e. simply) inherited, but rather, are conditioned by a number of alleles at several loci (i.e. quantitative inheritance). Utility of molecular markers for construction of linkage maps and the detection of quantitative trait loci (QTLs) for such complex traits is a common approach that is being used to identify useful physiological traits, understand their genetic basis and manipulate them in breeding programs.

To date, QTLs have been identified for several different traits in mungbean such as bruchid resistance (Young *et al*. 1992; Chen *et al*. 2012), powdery mildew resistance (Chaitieng *et al*. 2002; Kasettranan *et al*. 2010), hard-seededness (Humphry *et al*. 2005), morphological and agronomical traits (Isemura *et al*. 2012; Kajonphol *et al*. 2012) mostly using RFLPs (Restriction Fragment Length Polymorphism), RAPDs (Random Amplified Polymorphic DNA), AFLPs (Amplified Fragment Length Polymophism) and SSR (Simple Sequence Repeat) markers. In soybean, extensive QTL studies have been undertaken for various traits including flowering time, maturity and photoperiod insensitivity (Tasma *et al*. 2001), seed quality (Chapman *et al*. 2003), leaflet types (Kim *et al*. 2005), cyst nematode resistance (Guo *et al*. 2005), water-logging and chilling tolerance (Cornelious *et al*. 2005; Funatsuki *et al*. 2005), nodulation and shoot mass (Nicolas *et al*. 2006), pod and yield related traits (Panthee *et al*. 2007; Guang-yu *et al*. 2011), pod dehiscence (Luo *et al*. 2012) and relative water content and canopy-wilting traits associated with drought (Abdel-Haleem *et al*. 2012; Virginia *et al*. 2012).

Diversity Arrays Technology (DArT) is a molecular technique that has been successfully developed for various plant species, including rice (Jaccoud *et al*. 2001), barley (Wenzl *et al*. 2004), *Arabidopsis* (Wittenberg *et al*. 2005), cassava (Xia *et al*. 2005), pigeonpea (Yang *et al*. 2006), sorghum (Mace *et*

al. 2008) and *Eucalyptus* (Sansaloni *et al*. 2010). DArT markers have a number of advantages over other molecular markers, such as independence from DNA sequencing, low cost, expandable genetic scope, high-throughput of whole genome profiling and an 'open source' (i.e. freely available) technology (Huttner *et al*. 2004; Kilian *et al*. 2005). DArT markers have also been applied to genetic diversity and mapping studies (Wittenberg *et al*. 2005; Wenzl *et al*. 2004, 2006; Semagn *et al*. 2006c; White *et al*. 2008; Alheit *et al*. 2011; Tyrka *et al*. 2011; Raman *et al*. 2012; Zhang *et al*. 2012). DArT has potential application for a number of plant species, particularly for crops with low levels of past breeding investment, like mungbean.

When this research was initiated, two sets of germplasm that had been shown in previous studies to exhibit genetic diversity for several physiological traits were available for study. The first comprised early generation (F_3) hybrids from four mungbean populations that had been generated to enable the inheritance/ heritability of cultivated and wild traits to be studied (Nguyen 2011). The second comprised later generation hybrids (F_4 or F_5) between soybean genotypes that had been shown in another study (James 2004) to differ in physiological responses to severe drought. Given the existing knowledge that was available on physiological diversity within these two sets of germplasm, it was considered that these two populations could likely provide a useful means for testing the potential application of DArT markers in soybean and mungbean.

In the following chapter, the current state of knowledge concerning the genetic improvement of legumes, particularly of mungbean and to a lesser extent soybean, is briefly reviewed. The use of physiological traits in plant breeding is considered, with some emphasis on the traits which have been shown to vary in the two germplasm sets of interest. The use of molecular markers to identify and manipulate physiological traits is also briefly reviewed, with specific emphasis on the use to date of DArT markers. The use of markers for other purposes such as assessing genetic diversity and mapping linkage groups is also briefly reviewed, along with factors that have been shown in previous studies to affect the success or otherwise of marker studies. The broad aim of the review is to provide the background to the research reported in this thesis.

CHAPTER 2. LITERATURE REVIEW

2.1. Current status of genetic improvement of grain legumes

As previously noted, the genetic improvement of grain legumes generally lags behind cereals notwithstanding their importance to agriculture, the environment, and to animal and human nutrition (Borlaug 1973; Summerfield and Lawn 1987; Graham and Vance 2003; Wojciechowski *et al.* 2004). While cereal yields have increased greatly, grain legumes have shown only marginal improvement (Russell *et al*. 1989; Jin *et al*. 2010; Fan *et al*. 2011). For example, the average yield of food legume crops globally was barely 800 kg/ha in late 2000s while that of cereal crops in 2006 – 2008 was 3500 kg/ha (Akibode and Maredia 2011). In China, from the year 1961 to 2009, the average productivity of rice increased from 2041 to 6585 kg/ha (3.2 fold) and of wheat from 557 to 4739 kg/ha (8.5 fold) (Fan *et al*. 2011). Meanwhile, from 1950 to the 1990s, the soybean annual yield increase averaged 13.4 kg/ha (Jin *et al.* 2010). In the USA, from 1950 to the 1980s, average soybean yield increased from 1972 to 2609 kg/ha, an annual gain of 14 kg/ha (Ustun *et al.* 2001). In some Asian countries, average soybean and mungbean yields were quite low. For example, in India, the average yield for mungbean was 400 kg/ha in2011 (Nair *et al*. 2013) and for soybean, 570 to 1120 kg/ha (in the period 1994 – 1997) (Singh *et al*. 2006).

Various constraints may explain the slower rates of legume improvement. Legumes are often grown on marginal land and at non-optimal times because of the general view that legumes are secondary crops to cereals (Summerfield and Lawn 1987). Global research investment and resources devoted to grain legumes are also lower than for staple cereals (Akibode and Maredia 2011). Consequently, fewer studies have been conducted and fewer scientific papers published on grain legumes (Russell *et al*. 1989; Gepts *et al*. 2005).

In addition, constraints to increasing grain legume yields arise due to genotype and environment (G x E) interactions, multiple environmental stresses, limited genetic diversity in breeding and limited screening methods for precise phenotyping of target traits (Kumar *et al*. 2011). Large G x E interaction is a major concern because it slows down the progress in genetic improvement in yield and yield stability (Kumar *et al*. 2011). In addition, most grain legumes are grown as part of a complex farming system and frequently in areas where, or during parts of the year when only limited water is available. Therefore, the process of yield improvement needs to take into account unfavourable environmental conditions and low financial inputs (Russell *et al*. 1989). For soybean, there is also sometimes a trade-off between increased seed yields and improved seed oil and protein contents (Russell *et al*. 1989).

Legumes encounter multiple biotic and abiotic stresses. Examples of biotic stresses are diseases e.g. powdery mildew (mungbean, soybean and pea), mosaic yellow virus, *Cercospora* leaf spot (mungbean), wilt, asochyta blight (lentil, chickpea), *Phytophthora* root rot, as well as insect and nematode pests e.g. cyst nematode (soybean) (Bilyeu *et al*. 2010; Kumar *et al*. 2011; Nair *et al*. 2013). Major abiotic stresses for legumes grown in commercial production include drought, flooding, temperature extremes, and edaphic (i.e. soil-related) issues such as fertility, and toxicities e.g. aluminum and salt. Varieties resistant to more than one stress are therefore often required to ensure stable productivity. However, introgression/pyramiding of several genes to develop such superior genotypes is challenging, time consuming and can be problematic due to negative linkage between the resistant genes and desirable agronomic traits.

In many legumes including soybean and mungbean, studies that have used molecular markers to assess genetic diversity have concluded that the genetic base of cultivars is often narrow (e.g. for soybean – Satyavathi *et al*. 2006; Hwang *et al*. 2008; Wang *et al*. 2008; for mungbean – Yang 1996; Sangiri *et al*. 2007; Tantasawat *et al*. 2010; Datta *et al*. 2012; and for pigeon pea – Yang *et al*. 2006). The narrow range of germplasm that has been employed in cultivar development has inevitably limited the sources of unique traits and constrained the genotypic variation available for use in yield improvement.

Several traits, especially traits such as disease resistance and physiological traits like drought tolerance are difficult to screen for, because of G x E interaction (Genotype x Environment interaction), because their measurement is imprecise and/or time consuming or sometimes relies on visual observations. Thus, simple, precise and repeatable techniques for their measurement are needed. To date, molecular marker technology has not been a significant part of conventional legume breeding programmes. However, it would be expected to bring advances in genetic improvement in legumes comparable with those in cereals (Gupta *et al*. 2010; Kumar *et al*. 2011).

Worldwide, much less research attention has been given to mungbean than to soybean, due to its lesser economic importance. For example, in Australia, the development and growth of the mungbean industry have been limited because of its lower seed yields and protein content relative to other grain legumes such as lupins and soybean (Lawn *et al*. 1988a; Chauhan *et al*. 2010) and the perception of mungbean as a low-yielding, high-risk crop when grown after a winter cereal (Lawn and Russell 1978). In addition, mungbean yield is often unstable over locations and seasons due to its susceptibility to environmental stresses, pests and diseases (Kumar *et al*. 2011; Nair *et al*. 2013).

The primary objectives of mungbean genetic improvement have included high and stable yield, concurrent with resistance to pests such as bruchids and pod borers, and diseases like *Cercospora* leaf spot, powdery mildew and yellow mosaic virus (Nair *et al*. 2013). Other general objectives for mungbean improvement are reduced sensitivity to photoperiod and temperature, early and synchronous flowering, tolerance to lodging and drought, reduced weather damage to seeds, and less

pod shattering (Shanmugasundaram - http://203.64.245.61/fulltext_pdf/eam0117.pdf; Nair *et al*. 2013).

Specific breeding objectives differ from country to country and region to region. For example, mungbean varietal improvement programs in India have focused on increasing yield with compact plant types, high harvest-index (HI), reduced photoperiod sensitivity, early maturity and more determinate growth habit (Jain 1971, 1975; Tickoo *et al*. 1996) and on increasing disease and insect resistance (Singh 1996). In Thailand, the objectives of mungbean breeding were high and stable yield, and resistance to the major local disease and insect pests (Pichitporn *et al*. 1991). In Australia, mungbean improvement was initially constrained to the introduction and testing of overseas varieties with high and stable yields and traits suitable for mechanized production such as resistance to lodging and to pod shattering. Subsequently, mungbean breeding has targeted improved yield and reliability through utilization of mungbean genetic resources, improved climatic/regional adaptation and resistance to new foliar diseases and to weather damage (Lawn *et al*. 1988a; GRDC Report 2011).

Some successes in mungbean improvement have been achieved. For example, improved mungbean varieties out-yielded the local variety by 70% in Afghanistan (Rizvi *et al*. 2012) and heterosis effects of up to 200% have been reported (Tickoo *et al*. 2006). Lines resistant to powdery mildew, bacterial blight and *Cercospora* leaf spot have been achieved (GRDC Report 2011). Attempts were made to transfer hardseededness – a major character potentially conferring weather damage resistance – from the wild progenitor to the cultivated species and also to break the association with small seed size (Imrie *et al*. 1988). In Australia, newly released varieties were broadly better adapted and some of the improved lines which were earlier maturing may also exhibit improved drought tolerance (GRDC Report 2011).

Among tropical grain legumes, soybean is a “fast – runner” crop because of the dramatic increase in production in response to rising demand for vegetable protein and oil (Hartman *et al*. 2011) and because of an increase in research and breeding devoted to the crop (Summerfield and Lawn 1987; Manavalan *et al*. 2009; Chan *et al*. 2012; Yamanda *et al*. 2012). There was sustained exponential growth in world soybean production between 1922 and 1977, mainly due to increased areas sown, but also due to some gains in yields. In particular, world soybean production from 1949 to 1979 increased by 700%, with an average increase of 23.3% per year (Shurtleff and Aoyagi 2007). Soybean production and areas sown doubled between 1990 and 2012, respectively to 253 million tonnes and 106 million hectares (FAOSTAT 2012). Compared to mungbean, extensive studies have been undertaken on soybean germplasm, agronomic traits, pest and disease resistance, abiotic tolerance and its genome, notably by USA scientists. The number of soybean breeders in the USA, for example, increased from about 25 in 1976 to 400 in 1982 (Summerfield and Lawn 1987).

The primary objectives of soybean improvement have changed over time. Initially, soybean improvement mainly aimed at high yielding cultivars with high seed protein and oil contents (Morse

et al. 1949). Over subsequent years, additional emphasis was placed on improved seed size, seed quality, especially protein and oil quality, nodulation and nitrogen fixation, increased resistance to pests, diseases, lodging, shattering, mineral deficiencies, toxicities and herbicide injury; and improved resistance to abiotic stresses such as drought, chilling and salinity. These diverse objectives have been addressed variously by screening, hybridization, mutation and genetic engineering, primarily within the soybean primary gene pool (Singh and Hymowitz 1999; Dita *et al*. 2006; Vuong *et al*. 2007; Chung and Singh 2008; Manavalan *et al*. 2009).

For example, soybean lines were isolated with increased tolerance for sulfonylurea herbicides following chemical mutagenesis (Sebastian *et al*. 1989). γ-ray irradiation was used to break linked genes that cause grassy-beany flavour in soybean and soybean products. Genetic engineering is a useful tool to broaden the genetic base of crops by overcoming the genetic barriers in very distant crosses. A soybean line tolerant to glyphosate was developed using the Agrobacterium-mediated gene transfer method (Padgette *et al*. 1995). The γ-zein gene from maize has been introduced into soybean and recovered in transgenic lines which showed higher percentages of alcohol extractable proteins (from 2.54 to 6.49% of total seed proteins) than in the non-transformed control lines (0.35%) (Li *et al*. 2005).

Breeding of soybean in less well watered environments, such as in rainfed regions in Australia, is a relatively recent phenomenon. As soybean was originally domesticated in and adapted to relatively humid environments, it is relatively poorly adapted to water-scarce conditions. Soybean growth, development and yield were maximized when the crop was well-irrigated (Garside *et al*. 1992a). Indeed, substantial yield increases were possible with saturated soil culture (Garside *et al*. 1992b). Soybean is more sensitive to water deficit than other grain legumes such as cowpea, black gram and pigeonpea (Sinclair and Ludlow 1986). Studies have reported that transgenic soybean plants overexpressing an *Arabidopsis* gene, P5CR, showed greater tolerance to drought stress (de Ronde *et al*. 2004a, 2004b; Kocsy *et al*. 2005).

2.2. Role of physiological understanding in genetic improvement

Crop improvement involves two components: agronomy and plant breeding. Agronomy involves the manipulation of the crop environment to maximize the expression of genetic potential. Plant breeding involves the manipulation of the plant genetic make-up to maximize potential and minimize environmental constraints to that potential. Plant breeding variously involves germplasm screening to identify desirable plant phenotypes for use as parents for hybridization or gene transformation, selection of the desired recombinant progeny, and the regional evaluation and ultimate release of elite genotypes as improved cultivars. Both agronomy and plant breeding components usually occur concurrently, and both can be assisted by physiological understanding which can provide biological interpretations of how plants respond to environmental factors, and how different genotypes affect that response (Lawn and Imrie 1994a).

By identifying physiological and environmental constraints to productivity, product quality or adaptation, breeding objectives and selection criteria both direct and indirect can be clearly stated in terms of specific physiological traits (Lawn and Imrie 1994a; Jackson *et al*. 1996; Araus *et al.* 2008; Lawn and James 2011; Tausz *et al*. 2011). For example, wheat yield potential could be increased by 50% or more – at least theoretically – through the genetic improvement of radiation use efficiency (RUE). Attempts to increase RUE would then focus on increasing the efficiency of the rate-limiting enzyme Rubisco, introduction of C4-like traits such as CO_2 concentrating mechanisms, and improvement of light interception and photosynthesis at the spike and whole canopy levels (Reynolds *et al*. 2012). Productivity improvement of tropical grain legumes can be obtained through selection of genotypes which possess higher HI, shorter growth duration, reduced sensitivity to photothermal conditions and more synchronous reproductive ontogeny (Lawn 1988b, 1989; Lawn and James 2011; James and Lawn 2011). In mungbean, through identifying growth limiting characters, Mondal *et al*. (2012) concluded that high yielding mungbean varieties should possess large leaf area, high total dry mass production, and superior crop growth rate at all growth stages, and high relative growth rate and net assimilation rate at the vegetative stage.

Physiological understanding can help identify specific environmental factors which limit plant performance. Therefore, the identification of key constraints or key agro-climatic factors for which genetic variation in response exists, can be useful for choosing test environments for selection trials or screening. For example, photoperiodic and temperature effects are largely responsible for differences in time to flowering between tropical and temperate soybean genotypes (Lawn and Byth 1973). Hence, soybean improvement for tropical and subtropical Australia should take into account the adaptation to warm temperature and relatively short photoperiods (Lawn and Imrie 1991). In pearl millet, the existence of genotypic differences in yield in response to drought stress during the flowering and grain-filling periods indicated that selection for drought tolerance was possible (Fussell *et al.* 1991).

Physiological understanding can be particularly helpful in constructing dynamic and complex plant ideotypes – model plants that have a combination of desirable traits which are suited to the resources, constraints and risks of target production environments (Donald 1968). For example, crops adapted to the intermittent drought conditions of northern Australia should have mechanisms such as dehydration avoidance or physiological traits such as longer leaf maintenance which favour recovery following stress (Lawn 1988a). James *et al*. (2008b) suggested that a drought tolerant soybean ideotype would combine three traits (high osmotic adjustment, low epidermal conductance and low critical relative water content) which are conducive to longer leaf survival during drought and faster recovery capacity after rain.

Physiology is believed to be of potentially greatest value in identifying putatively useful traits that are complex, are affected by G x E interaction and/or collectively constitute minor traits or non-additive effects. For example, G x E for drought resistance is typically large, complex and non-systematic,

with other contributory components such as soil, atmosphere and crop microenvironment (Lawn 1988a; Hugh 2003; Cattivelli *et al*. 2008; Ashraf 2010; Sinclair 2011; Tuberosa *et al*. 2012). In grain legumes, a variety of physiological traits putatively associated with drought stress responses which act "in concert" has been identified e.g. water use efficiency, stomatal conductance, epidermal conductance, leaf area adjustment, root density and depth, osmotic adjustment and critical relative water content (Lawn and Imrie 1991; James 2004; Hufstetler *et al.* 2007; Lawn and Likoswe 2008; Manavalan *et al*. 2009; Gilbert *et al*. 2011). Hence, selection for drought adaptation should not only take into account individual traits. Weathering resistance in mungbean illustrated large G x E interaction with environmental factors such as humidity, temperature and rainfall. Physiological analysis was used to break down weathering resistance mechanisms into several putative traits, consisting of hardseededness, pod wall thickness and density, and phenological avoidance of wet weather, which were more easily used as a basis for screening and selection (Imrie *et al*. 1988).

Although physiological understanding is useful and will remain a cornerstone for crop genetic improvement, there are many shortcomings. Measurement of many physiological traits is laborious and time consuming and may require expensive equipment and/ or specialized expertise (Jackson *et al*. 1996; Araus *et al*. 2008). The ability to handle large amounts of data or samples to obtain high throughput phenotyping may be constrained (Cabrera-Bosquet *et al*. 2012). Therefore, physiological research on more than a few samples/ genotypes is often costly. Many physiological studies have suffered from one or more constraints: (i) they investigated a limited range of genotypes; (ii) they were conducted in isolation from the key objectives of the breeding programs; or (iii) they were conducted with limited or no information on the heritability or the efficacy of the putative trait of interest. In addition, expression of many physiological traits can only be observed in specific stages of plant development, reducing selection efficiency; or alternatively, trait expression is dependent on environment e.g. drought stress response traits. Collectively, these deficiencies have restricted the application of outputs from physiological studies within breeding programs (Lawn 1988a, 1988b; Jackson *et al*. 1996) or even resulted in them being ignored (Cabrera-Bosquet *et al*. 2012).

2.3. Potential utility of wild germplasm

Wild germplasm is a potentially important source of traits for crop genetic improvement, especially traits of adaptive significance (Hajjar and Hodgkin 2007). This is because the accumulation of genes for tolerance to stresses is likely to have been induced by the persistent, long-term exposure of the wild materials to the challenges of the natural environments where they are adapted.

The total number of mungbean accessions held in *ex-situ* collections worldwide is 43,027 (Nair *et al*. 2013), which is less extensive than for soybean which has 170,000 accessions over 70 countries (Carter *et al*. 2004). Germplasm collections of the putative wild progenitor of mungbean and its wild relatives, conducted by several scientists (Lawn and Cottrell 1988; Tomooka *et al*. 1992; Tomooka *et al*. 2006a, 2006b; Vaughan *et al*. 2006), have expanded the extent of *Vigna* genetic resources. A

collection of five *Vigna* species (*V. radiata*, *V. vexillata*, *V. luteola*, *V. marina* and *V. lanceolata*) from Australia and nearby islands of Indonesia and Papua New Guinea and India has been assembled and consists of more than 400 accessions, of which >120 accessions are *V. radiata* ssp. *sublobata* (Lawn and Watkinson 2002). The wild mungbean hybridizes readily with the cultivated mungbean, with normal inheritance for a range of qualitative and quantitative traits (Singh *et al*. 1983; James *et al*. 1999; Nguyen 2011). Other wild relatives of mungbean such as *V. umbellata*, *V. aconitifolia* and *V.trilobata* hybridise to some extent with mungbean (Lawn and Rebetzke 1991; Bharathi *et al*. 2006; Palmer *et al.* 2002). This suggests that wild relatives potentially can be used to augment the gene pool of cultivated mungbean.

In soybean, *G. soja* belongs to primary gene pool of soybean and as the annual wild progenitor of cultivated soybean potentially provides a useful source of new genetic variability. For example, highly productive and high-protein lines were derived from soybean and *G. soja* hybrids (Hartwig 1973). *G.soja* was also successfully used as a non-recurrent parent for the development of small seeded cultivars which are used for sprouts and fermented Japanese products (Carter *et al*. 1995). The 26 wild perennial species of the subgenus *Glycine* with about 3500 accessions are indigenous to Australia and extremely diverse morphologically, cytologically and genomically. Moreover, they harbour a number of potentially useful agronomic traits such as resistance to soybean rust, soybean brown spot, powdery mildew, phytophtora root rot and soybean nematode as well as tolerance to certain herbicides (Riggs *et al*. 1998; Singh and Hymowitz 1999; Chung and Singh 2008).

Wild relatives and landraces are also useful in molecular marker and mapping studies because they provide high levels of polymorphism for study. For example, in mapping studies such as construction of genetic linkage maps and QTL analysis, the most important step is the selection of the appropriate parent lines to create segregating populations for traits of interests (Tuberosa 2012). The parents should be genetically divergent enough to exhibit sufficient polymorphisms and at the same time should not be so far apart so as to cause sterility of the progeny (Collard *et al*. 2005; Dixon *et al*. 2007; Würschum 2012). As noted previously, the genetic bases of both soybean and mungbean crops globally are narrow. For example, only six ancestors constituted more than half the genetic base of North American soybean cultivars released between 1947 and 1988 (Gizlice *et al*. 1994). The soybean genetic base in Australia is similar to or narrower than that of North America; Australian cultivars are either direct introductions or are largely derived from hybrids between cultivars from the United States (Rose 1988; Desborough and Rose 1990, 1992; Lawn and Imrie 1994b; Andrews and Rose 1996). By contrast, population genetic analysis based on the whole-genome sequencing data revealed that wild soybean possesses a much higher diversity (Lam *et al*. 2010). Similarly, more genetic polymorphisms were detected in the wild than that in cultivated mungbean accessions (Sangiri *et al*. 2007).

Tanksley and McCouch (1997) pointed out the genetic bottlenecks imposed on crop plants during domestication, in which allelic variations of genes originally found in the wild are gradually lost

through domestication and breeding. Therefore, it is suggested that the continued sampling of wild germplasm would result in new gene discoveries and use. For example, improvement in red fruit colour in tomato (attributable to the pigment lycopene) was achieved by the introduction of genes from the wild that can enhance earlier steps in the biosynthetic pathway leading to lycopene (Benarcchi *et al*. 1998). In addition, with the aid of technologies and discoveries that make DNA analysis and manipulation possible, differences between loci from the wild type and domesticated traits and chromosomal position of wild alleles that have been transmitted into the progeny can be revealed. A study of QTL mapping of domestication-related traits in soybean (DRTs) (e.g. large seed, loss of pod dehiscence and hard seededness) using a population derived from a cultivated and wild soybean showed that most of the DRTs were conditioned by one or two major QTLs and a number of genotype-dependent minor QTLs (Liu *et al*. 2007). Other findings indicated that the useful QTLs from the wild soybean *G. soja* such as the QTLs for high protein content (Sebolt *et al*. 2000), soybean cyst nematode resistance (Wang *et al*. 2001) and yield (Concibido *et al*. 2003) were already present in the *G. max* germplasm and/ or mapped in cultivated soybean populations. These indicated that some of the useful genes from the wild soybean, as exemplified by Wang *et al*. (2004), have been repeatedly introduced into the cultivated germplasm pools without marked obstacles. Similarly, a study using a mapping population derived from wild and cultivated mungbean identified 105 QTLs and genes for 38 DRTs which were distributed across seven out of 11 linkage groups (Isemura *et al*. 2012). The accumulation of many mutations with large and/or small contribution has contributed to the differentiation between wild and cultivated mungbean.

2.4. Some physiological traits of interest in mungbean and soybean

2.4.1. Useful and novel physiological traits in wild mungbean

2.4.1.1. Seed traits

Seed characteristics of mungbean, especially grain colour, size and quality, can have a large impact on market value. The importance of seed size (large/medium/small), seed colour (green/yellow) and seed lustre (shiny/dull) varies according to regional preferences and depends on the form in which mungbean is consumed. For example, some of the yellow-seeded types are preferred by consumers in countries such as Sri Lanka and the Philippines while medium-sized and dull-seeded types are respectively preferred for sprout production or for soups (Nair *et al*. 2013). Seed size, a primary component of the yield, is considerably smaller in wild mungbean accessions, especially accessions from Austronesia where seed size was only about 20% that of some cultivars (Lawn and Rebetzke 2006).

Seed protein content also varied among wild accessions from 26.2 – 32.4% (Lawn and Rebetzke 1991). Austronesian wild accessions had a wide range in protein concentration and about half the accessions had seed protein contents matching or exceeding those of the Australian cultivar Satin (Lawn and Rebetzke 2006).

Hardseededness may be useful in contributing to resistance to weather damage as it can protect mature seeds from moisture and sprouting (Lawn *et al*. 1988b). However, the use of this trait in mungbean cultivars is difficult because of variable levels of hard seed (usually in the range 0 – 70%), even within plants. In contrast, hardseededness in the wild subspecies is durable and near absolute, which can keep the seeds viable for more than 20 years. In addition, measuring this trait is difficult as the classification of seedlots into "hard" and "soft" is somewhat subjective.

Hardseedednees was suggested to be a dominant trait as it expressed in the F_1 from wild subspecies x cultigen hybrids, with possibly only a single major gene conferring hardness, as the F_2 progeny segregated very close to 3:1 ratio (Singh *et al*. 1983; Lawn *et al*. 1988b). Heritability of the trait was high in both field and glasshouse condition (0.89 – 0.99) (Humphry *et al*. 2005; Nguyen 2011).

In general, the magnitude of genetic effects on seed traits is much greater than G x E effects. For example, the expression of seed protein content was stable across environments (locations and sowing dates) and independent of seed size (Lawn and Rebetzke 1991, 2006). Both seed size and protein content were suggested to be under the control of non-additive gene action (Barad *et al*. 2008). However, Zubair *et al.* (2007) reported additive gene action for seed size.

2.4.1.2. Phenological traits

Mungbean production can be expanded by the use of short-duration varieties to fit into cropping systems and relatively longer duration varieties for regions where cropping duration is not a constraint (Nadarajan and Gupta 2010). There were variations in flowering, duration of reproductive growth and growth cycles among wild mungbean accessions (Lawn and Rebetzke 2006). Time to flower of wild mungbean is in the range of 47 – 121 days (Lawn and Rebetzke 1991). Most accessions flowered early when sown during short days, which was consistent with quantitative short day response in mungbean. However, one accession, ACC 1 collected near Mackay (Queensland, Australia) was recorded to have a long time to flower (mean of 121 days across environments and never shorter than 98 days) and appeared to be photoperiod insensitive. Rebetzke and Lawn (2006a) hypothesised this response may have been due to a long- juvenile (LJ) trait analogous to that in soybean (Hinson 1989). In tropical soybean, the LJ has been employed to reduce photoperiod sensitivity and to broaden adaptation across latitudes and seasons (James and Lawn 2011; Lawn and James 2011). A late flowering trait may also be potentially useful in forage type mungbean cultivars, or those used as cover crops, where vegetative vigour is more important than seed.

Nguyen's study (Nguyen 2011) using F_1 hybrids and backcrosses between the wild accession ACC 1 and two cultivars (Berken and Kiloga) suggested both additive and dominance effects for the late flowering trait. Some other studies also reported both additive and dominance components for days to maturity (Malik and Singh 1983; Khattak *et al.* 2001; Rehman *et al*. 2010). However, others suggested the incidence of additive gene effects only (Zubair *et al*. 2007; Barad *et al*. 2008). Again, a

difficulty in the evaluation of phenology traits is that all phenological phases are strongly affected by E and G x E interactions as well as G alone (Lawn and Rebetzke 2006).

2.4.1.3. Perenniality trait

Perenniality is a novel trait which appears to be unique to Australian accessions of wild mungbean, including ACC 87, obtained from coastal-subcoastal, speargrass-dominant woodlands of North East Queensland (Rebetzke and Lawn 2006c). Perenniality is characterized by fleshy, tuberous and shallow root systems. The variation in gross root morphology and phenology was relatively small. However, there were differences among accessions in the production of seed, tuberised root, and total plant biomass. It was suggested that the perenniality trait could facilitate rapid plant re-growth following early summer rainfall, especially where dry-season fire has removed previous-season above-ground growth and may enable plant survival under environmental stresses. The trait is of potential interest for the development of perennial grain crops for sustainable cropping systems (Jackson and Jackson 1999; Scheinost *et al.* 2001), or alternatively, for the development of a forage legume adapted to the seasonally arid coastal and sub-coastal grasslands of northern Australia. The first inheritance study of perenniality on F_2 and backcross populations suggested that the trait was conditioned by two complementary dominant genes (Nguyen *et al*. 2012).

2.4.2. Plant physiological traits in soybean in response to water stress

Water stress is considered one of the most important factors limiting plant performance and yield stability worldwide, including soybean production (Manavalan *et al*. 2009; Mir *et al*. 2012, Parcell and Cain 2013; Ku *et al*. 2013). Water stress is defined as water shortage which is disruptive to the normal physiological function of the plant (Kramer 1980). In agriculture, drought refers to the condition in which the amount of water available through rainfall and/ or irrigation is insufficient to meet the transpiration needs of the crop (Tuberosa 2012). Breeding for drought tolerance is becoming important due to global climate change, land degradation and decline in water quantity and quality (Salekdeh *et al*. 2009)

Many physiological traits for which expression is associated with plant adaptability to drought-prone environments have been identified in various crops (Ludlow and Muchow 1990). Traits that have been variously proposed as important in terms of drought tolerance include leaf elongation and membrane stability in rice (Praba *et al*. 2009) and leaf water potential and stay-green in wheat (Meyer and Green 1981; Christopher *et al*. 2008). In soybean, traits of interest include leaf water potential (Meyer and Boyer 1981), water use efficiency (Spetch *et al*. 2001; Hufstetler *et al*. 2007), stomatal conductance (Muchow 1985; Sinclair and Ludlow 1986), osmotic adjustment, relative water content, epidermal conductance (Paje *et al*. 1988; James 2004) and leaf area maintenance (James *et al*. 2008b; Lawn and Likoswe 2008) (see also reviews by Ludlow and Muchow 1990; Manavalan *et al*. 2009; Salekdeh *et al*. 2009; Tuberosa 2012; Passioura 2012; Ku *et al*. 2013).

All aspects of plant growth can be adversely affected by water stress, with the size of the effects dependent on the timing, severity and duration of the stress. This review will focus only on traits that have a primary impact on plant survival during the late stages of drought, through their effects on plant water status and leaf area maintenance. These traits were among those known to vary in the germplasm available for study.

2.4.2.1. Plant water status

Measurement of plant water status is difficult as it can be affected by complex factors including physical and chemical properties of plant tissue and time and plant part being measured (Hanks and Nimah 1988). Many traits can be indicators of plant water status such as water potential, relative water content (RWC), tissue water content and fresh weight (Hsiao 1973). Other measurements including stomatal resistance, transpiration rate, net photosynthesis rate, canopy temperature, canopy minus air temperature, crop water stress index and visual leaf rolling score are also used as indicators of crop water status (O'Toole *et al*. 1984). Sometimes, leaf thickness, stem diameter and pod thickness can also be useful indicators for evalutation of water stress tolerance (Kozlowski 1968; Ohashi *et al*. 2009).

Two commonly used physiological traits, relative water content (Hsiao 1973) for plant water status and epidermal conductance (Sinclair and Ludlow 1986; Paje *et al*. 1988) for rate of water loss, are discussed in detail below.

Relative water content (RWC)

In the mid 1980s, relative water content (RWC) was proposed as an alternative measure of plant water status instead of water potential (Flower and Ludlow 1986). RWC given in percentage is the water content relative to the water content of the same tissue at full turgor. In other words, it indicates the balance between water absorbed by the plant and water consumed through transpiration.

Maintenance of a relatively high RWC during water stress may be an indicator of drought tolerance. For example, Schonfeld *et al*. (1988) showed that wheat cultivars having high RWC were more resistant to drought stress. RWC has been used as a screening tool for drought tolerance selection in several crops such as wheat and cotton (e.g. Larbi 2004; Farshadfar *et al*. 2011; Ananthi *et al*. 2013). Critical relative water content (RWC_C) – the RWC at incipient leaf death – can also be another useful indicator for plant water status, as plants that have a lower RWC_C are by definition more tolerant to desiccation than plants with high RWC_C.

Changes in RWC under stress are more significant than under normal conditions (Ananthi *et al*. 2013). Generally, RWC declines as water stress increases, but the responses to water stress vary greatly among species and even genotypes. For instance, based on RWC_C, substantial variation in dehydration tolerance was detected among four legume species which were ranked in order pigeonpea

> cowpea > mungbean > soybean (Sinclair and Ludlow 1986). James *et al.* (2008a) reported 12 (65 – 87%) and 11 (40 – 51%) percentage point ranges in RWC and RWC_C respectively among 58 soybean genotypes. Some wild *Glycine* species had RWC_C substantially lower than the soybean accessions (James *et al*. 2008a). Broad-sense heritability for RWC of severely stressed plants ranged from 40% to 74%, and was statistically significant ($P < 0.05$) for some hybrid populations (James *et al*. 2008c).

RWC has several disadvantages as a drought stress response trait. RWC reflects changes in both cell volume and turgor but its relationship with physiological processes is ambiguous. RWC can also vary within plants with age and habitat, and among species. Other disadvantages include insensitivity of RWC to water deficits at high water potential values and errors due to measurement technique (Barrs 1968). In addition, RWC cannot be easily related to soil water status, except that when plant available water has been depleted and permanent wilting occurs.

Nevertheless, RWC is a convenient and inexpensive measurement (Kramer 1988). RWC appears to be independent of osmotic adjustment and seems to effectively represent the water status of moderate to severely stressed leaves (Flower and Ludlow 1986). RWC_C provides an especially useful descriptor of desiccation tolerance because of its stability for a given genotype across a wide range of water stress, temperatures and vapour pressure deficits (Ludlow 1987).

Epidermal conductance (g_e)

Epidermal conductance (g_e) is the rate of evaporative water loss from the leaf after stomata have closed. Total conductance is the sum of water lost through both cuticular and stomatal leakages which occur in parallel (Holmgren *et al*. 1965). Cuticular conductance is generally a negligible fraction of total conductance when the stomata are open. However, in water-stressed leaves in which the stomata have closed, the cuticular component may exceed stomatal leakages (Boyer *et al*. 1997). Under severe water stress, stomatal closure is maximized and g_e controls the rate of water loss, which progresses until the critically low water content is reached and the leaf tissue dies. Although the rate of epidermal water loss is small, the water loss can be substantial relative to the amount of water available to severely stressed plants (Sinclair and Ludlow 1986).

Some studies have shown that low g_e is associated with increased drought tolerance and prolonged plant survival during severe water deficits. In a comparison of four grain legume species, soybean had the highest conductance, followed by black gram, cowpea and pigeonpea, and high conductance was associated with shorter survival duration (Sinclair and Ludlow 1986). Species adapted to arid environments tend to have low g_e (Riederer and Schreiber 2001; Smith *et al*. 2006).

Epidermal conductance is a reliable and stable physiological trait for drought assessment or indicator of plant water stress response. Firstly, it has been effectively used for drought evaluation in various species such as sorghum (Muchow and Sinclair 1989); durum wheat (Araus *et al*. 1991) and *Ficus* tree (Hao *et al.* 2010). Secondly, genotypic variation in g_e exists in a number of crops and can be

consistent across environments. In soybean, significant genotypic variation in g_e was found among 74 accessions (Paje *et al*. 1988). Although absolute g_e values were sensitive to temperature, relative humidity and water stress, the genotypic differences were consistent in different environments. For instance, there was a considerable agreement in g_e rankings for the same soybean accessions between studies by Paje *et al*. (1988) and James *et al*. (2008a). It was also suggested that genotypic rankings for g_e of stressed plants were more consistent than that of well-watered plants. Finally, g_e is not affected by leaf position and genotype x leaf position interactions (Hufstetler *et al*. 2007).

Selection for lower epidermal conductance could allow improvement in leaf survival, so that sufficient leaf area remains available for plant recovery after the stress is relieved (Paje *et al*. 1988). A significant negative correlation between g_e and water use efficiency under drought again suggest that lower g_e is a desirable trait for drought resistance (Hufstetler *et al*. 2007; Fish and Earl 2009). g_e values of perennial wild-type soybean were lower than those of various soybean cultivars and the annual wild soybean *G. soja* (James *et al*. 2008a). Among soybean genotypes, tropically adapted genotypes expressed the lowest g_e compared with the genotypes adapted to temperate regions. Selection pressure for survival under drier environments in tropical genotypes may result in the adaptability of the tropical genotypes. Broad-sense heritability estimates of g_e in 10 hybrid populations were moderate to high, suggesting the trait was moderate to highly heritable (James *et al*. 2008b).

2.4.2.2. Leaf area maintenance

Senescence that leads to leaf death and a decrease in leaf area is a highly regulated physiological process and can be induced by environmental stresses (Munne-Bosch and Alegri 2004). In legumes, water deficit slows down the production of new leaves and reduces leaf area by accelerating the rate of senescence, from oldest to youngest leaves. In soybean, water deficits lead to the firing of older leaves as water stress develops, whereas cowpea and pigeonpea respectively have the capacity for paraheliotropic leaf movement and leaf rolling (Lawn 1982; Muchow 1985). In a comparison of the response to terminal water deficit stress among cowpea, pigeonpea and soybean, the rate of senescence of the lower leaves was most rapid in soybean and slowest in cowpea (Likoswe and Lawn 2008).

Leaf area retention can be an informative indicator of plant water status as it is a contributory factor to plant recovery when stress is relieved. For instance, in soybean there is genotypic variation for leaf area maintenance during severe water stress (Lawn and Likoswe 2008), and plants with higher capacity for leaf survival had higher capacity for rapid recovery when stress was relieved. Oya *et al.* (2004) showed that soybean cultivars with higher drought tolerance maintained a larger leaf area during the stress period. Transgenic tobacco plants expressing delayed leaf senescence showed extreme drought tolerance and vigorous growth after a long drought period compared with the control plants (Rivero *et al*. 2007).

2.5. Utility of molecular markers in targeting physiological traits

In addition to the shortcomings of studying physiological traits mentioned earlier, various practical problems have confounded the successful application of physiological understanding in genetic improvement programs. Such problems include a lack of suitable genetic variants, interrelationships among traits and the "tyranny of numbers" (Marshall 1991). Developing uniculm cultivars in the small-grained cereals was difficult, for example, because of a lack of genetic variants. Multiple awns in barley did not contribute to improved yield because of associated negative pleiotropic effects including fewer kernels per head and reduced kernel weight (Rasmusson 1991). Increases in leaf stomatal frequency were off-set by reductions in stomatal size, which was an explanation for the absence of correlation between stomatal frequency and leaf conductance (Jones 1977). Hence, it can be a challenge to identify physiological traits worthy for selection. Even then, the desired yield improvements may not be achieved via breeding for ideotype traits or may be achieved only after considerable effort. Moreover, screening for traits controlled by more than two genes may require a larger number of recombinant individuals, which makes the case more complex and difficult (Marshall 1991 - "tyranny of numbers").

Recent developments in molecular tools and novel biotechnology based approaches offer the promise of providing additional information on the genetic basis of desirable traits, the nature of linkages with other traits, and likely changes under selection and so may assist major yield advances in crop improvement in the future (Edmeades *et al*. 2004; Dreisigacker 2012). For example, molecular genetic markers can alleviate the problems of working with physiological traits by offering easier manipulation of complex and difficult-to-measure traits. They can define the presence of "non-active" genes, identify where traits are controlled by several genes and reduce the need for elaborate and expensive screening procedures. Molecular genetic markers also potentially allow plant genotyping at almost any stage of development with small quantities of tissue and non-destructive sampling methods (Lawn and Imrie 1994a). Thus, they offer the possibility for adoption of new biotechnology discoveries into current plant breeding strategies (Gupta *et al*. 2010).

Various applications of molecular genetic markers include: (1) uncovering phylogeny and population structure of crop germplasm and characterizing or identifying genetic variation in parental germplasm (e.g. Cronk *et al*. 2006; Wang *et al*. 2008; Javadi *et al*. 2011; Datta *et al*. 2012; Raman *et al*. 2012); (2) identifying gene functions via quantitative trait loci or association mapping (e.g. Chen *et al*. 2007c; Abdel-Haleem *et al*. 2012; Isemura *et al*. 2012; Kajonphol *et al*. 2012) which in turn are implemented as marker-assisted selection (MAS) (e.g. Shen *et al*. 2001; Steele *et al*. 2006, 2013; Ribaut and Ragot 2007; Ma *et al*. 2010; Varshney *et al*. 2013); (3) linking genomes of related species via comparative mapping for gene discovery and comparisons of gene functions (e.g. Choi *et al*. 2004; Laurie *et al*. 2004; Zhu *et al*. 2005; Schlueter *et al*. 2008; Jung *et al*. 2012).

The utilization of molecular genetic markers in identifying genes or qualitative and quantitative trait loci (QTLs) associated with physiological traits, with specific emphasis on DArT markers, and on mungbean and soybean is described in the following sections.

2.5.1. QTLs – some basic concepts

Many important traits in crop plants, such as yield, quality, disease resistance or abiotic stress tolerance are quantitative and controlled by many genes, known as polygenes (Tanksley 1993; Mackay *et al*. 2009). Uncovering the genetic basis of these quantitative traits is of prime importance for plant breeding, genomics and germplasm utilization (Asıńs 2002).

A quantitative trait locus (QTL) is defined as "a region of the genome that is associated with an effect on quantitative trait" (Gelderman 1975). QTL analysis provides information about the nature of QTLs, such as where they are, what they do and how they act and interact (Kearsey and Farquhar 1998).

Genetic markers

Genetic markers are specific locations on a chromosome and serve as landmarks for genome analysis (Kumar 1999). Morphological, biochemical and DNA markers are three basic types of genetic markers (Collard *et al*. 2005; Segman *et al*. 2006a). Some group biochemical and DNA markers in the same category as molecular markers.

The emergence of new technologies and tools has facilitated the development of different DNA marker systems, which can be categorized based on their modes of polymorphism revelation: PCR-based polymorphisms, hybridization-based polymorphisms and DNA sequencing (Semagn *et al*. 2006a; Bilyeu *et al*. 2010; Jonah *et al.* 2011). DNA markers can be codominant or dominant based on whether they can distinguish between homozygotes and heterozygotes. DNA markers are not affected by either environment or plant developmental stage (Winter and Kahl 1995).

Various molecular marker systems have been continuously developed since the 1980s and improved over the last two decades to provide easy, fast and automated assistance to scientists and breeders (Semagn *et al*. 2006a; Gupta *et al*. 2008; Dreisigacker 2012). Restriction fragment length polymorphisms (RFLPs) were the first widely used DNA marker type, when hybridization technology and gene-derived probes (gene segments and/ or cDNAs) were first available (Botstein *et al.* 1980). Since then, amplified fragment length polymorphisms (AFLP) (Zabeau and Vos 1993; Vos *et al*. 1995) and random amplification of polymorphic DNA (RAPD) markers (Williams *et al*. 1990) (PRC-based polymorphisms) using degenerate or random primers, were introduced to cover the entire genome in a cost-effective manner. As DNA sequencing technology advanced in the mid-1990s, simple sequence repeats (SSRs) became preferred over AFLP and RAPD because they were co-dominant and more polymorphic and have high degree of amplification and reproducibility (Akkaya *et al*. 1992; Weising *et al.* 1992, 1998). Two marker systems, diversity array technology (DArT)

(Jaccoud *et al*. 2001) and single nucleotide polymorphisms (SNPs) (Jordan and Humphries 1994) which both provide high-throughput of the whole genome profile inexpensively have been developed and are becoming more widely used in studies of various species.

In addition, there are also various PCR-based polymorphisms such as ISSR (inter-simple sequence repeat amplification) (Zietkiewcz *et al*. 1994), PCR-SSCP (single-strand conformation polymorphism) (Orita *et al*. 1989), STMs (sequence-tagged microsatellite sites) (Beckmann and Soller 1990; Hearn *et al*. 1992), STS (sequence tagged site), CAPs (cleaved amplified polymorphic regions) (Konieczny and Ausubel 1993) and SCARs (sequence characterized amplified regions) (Paran and Michelmore 1993) (see the review by Semagn *et al*. 2006a).

Linkage maps

Linkage maps provide the position of and relative genetic distances between markers along chromosomes. Distance along a linkage map is expressed in centi-Morgan (cM), a unit which is converted from the Kosambi recombination fraction (Kosambi 1944) or Haldane mapping functions (Haldane 1919). Kosambi's mapping function allows some interference – the effect in which the occurrence of a crossover in a certain region reduces the probability of a crossover in the adjacent region (Kosambi 1944). Linkage between markers is usually expressed as the logarithm of the ratio of linkage versus no linkage, the so-called logarithm of odds (LOD). Normally, a maximum LOD of 3 – linkage is 1000 times more likely than no linkage – is required for linkage assertion (Morton 1955).

The principle of linkage map construction is the segregation of genes and markers via chromosome recombination during meiosis. In other words, a linkage map is based on the analysis of many segregating markers. Linked markers are grouped into linkage groups which indicate chromosome segments or entire chromosomes, but are not necessarily evenly distributed. The linkage map serves many practical biological purposes such as identifying the chromosomal regions containing genes and QTLs associated with target traits (Cheema and Dicks 2009).

Principles of QTL mapping

The principle of QTL analysis is the detection of associations between phenotype and genotype of markers based on correlative statistics. A mapping population is partitioned into different genotypic classes based on the presence or absence of a particular marker locus. A significant difference between mean trait values of classes indicates the association between the marker and the QTL (Kearsey 1998; Collard *et al*. 2005). This is because markers and QTLs with tight link will be inherited together in the progeny, leading to linkage disequilibrium. Then, the mean of the class with the tightly linked marker will be significantly different to that of the class without the marker. In contrast, markers and QTLs with loose or no linkage will independently segregate – linkage equilibrium – and the means of the classes will not be significantly different.

Major objectives of QTL mapping in plants are to increase the biological knowledge of the inheritance and genetic architecture of traits and to identify markers that can be used as indirect selection tools in breeding programs (Semagn *et al*. 2010).

2.5.2. Design of QTL mapping experiments to target physiological traits

2.5.2.1. Choice of mapping population

QTL mapping requires a segregating plant population derived through sexual reproduction. Preferably, selected parents should be highly contrasting phenotypes for the traits of interest (e.g. highly resistant and susceptible lines). In addition, the parents should be genetically divergent to enhance the identification of as many polymorphic markers as possible across the whole genome (Kumar 1999; Collard *et al*. 2005).

The choice of mapping population greatly depends on the intention of the experimenter, trait complexity, the timeframe as well as the resources available for undertaking QTL analysis (Semagn *et al*. 2010; Würschum 2012). Segregating populations can be an F_2 or F_2-derived population, backcrosses, recombinant inbred lines (RILs), near isogenic lines (NILs), backcross inbred lines (BILs) and double haploid (DH) populations. $F_{2:3}$ families allow the identification of additive and dominance gene action at specific loci. However, if DNA gets exhausted when F_2 or $F_{2:3}$ and backcross populations are used for mapping, a new segregating population will need to be regenerated for re-determination of both phenotype and genotype.

RILs developed from inbreeding of individual F_2 plants consist of a series of homozygous lines. NILs are obtained through repeated backcrossing and may also be referred to as BILs (Monforte and Tanksley 2000; Blanco *et al*. 2006; Jeuken *et al*. 2008). These populations consist of lines containing a single or a small number of genomic introgression fragments from a donor parent. Thus, only additive gene action can be determined (Kumar 1999). The main disadvantage of RILs, NILs and BILs is the time requirement for obtaining the desired generations (i.e. six to eight generations) (Robin *et al*. 2003; Collard *et al*. 2005; Tang *et al*. 2008) and limited number of effective meiotic recombination events (Keurentjes *et al*. 2011). DH populations are usually quicker to generate than RILs and NILs but are only possible in several species amenable to tissue culture, such as wheat, rice and barley. The major advantage of RILs, NILs, BILs and DH populations is the ability to conduct replicated experiments at several locations and in multiple years as the genotypes can be multiplied and reproduced without genetic change, resulting in improved statistical power (Semagn *et al*. 2010).

2.5.2.2. Size of mapping populations

The size of mapping populations is another key factor affecting the power of QTL detection. Theoretically, using 100 progeny could greatly overestimate phenotypic variances associated with a correctly identified QTL, whereas increasing the population size to 500 would lead to only a slight overestimate. A population size of 1000 would provide an estimate of the magnitude of the

phenotypic effect close to the actual value (Beavis 1998). This phenomenon has been called the Beavis effect.

In practice, many field studies have supported the theory of Beavis effects. For instance, the number of QTLs detected for barley stripe rust resistance increased from one to seven when the size of double haploid populations increased from 50 to 409 individuals (Vales *et al*. 2005). Similarly, as the population size increased from 122 to 976, the number of QTLs detected for plant height and grain yield in maize increased from 0 to, respectively, 14 and 7 (Schon *et al*. 2004). The number of QTLs detected and the proportion of genotypic variance explained by QTLs generally increased more with increasing the population size than with increasing the number of testing environments (Schon *et al*. 2004). Large mapping population sizes can also enable the detection of QTLs that have small effects on traits of interest. However, population sizes of > 500 are not practicable as they are laborious and costly for phenotyping and genotyping, and it is more useful to detect QTLs with major effects (Jeuken *et al*. 2008). A mapping population size of 50 – 300 individuals, combined with reasonably reliable phenotypic data and inter-marker spaces on the linkage map about 10 – 15 cM apart, together with an appropriate statistical method, will usually lead to the identification of QTLs (Bernardo 2008). However, with only 50 individuals, QTL effects would have to be very large to be detected and considered as useful QTLs.

2.5.2.3. Collection of phenotypic data

Good phenotyping means the collection of accurate and relevant data. Precision/ accuracy is required to minimize the experimental "noise" introduced by uncontrolled environmental and experimental variability. Relevance refers to the meaning of the data from a biological and an agronomic standpoint (Tuberosa 2012).

The basic phenotypic data required for QTL mapping are estimates of the phenotypic performance of individuals across environments. The power of QTL detection depends upon the sample size, the heritability of the trait, the genetic dissimilarity among progenies, the effect of the QTLs, and the environment used for phenotypic evaluation (Semagn *et al*. 2010; Würschum 2012). Although current molecular tools can provide high throughput with low cost genotyping, phenotyping imposes limits on sample sizes due to its laborious, time-consuming and high cost nature. High trait heritability is a prerequisite for reliable QTL results and low bias in the estimation of QTL effects.

Phenotypic data can be pooled over locations and replications for a line. It is also desirable to have replicated trials across locations for detection of QTL x environment interaction, if any. In addition, QTL analysis from multiple populations – a means of increasing the population size – provides evidence for consistency in the existence of QTLs in independent studies, as well as for detecting differences in QTL effects among different populations. It is suggested that pooled analysis from genetically similar populations may have higher power of QTL detection than single-population based analyses (Guo *et al*. 2006; Negeri *et al*. 2012).

2.5.2.4. Statistical methods for QTL mapping

The various mathematical methods for QTL mapping can be grouped based on their requirements for genetic maps: (a) those methods that do not require prior genetic linkage map construction (e.g. single marker analysis (ANOVA), statistical machine learning method, partial least squares regression); and (b) those methods that require a genetic map for the population (e.g. simple interval mapping, composite interval mapping, multiple interval mapping) (e.g. see the review by Semagn *et al*. 2010).

Single marker analysis is the simplest method for QTL detection, in which a t-test, analysis of variance (ANOVA) and linear regression are included, but which does not require a complete linkage map. However, the two major limitations of this statistical method are (i) the reduced chance of detecting a QTL that is further apart from the markers and in identifying associations with other QTLs and (ii) the underestimation of QTL effects because of recombination (Tanksley 1993; Collard *et al.* 2005).

Statistical machine learning (SML) divides data into independent training and tests subsets for model induction and evaluation, respectively. The contribution of each marker is determined during a recursive feature elimination procedure and the change in variance explained after each elimination is assigned to the marker that was removed. SML provides better estimates of QTL effects, greater precision and can identify markers linked to QTLs without a genetic map (Bedo *et al*. 2008).

The simple interval mapping (SIM) method makes use of linkage map information and evaluates the association between trait values and the target QTL in the intervals between adjacent pairs of linked markers (Lander and Bostin 1989). Using tightly linked markers for analysis will compensate for recombination between markers and QTLs, which is the major shortcoming of single marker analysis. However, SIM cannot determine genetic variance due to other QTLs if more than one QTL is segregating in a cross.

Composite interval mapping (CIM) analysis combines interval mapping with multiple regression analysis. In other words, linked markers treated as cofactors are incorporated into a multiple regression model (Zeng 1994). Thus, CIM takes into account the genetic variation due to other QTLs, resulting in more precise QTL mapping and greater power of QTL detection.

CIM is further extended to other approaches including so-called multiple interval mapping (MIM) and inclusive composite interval mapping (ICIM). MIM uses multiple marker intervals to fit multiple QTL models (Kao *et al*. 1999). In ICIM, all marker information is simultaneously considered and phenotypic values are adjusted by all markers retained in the regression equation except for the two flanking markers of the current mapping interval. Therefore, marker selection is conducted only once, which makes ICIM simpler and allows faster convergence speed than CIM (Li *et al*. 2007; Wang *et al*. 2011a).

However, there are always errors in any QTL analytical approach. As with any statistical test, two types of error, Type I and Type II, can occur in QTL mapping (Li *et al.* 2007; Li *et al*. 2012a). (1) A Type I error is a false positive, in which a segregating QTL is detected when in fact it is not present. (2) A Type II error is a false negative, in which a QTL is not detected when it actually exists. These two types of errors can be controlled by proper critical values (Type I) and determined by the experimental design and the size of the QTL effect (Type II). Statistically, an efficient QTL mapping method should have high detection power and low false discovery rate.

2.5.2.5 Variation in estimates of QTL effects

A major limitation of QTL mapping in a segregating population is that the detected QTLs and their estimated effects are generally only specific to that population (Holland 2007; Bernardo 2008; Negeri *et al*. 2011; Ding *et al*. 2011; Li *et al*. 2012b). This makes QTLs often not transferable to other populations and complicates comparison across studies. The discrepancies in QTLs identified in different mapping populations can result from the confounding effects of different environments and experimental procedures. Other causes are true genetic differences due to heterogeneity (Holland 2007), multiple alleles at a QTL locus and differences in allele frequencies between populations (Steinhoff *et al*. 2012). Thus, the combined analysis of multiple mapping populations which are connected by common parents, evaluated in common environments, and genotyped using a common set of genetic markers, is suggested to minimize these problems, to increase the power and precision of QTL detection and to provide comparable allele effects across founder parents (Coles *et al*. 2010; Negeria *et al*. 2011)

2.6. Diversity Array Technology (DArT)

As discussed above, there are various types of molecular markers which have been successfully applied to many agricultural species. However, the key limitation of existing markers is their high cost, as a sufficiently large number of polymorphic markers needs to be screened for whole genome coverage, especially for species/ crops with no available molecular data (called ‘orphan’ crops – Kilian *et al*. 2005). Other limitations include sequence dependence, limited throughput because of electrophoresis and gel reliance, and labour-intensity (Huttner *et al.* 2004).

Diversity Array Technology (DArT) was reported in 2001 (Jaccoud *et al.* 2001) and since then, has been successfully applied to different crops including some orphan crops. DArT is a sequence-independent, high throughput, reproducible and cost effective method which has been useful for genetic diversity studies, linkage map construction, QTL analysis as well as marker-assisted selection development, especially in orphan crops.

2.6.1. DArT – basic principles

DArT markers – hybridization-based markers – are polymorphic segments of genomic DNA that are present in a defined genomic representation. These markers are biallelic (either dominant or co-dominant) (Huttner *et al.* 2004). DArT uses microarrays to detect DNA polymorphisms at several hundred genomic loci in a single assay.

DArT methodology involves initial development of a discovery array, followed by a genotyping array for identification of polymorphic DArT markers. Generation of a discovery array involves complexity reduction of a metagenome sample (a pool of genomes representing the germplasm of interest) which includes restriction enzyme (RE) digestion (usually with *Pst*I and a frequent cutter), adapter ligation and amplification of adapter-ligated fragments for obtaining representations. The representations are then cloned and the individual clones are amplified and spotted onto microarray slides to serve as probes for a DArT array (Jaccoud *et al.* 2001; Kilian *et al*. 2005). The level of genetic diversity within the metagenome pool used for generation of the discovery array affects the efficiency of the DArT method.

For the genotyping array, representations of individual genomes/ tested samples are prepared using the same complexity reduction method, followed by labelling with different dye (Cy3- or Cy5-labelled random decamers) and hybridizing to the microarray slides. The slides are then scanned for fluorescence intensity. If there are significant differences in hybridization signal intensity among tested samples, the clones are polymorphic. The in-house software DArTsoft is used for the analysis of hybridization intensities and conversion to scores.

2.6.2. Current status of DArT and its application in different crops

DArT markers have been developed for variety of species, from the model plant *Arabidopsis* (Wittenberg *et al*. 2005) to crops such as rice, wheat, barley, sorghum (e.g. Wenzl *et al*. 2004; Akbari *et al*. 2006; Xie *et al*. 2006; Hearnden *et al*. 2007), cassava (Xia *et al*. 2005), sugarcane (Heller-Uszynska *et al*. 2011), bananas (Huttner *et al*. 2007; Kilian 2007; Risterucci *et al*. 2009), *Eucalyptus* (Sansaloni *et al*. 2010) and hop (Howard *et al*. 2011). Rice, a major cereal crop with a relatively simple genome, was used as model for the establishment of the DArT concept (Jaccoud *et al.* 2001).

Among legumes species, DArT was successfully applied to pigeonpea (Yang *et al*. 2006), chickpea and groundnut (Varshney *et al*. 2010; Thudi *et al*. 2011) and lupin (Vipin personal communication, 2012).

DArT has proved to be a genotyping technology applicable to any genome or complex genome mixture, to provide genetic differentiation in plant diversity studies and to improve the genomic coverage in genetic maps.

2.6.2.1. DArT in genetic diversity studies

DArT has demonstrated its advantages in genetic diversity studies in terms of speed of marker discovery and analysis and high-throughput. For instance, the number of fragments in representations was in the range of 8,600 to 17,000 when three combinations of restriction enzymes and 22 cassava genotypes were used for development of the discovery array. Then, the DArT genotyping array detected approximately 1000 polymorphic clones, suggesting an effective method for exhaustive fingerprinting of a germplasm collection (Xia *et al.* 2005). In pigeonpea, using 96 accessions and eight complexity reduction methods, DArT generated from 768 to 1536 clones and of these, nearly 700 were polymorphic (Yang *et al.* 2006). In rapeseed, 1547 DArT markers out of 11,520 clones were generated by two complexity reduction methods and 107 were polymorphic.

Levels of genetic diversity revealed by DArT markers in a number of crop species have been consistent with available phylogeny information or with those revealed by other markers. For example, genetic relationships among 96 *Cajanus* accessions revealed by DArT markers were consistent with the available information and systematic classification (Yang *et al*. 2006). The discrimination of 89 rapeseed genotypes based on DArT markers was also consistent with their phenology, genetic lineage and origin (Raman *et al*. 2012). In rice, DArT markers clustered 17 of 24 genotypes into groups which corresponded with SSR analysis results (Xie *et al.* 2006). This was also the case when similar clustering patterns of 436 cassava accessions from Africa and Latin America were revealed by both SSR and DArT markers (Hurtado *et al.* 2008).

Using DArT as a high-density genotyping method also allows direct comparison of genetic diversities at different levels (e.g. national, regional or individual genome level), and over time. For instance, wheat germplasm is most diversified in Australia, followed by the United States (US) and the United Kingdom (UK) (White *et al.* 2008). There were also significant genetic distances between wheat varieties between countries. The diversity change over time remained relatively constant in Australia and UK while there was an upward trend in the US germplasm. Even closely related cultivars could be distinguished (Akbari *et al*. 2006).

2.6.2.2. Use of DArT in construction of genetic linkage maps and QTLs studies

DArT markers have been used in the construction of genetic linkage maps in different species such as barley (e.g. Alsop *et al.* 2007, 2011; Steffenson *et al.* 2007; Wenzl *et al.* 2004, 2006; Hearnden *et al.* 2007), wheat (e.g. Akbari *et al.* 2006; Semagn *et al.* 2006c; Crossa *et al.* 2007; Zhang *et al*. 2012), sorghum (Bouchet *et al.* 2007; Mace *et al.* 2008), triticale (Alheit *et al*. 2011) and rapeseed (Raman *et al*. 2012). Among legumes, so far DArT markers have only been used for mapping pigeonpea (Yang *et al*. 2011), chickpea (Thudi *et al*. 2011) and lupin (Vipin personal communication, 2012).

DArT markers have produced broad covering and medium to high density maps which are equivalent to other marker-based maps or with even better coverage. For instance, a barley genetic map

containing 385 unique DArT markers was constructed with 7 linkage groups (LGs) of a total of 1,137 cM (Wenzl *et al.* 2004). The quality of this DArT map in terms of genome coverage, length, and double crossing over events was equivalent to that of RFLP based-maps. A single DArT assay in barley could cover more than 98% of the map as DArT markers were less clustered than other markers such as SSRs (Hearnden *et al.* 2007) and only tended to moderately cluster around centromeres (Akbari *et al.* 2006). In sorghum, 596 DArT markers constructed a medium-density genetic linkage map with even distribution over the genome and mapped to all 10 chromosomes (Mace *et al.* 2008). In wheat, DArT markers were less clustered than non-DArT markers in centromeric regions and uniquely detected on two small linkage groups of wheat chromosomes (Akbari *et al.* 2006).

However, several studies have indicated that the inclusion of DArT markers with other markers such as SSRs, RFLP, STS and ETS-SSRs (Expressed tagged sites – Simple sequence repeats) can provide a higher level of genomic coverage, including the telomeric regions - regions of repetitive DNA at the end of chromosomes. For instance, the addition of RFLP, STS, and SSR to DArT markers enhanced barley genome coverage from 1,137 cM (Wenzl *et al.* 2004) to 1,161 cM (Wenzl *et al.* 2006). Another barley linkage map with a high level of genomic coverage including the telomeric regions resulted largely from the inclusion of DArT and EST-SSRs markers (Hearnden *et al.* 2007). A combination of DArT, RFLP, SSR and AFLP markers expanded the wheat genetic map to 2,937cM compared with 2,383cM using a single DArT assay (Akbari *et al.* 2006).

Because DArT markers provide thorough coverage and high-dense genetic maps, they increase the power of QTL detection. For instance, DArT markers were used for QTL mapping of *Fusarium* head blight in barley and proved extremely useful in adding a large number of markers (>200) in very little time (Rheault *et al.* 2007). A large number of DArT markers widely spread across all chromosomes were detected to be significantly associated with stem rust (63), yellow rust (122), leaf rust (87), powdery mildew (61) and yield-related traits (213) in wheat. Most of these markers were in the same reported genomic regions or were associated with QTLs influencing the same traits (Crossa *et al.* 2007). Five QTLs associated with spikelet number and length and 1000-grain weight mapped on the linkage map constructed of DArT and SSRs (Zhang *et al*. 2012). Interestingly, DArT markers have been found very useful for monitoring genomic introgression in the cultivated species of pigeonpea from the wild species (Mallikarjuna *et al*. 2011).

2.7. Genomic resources in mungbean and soybean

There are limited genomic resources available for mungbean, reflecting the fact that there have been fewer than two published papers per year in the field of genomics in mungbean (Somta and Srinives 2007) and the first genetic map was only reported in 1992 (Young *et al*. 1992). In contrast, tremendous advances in all aspects of genomic research have been made in soybean (as reviewed by Shoemaker *et al*. 2003; Bilyeu *et al*. 2010; Chan *et al*. 2012) with the first genetic map constructed in

1987 (Palmer and Kilen 1987). However, recently, the development pace of molecular markers and genomic resources in mungbean has progressed significantly (Lambrides and Godwin 2007; Somta and Srinives 2007; Kumar *et al*. 2011; Varshney *et al*. 2013).

2.7.1. Molecular markers and genotyping platforms

DNA markers are indispensable for genomic studies. Although a range of marker systems including hybridization-based Diversity Array Technology (DArT) and sequence-based markers such as single nucleotide polymorphisms (SNPs) have been available (Semagn *et al*. 2006a, 2010), only several were developed specifically for mungbean. RFLPs, RAPDs and AFLPs were commonly employed in the first mungbean genomic studies (e.g. Young *et al*. 1992; Fatokun *et al*. 1993; Lambrides *et al*. 2000; Humphry *et al*. 2002, 2005; Chen *et al*. 2007a). Other markers recently developed for mungbean have involved limited numbers, such as SSRs of 20 (Kumar *et al*. 2002a, 2002b; Gwag *et al*. 2006), 12 (Somta *et al*. 2008) and 1493 (Tangphatsornruang *et al*. 2009) or ESTs (Expressed sequence tags) of 489 (Chen *et al*. 2008b). Other SSRs from legume species such as azuki bean, cowpea, common bean and soybean were also used in mungbean (e.g. Isemura *et al*. 2012). Moe *et al*. (2011) was the first to report the development of SNPs for mungbean.

In soybean, the development of molecular marker systems has been closely associated with DNA manipulation technologies at any given time. Similar to mungbean, RFLPs, RAPDs and AFLPs were the first generation of molecular markers developed and used in various soybean studies (e.g. Keim *et al*. 1990; Shoemaker and Specht 1995; Mian *et al*. 1998b; Cregan *et al*. 1999; Song *et al*. 2004; Matsumura *et al*. 2008), then later came SSRs (e.g. Chen *et al*. 2007c; Hwang *et al*. 2008; Wang *et al*. 2008). More recently, SNPs have also been developed (e.g. Zhu *et al*. 2003; Van *et al*. 2005; Varala *et al*. 2011). The year 2010 has signified a major break-through in soybean genome research with the first published report of an assembled reference genome of cultivated soybean (Schmutz *et al*. 2010). This was followed by reports on the re-sequencing of 17 wild and 14 cultivated soybean genomes (Kim *et al*. 2010; Lam *et al*. 2010).

2.7.2. Genetic maps and QTL mapping

In mungbean, only a few genetic linkage maps have been published (e.g. Young *et al*. 1992; Menancio-Hautea *et al*. 1993; Boutin *et al*. 1995; Lambrides *et al*. 2000; Humphry *et al*. 2002; Zhao *et al*. 2010; Kajonphol *et al*. 2012; Isemura *et al*. 2012). These maps were constructed from single F_2 or recombinant inbred line (RIL) populations from crosses between cultivated and wild parents, such as VC3980 x TC1966 (wild from Madagascar), Berken x ACC 41 (wild from Australia), JP229096 x JP211874 (wild from Myanmar) and KUML29-1-3 x W021 (wild from Australia). The population size ranged from 58 to 250 plants. The maps differed in length (691.7 – 1831.8 cM), in numbers of markers (102 – 430), in numbers of linkage groups (LGs) (11 – 14) and in adjacent marker distances

(1.78 – 10.2 cM). Only two maps, by Kajonphol *et al*. (2012) and Isemura *et al*. (2012), resolved 11 LGs which is the haploid chromosome number of mungbean.

Genes or QTLs associated with traits encompassing insect pests, diseases and seed-related characters in mungbean were mapped. Examples were QTLs conditioning bruchid resistance (Young *et al*. 1992; Chen *et al*. 2007a), powdery mildew resistance (Chaitieng *et al*. 2002; Humphry *et al*. 2003; Kasettranan *et al*. 2010), and hardseededness and seed size (Humphry *et al*. 2005). More recent are QTLs for mungbean yellow mosic virus (Chen *et al*. 2012), for various agronomic characters (Kajonphol *et al*. 2012) and for domestication-related traits (Isemura *et al*. 2012).

The genomic study of soybean began with tremendous efforts in building linkage maps and physical maps. Genetic maps were derived from one or more than one genetic population. Various segregating population types were used such as F_2, RILs, NILs and backcrosses (Keim *et al*. 1990; Cregan *et al*. 1999; Song *et al*. 2004; Choi *et al*. 2007). A large number of markers (11 – 2977) mapped on genetic maps with various lengths (1056 – 3771 cM) and number of LGs (4 – 35) (as reviewed by Bilyeu *et al*. 2010). It is suggested that the consensus linkage map of soybean has already been saturated.

Physical maps were also developed (Wu *et al*. 2004, 2008a; Shoemaker *et al*. 2008). By integrating available genetic maps and physical maps, the Soybean Consensus Map 4.0 with a resolution of 0.6 cM interval was built (Hyten *et al*. 2010b; Table 2.1). The whole-genome sequencing of soybean cultivar Williams 82 was performed using Sanger's method (Schmutz *et al*. 2010). Sequencing information together with marker information of the integrated maps (Song *et al*. 2004; Choi *et al*. 2007; Hyten *et al*. 2010a, 2010b; Lee *et al*. 2013) successfully assembled 950 Mbp (Megabase pair), representing 85% of the predicted 1115 Mbp of the soybean genome (Chan *et al*. 2012).

According to Bilyeu *et al*. (2010) and based on data collected in the SoyBase database (Grant *et al*. 2008), since the publication of the first soybean molecular linkage map in 1990, over 270 QTL mapping studies have been published and over 1,100 QTLs have been identified for over 85 traits up to the year 2007. Resistance to soybean cyst nematode (SCN) was the most studied trait followed by protein concentration, oil content, seed weight, plant height, and yield. Examples of QTLs associated with different traits in soybean include: agronomic traits such as plant height, leaf length and width (Orf *et al*. 1999; Kim *et al*. 2005; Chen *et al*. 2007c; Panthee *et al*. 2007); pod shattering (Funatsuki *et al*. 2006; Luo *et al*. 2012); yield-related traits including pods per plant, seed weight per plant, 100 seeds weight and yield (Chapman *et al*. 2003; Chen *et al*. 2007c); resistance to *Phytophthora sojae* (Burnham *et al*. 2003) and soybean cyst nematode (Heer *et al*. 1998; Concibido *et al*. 2004); and physiological traits such as salt tolerance (Chen *et al*. 2008a) and drought tolerance (Mian *et al*. 1996, 1998a; Specht *et al*. 2001; Du *et al*. 2009; Abdel-Haleem *et al*. 2012; Virgina *et al*. 2012). The accumulation of available genomic data in soybean and the addition of more user-friendly and high-throughput markers will intensify and increase the number of soybean studies in the coming years.

Table 2.1. Summary data for Soybean Consensus Map 4.0 (Hyten *et al.* 2010b; http://soybase.org/sbt/) including chromosome/ linkage group lengths, numbers of markers and average recombination rates

Chr. = Chromosome; LG = Linkage group

Chr.	LG	Length (cM)	Physical length (Mbp)	Number of markers	Average recombination rate (cM/Mbp)♪
1	D1a	98.4	55.2	226	1.8
2	D1b	140.6	51.6	249	2.7
3	N	99.5	47.5	249	2.1
4	C1	112.3	49.2	258	2.3
5	A1	86.7	41.7	269	2.1
6	C2	136.5	50.6	313	2.7
7	M	135.1	44.4	245	3.0
8	A2	146.7	46.8	384	3.1
9	K	99.6	46.8	277	2.1
10	O	132.9	50.9	275	2.6
11	B1	124.2	39.0	175	3.2
12	H	120.5	40.0	230	3.0
13	F	120	44.2	331	2.7
14	B2	108.2	49.5	230	2.2
15	E	99.9	50.5	292	2.0
16	J	92.3	37.3	286	2.5
17	D2	119.2	41.8	273	2.9
18	G	109.9	62.3	418	1.8
19	L	101.1	50.5	245	2.0
20	I	112.8	46.6	275	2.4
Total/Average		**2296.4**	**946.2**	**5500**	**2.5**

♪: Average recombination rates were obtained by dividing the total linkage distance (cM) by the total physical length (Mbp) for each linkage group (Lee *et al.* 2013). These estimates were not adjusted for differences in marker density.

The major limitation to QTL discovery in general, and in mungbean and soybean in particular, is the difficulty in obtaining accurate measurement of traits with low heritability. In addition, the narrow genetic base of the parents used to create genetic populations for study can influence the effectiveness of markers in polymorphism detection. Most of the QTLs detected in mungbean and soybean have not been confirmed by separate studies. However, in soybean, independent mapping studies with populations developed from different parents have frequently identified QTLs for the same trait in a similar region on the integrated linkage map. For example, a QTL on LG G for cyst nematode resistance was identified in 14 mapping populations (Concibido *et al.* 2004). The consistency of QTL location across mapping populations in independent studies indicates the existence of a 'real' QTL.

2.7.3. Marker-assisted selection (MAS)

Marker-assisted selection (MAS) refers to the integration and use of molecular markers and the results of QTL mapping into selection/ breeding practices for developing superior lines with enhanced biotic

or abiotic stress tolerance and improved yield. MAS has became routine in crop improvement because it is known to help breeders to increase efficiency and precision of selection, and especially, to select favourable combinations of genes in early generations without having to depend on generating the phenotype (Kelly *et al*. 2003; Kumar *et al.* 2011; Varshney *et al*. 2013). Important uses of MAS include: (i) selection of genetically diverse parents for making crosses; (ii) germplasm characterization and verification, including confirmation of hybrids derived from manual pollinations; (iii) marker-assisted introgression of useful genes; (iv) accurate selection of plants with genes conditioning low-heritability traits; (v) selection for resistance in the absence of a pathogen or pest; and (vi) gene pyramiding (Bilyeu *et al*. 2010).

In mungbean, for example, resistance to weather damage is conditioned by several seed and pod traits such as hardseededness and podwall density (Imrie *et al*. 1988). Incorporation of these traits or pyramiding of appropriate genes would be desirable for the development of a resistant ideotype. In addition, characteristics of the testa which are of maternal inheritance also influence seed appearance and weathering resistance. Thus, expression of seed phenotype can only be assessed when recombinant plants have grown through to maturity. The identification of commercially desirable traits such as shiny testa and solid green testa colour, which are recessive, would require additional selfed-generations. In these situations, MAS is able to identify specific genotypes at an early stage of development by using a small piece of cotyledon or seedling leaf tissue and/or without adding required generations (Lawn and Imrie 1994a).

In soybean, the long-juvenile trait showing a delayed flowering response under short day conditions is recessive (Ray *et al*. 1995). A selfed-generation in each backcrossing cycle is needed to recover the recessive homozygote for selection. Selection is complicated because repeated exposure of genotypes to relatively short photoperiods is required and the trait is difficult to reliably assess in segregating populations. Identification of generated markers, SCAR (sequence characterised amplified region) and RFLPs, linked to the long-juvenile locus proved to be useful for both the localisation of the gene associated with the character and for tagging the juvenile trait in soybean breeding programs (Cairo *et al*. 2002). MAS is also employed to screen and select for other traits such as a four-seeded pod trait (Zhu and Sun 2006) and resistance to lepidopteran pests (Walker *et al*. 2002) in soybean.

Many studies have identified molecular markers linked to QTLs conditioning stress tolerance, which opens the possibility of simultaneously transferring and pyramiding those QTLs into one improved cultivar (Dita *et al*. 2006; Cattivelli *et al*. 2008). For example, three QTLs were detected with strong effects on soybean traits (maturity, yield and other traits) in response to drought – a major constraint to soybean production (Specht *et al*. 2001). Ten QTLs associated with survival time of seedlings, percentage of seedling plant survival and visual salt tolerance ratings were detected (Chen *et al*. 2008a). Some of those QTLs were also co-located with resistance to soybean mosaic virus resistance and water stress.

For certain traits, MAS is quickly recognized as a cost-effective alternative to phenotypic selection. This is especially true if phenotyping requires expensive equipment and assays which are laborious and time-consuming or which can only be conducted at a certain stage of plant growth. For example, the cost per data point for MAS of soybean cyst nematode resistance (US$0.25–$1.00) was substantially less than the cost of phenotypic selection based on cyst counts (US$1.50–$5.00) (Concibido *et al*. 2004). Furthermore, MAS could be completed in 1 – 2 days whereas a longer time is required for phenotypic assays.

However, there are still major limitations to the practical application of MAS. The full complement of genes determining a trait is rarely known, particularly for traits with a low heritability. Most markers are near, but not within a QTL, so crossovers between the marker(s) and a QTL may occur. The success of MAS is dependent upon the initial quality of the phenotypic data and the QTL mapping studies. Therefore, verification of the target QTL for its magnitude of effects and accurate location on the genome is required to realize the potential of MAS (Kumar *et al*. 2011). Nevertheless, precision mapping and high-throughput MAS have been and will still be greatly aided by recent and ongoing technological advances with high-thoughput marker systems as DArT and SNP markers and genome sequencing.

2.8. Conclusion and objectives of this thesis

Improving mungbean and soybean productivity by developing varieties with desirable traits is the main goal of genetic improvement/ breeding programs. This requires long-term effort and a multidisciplinary approach. In both crops, one of the major constraints is limited genetic diversity in breeding programs. Wild species and landraces can be used to broaden the genetic base and so provide potentially useful sources of novel traits. However, in order to successfully introgress desirable traits into cultivars, it is necessary to understand the expression and inheritance of traits of interest. Molecular markers, genetic maps and QTL mapping are pivotal tools to dissect the genetic basis of traits.

Mungbean improvement is constrained by limited genomic resources. Only a few molecular marker systems have been developed so far. In contrast, all aspects of genomics in soybean have been more extensively studied. That said, one of the major and important objectives in current soybean improvement is breeding for drought tolerance through the identification of QTLs associated with different physiological drought stress responses. Although QTLs have been identified for several traits, only a few studies have attempted to detect QTLs conditioning plant water status traits (i.e. water use efficiency, relative water content and epidermal conductance). There have not been any studies on QTLs associated with epidermal conductance and leaf area maintenance in soybean under water stress.

DArT has been considered very useful for genetic studies because of its low cost, ultra-high-throughput and extensive genome-wide coverage. However, DArT markers have been developed and used in only a few legumes, including pigeopea, chickpea, groundnut and lupin. Given the advantages for DArT markers, the potential value of marker and related information for plant genetic improvement, and the general paucity of molecular marker and linkage group mapping information available for traits in mungbean, there is a strong case for research to develop and apply DArT marker protocol for mungbean. The case for analogous research in soybean is not as strong, given the extensive genomic research already undertaken on that species as outlined above. Nonetheless, given the paucity of information on molecular markers associated with drought stress response traits in soybean, there is a case for exploring the use of DArT markers for at least those traits in soybean. The concurrent development and application of DArT markers in both species should also provide opportunity for useful comparative study between these two legume species.

Based on the review, it was also concluded that, with some limitations which are discussed later, the two sets of germplasm that were available for study when the project commenced would provide a suitable basis for research. Accordingly, research was designed using these two germplasm sets to achieve the following overall objectives:

(i) To develop protocols for applying DArT markers in both soybean and mungbean. (This research and its outcomes are described in Chapter 3);

(ii) To evaluate the utility of DArT markers for detecting and mapping QTLs associated with diverse traits in mungbean. (The phenotypic evaluation of 54 traits is described in Chapter 4, while the QTL and mapping outcomes for these traits using DArT markers are reported in Chapter 5);

(iii) To evaluate the utility of DArT markers for identifying and mapping QTLs associated with drought stress responses traits in soybean. (This research and its outcomes are described in Chapter 6); and

(iv) To compare and contrast the utility of DArT markers in the two legumes species and to assess their likely utility for plant breeding. (This research is reported in Chapter 7).

CHAPTER 3. DEVELOPMENT AND INITIAL EVALUATION OF DIVERSITY ARRAY TECHNOLOGY (DArT) FOR SOYBEAN AND MUNGBEAN[1]

3.1. Introduction

As noted in Chapters 1 and 2, soybean and mungbean are important as annual, edible and nutritious seed crops for humans and animals (Singh and Singh 1992; Liu 1997) and have roles in sustainable agricultural systems (Graham and Vance 2003). While the genetic improvement of soybean has been more extensively studied than mungbean, genetic gains in both crops lag behind cereal crops (Summerfield and Lawn 1987; Zubair 2004; Singh 2005; Akibode and Maredia 2011). Mungbean still remains largely a village crop, with relatively few studies at the molecular level on its genetic diversity and useful traits (Khattak *et al*. 2008; Somta *et al*. 2008).

Also as noted previously, molecular markers provide a potentially useful tool for improving the rates of gain from plant breeding through their application to commonly accepted field techniques and the identification of markers associated with many physiological traits of interests to breeders (Lörz and Wenzel 2005; Kulwal *et al*. 2010), such as water use efficiency in soybean (Main *et al*. 1998) and seed weight in mungbean (Humphry *et al*. 2005). Of available types of molecular markers such as RFLPs, RAPDs, AFLP, SSRs and SNPs, DArT markers have a number of advantages (Kilian *et al*. 2005) (Chapter 2). The advantages make DArT potentially applicable to any species, regardless of how much DNA information is available (Huttner *et al*. 2004; Kilian *et al*. 2005).

DArT has been successfully developed for diverse plant species, including few legumes (reviewed in Chapter 2) but not yet for either soybean or mungbean. The successful application of DArT to pigeonpea (Yang *et al*. 2006) and chickpea (Thudi *et al*. 2011) suggested its application to other legume species such as soybean and mungbean is also feasible. This chapter reports the development of DArT arrays for soybean and mungbean, and an assessment of DArT markers in studies of genetic diversity within *Vigna*, and of the transferability of markers between soybean and mungbean, and between mungbean and other *Vigna* spp.

[1] A substantially similar version of this chapter was published as a peer-reviewed paper in the journal *Euphytica* (see Vu *et al.* 2012)

3.2. Materials and methods

3.2.1. Plant germplasm and sampling

The germplasm used to prepare the DArT soybean library/ array comprised five *G. max* cultivars and three related wild *Glycine* species (Table 3.1). The germplasm used for the mungbean library/array comprised two cultivars (ssp. *radiata*), two accessions of the wild progenitor (ssp. *sublobata*) and 22 accessions of more distantly related cultivated and wild *Vigna* species (Table 3.1).

Table 3.1. Germplasm used for DNA clonal library development for (a) Soybean and (b) Mungbean using DArT

Species and accession identity♪	Provenance / origin
(a) Soybean library (*Glycine* spp.)	
G. max cv. G2120	Cultivated variety, Asian Vegetable Research & Develop. Center
G. max CPI_A 26671	Cultivated variety, USA via Morocco
G. max cv. Valder	Cultivated variety, Australia
G. max AC Colibri	Cultivated natto variety, Canada
G. max OAC Morris	Cultivated oilseed variety, Canada
G. falcata ACC 648	Endemic wild perennial, Longreach, Australia
G. soja CPI 32899	Indigenous wild annual, China
G. tomentella	Indigenous wild perennial, Townsville, Australia
(b) Mungbean library (*Vigna* spp.)	
V. radiata v. *radiata* cv. Berken	Cultivated variety, USA
V. radiata v. *radiata* cv. Kiloga	Cultivated variety, USA
V. radiata v. *sublobata* ACC_B 1	Indigenous wild annual, Mackay, Australia
V. radiata v. *sublobata* ACC 87	Indigenous wild perennial, Townsville, Australia
V. lanceolata ACC 200	Endemic wild annual, Wyndham, Australia
V. lanceolata ACC 207	Endemic wild perennial, Dalby, Australia
V. lanceolata ACC 235	Endemic wild annual, Townsville, Australia
V. lanceolata ACC 251	Endemic wild perennial, Victoria River Downs, Australia
V. lanceolata ACC 257	Endemic wild perennial, Burdekin Valley, Australia
V. lanceolata ACC 296	Endemic wild perennial, Pentecost River, Australia
V. lanceolata ACC 905	Endemic wild perennial, Clermont, Australia
V. lanceolata X	F_1 hybrid, ACC 235 x ACC 200
V. lanceolata X	F_1 hybrid, ACC 251 x ACC 296
V. lanceolata X	F_1 hybrid, ACC 257 x ACC 235
V. lanceolata X	F_1 hybrid, ACC 296 x ACC 207
V. lanceolata X	F_1 hybrid, ACC 949 x ACC 235

♪: A = Commonwealth Plant Introduction number; B = Australian Native *Vigna* Collection accession number (Lawn and Watkinson 2002)

Table 3.1. Continued...

Species and accession identity	Provenance / origin
(b) Mungbean library (*Vigna* spp.) (cont...)	
V. mungo CPI 30067	Cultivated variety, Poona, India
V. mungo cv. Regur	Cultivated variety, Australia
V. *mungo* v. *sylvestris* ACC 701	Wild annual, Alotau, Papua New Guinea
V. *mungo* v. *sylvestris* ACC 703	Indigenous wild annual, India
V. *trilobata* ACC 702	Indigenous wild annual, Palanser, India
V. *trilobata* ACC 704	Indigenous wild annual, Mandi, Himachal Pradesh, India
V. *vexillata* ACC 390	Indigenous wild perennial, Harvey's Range, Australia
V. *vexillata* Jim 2	Cultivated variety, Bali, Indonesia
V. *vexillata* v. *macrosperma* CPI 69030	Semi-domesticate, Malakal, Sudan
V. *vexillata* X	F_1 hybrid, Jim 1A x ACC 390

Eleven *Vigna* spp accessions were selected for evaluation of their genetic diversity and apparent interrelations. The 11 accessions comprised four mungbean accessions (the two mungbean cultivars, Berken and Kiloga, and the two wild mungbean accessions, ACC 1 and ACC 87), four samples of *V. vexillata* (the wild accession ACC 390, *V. vexillata* v. *macrosperma* CPI 69030, the cultivated Bali accession Jim 2, and a cultivated Bali x wild hybrid, Jim 1A x ACC 390) and three wild accessions (ACC 207, ACC 235, ACC 296) of the Australian endemic taxon, *V. lanceolata* (see Table 3.1 for details).

Young fully expanded leaves were collected from vigorously growing seedlings, lyophilised at CSIRO Davies Laboratory in Townsville, and taken to the DArT laboratory in Canberra for DNA extraction and species library creation.

3.2.2. DNA extraction

Soybean DNA extraction followed standard DArT protocols (http://www.diversityarrays.com/sites/default/files/pub/DArT_DNA_isolation.pdf). The lyophilised leaf materials (approx. 0.03 – 0.1 g) were ground under liquid nitrogen to a fine powder and transferred to a 2 ml eppdendorf tube containing 1 ml of preheated 65^{o}C fresh buffer solution [(made of extraction buffer (0.35 M sorbitol, 0.1 M TrisHCl, 5 mM EDTA pH 8.0), lysis buffer (0.2 M TrisHCl, 0.05 M EDTA pH 8.0, 2 M NaCl, 2% CTAB), 5% (w/v) sarcosyl, 0.5 % (w/v) sodium disulfite and 2 % (w/v) PVP-40 (Sigma)]. The tubes were inverted every 20 min while being incubated at 65^{o}C for 1 hr. Samples were cooled to room temperature (RT) before 1 ml of a chloroform: isoamyl alcohol (24:1) mixture was added and mixed well for 30 min. Samples were then centrifuged at 10,000 x g at room temperature (RT) for 20 min, after which the aqueous phase was transferred to a fresh tube and mixed with the same volume of ice cold isopropanol. Tubes were inverted 10 times and centrifuged at 10,000 x g at RT for 30 min, after which the supernatant was discarded. The DNA pellets were washed by adding 2 ml of 70% ethanol and then centrifuged another

30 min at 10,000 x g at RT. The supernatant was again discarded and the pellets were dried in a 37°C incubator before being dissolved in 100 µl 1 x TE buffer (10 mM TrisHCl, 1 mM EDTA pH 8.0). DNA of soybean varieties AC Colibri and OAC Morris was isolated in Canada as described in Molnar *et al*. (2003).

A similar extraction protocol was followed for mungbean, with some minor modification as follows. After suspension of the ground plant material in 600 µl of fresh buffer solution and incubation at 65°C, 600 µl of saturated phenol solution (Sigma – Aldrich) and chloroform : isoamyl (24 : 1) mixture were added and mixed for 10 min. Tubes were then centrifuged at 10,000 x g at RT for 20 min. The aqueous phase containing DNA was transferred to new tubes and mixed with one volume of chloroform : isoamyl (24:1) mixture. Samples were again centrifuged at 10,000 x g at RT for 20 min. The aqueous phase was pipetted to new tubes and precipitated with 2 volumes of ice cold isopropanol. Samples were mixed gently, placed in a refrigerator to cool for 20 – 30 min while being inverted every 10 min, and then spun at 10,000 x g at RT for 30 min. After discarding the supernatant, the pellets were washed with 2 ml of 70% ethanol and dissolved in 20 µl 1 x TE buffer. In some cases, DNA repurification was undertaken using a DNA repurification kit [ZR - 96 DNA clean & concentratorTM - 5 (Zymo Research)] to obtain good DNA quality for genomic complexity reduction.

3.2.3. Genomic complexity reduction

Reduction of genomic complexity was achieved by digesting DNA extracts with various combinations of rare and frequent cutting restriction enzymes (RE), the ligation of an adapter to the ends created by the rare cutting RE, followed by amplification using primers specific to the adapter. Each RE combination consisted of the rare cutter *Pst*I and a frequent cutter. Initially, seven frequent cutters, *Taq*I, *BstN*I, *Alu*I. *Ban*II, *Hae*III, *Mse*I and *Msp*I, were tested on five soybean genotypes (G2120, CPI 26671, Valder, AC Colibri and OAC Morris) and four mungbean genotypes (Berken, Kiloga, ACC 1 and ACC 87). RE digestions were carried out simultaneously in the mixture containing 20 – 100 ng of DNA, 2 units of *Pst*I and 2 units of a frequent cutter. 0.05 µM of *Pst*I adapter (5'- CAC GAT GGA TCC AGT GCA - 3' annealed with 5'- CTG GAT CCA TCG TGCA - 3') was simultaneously ligated with 6 units of T4 DNA ligase (NEB) (Wenzl *et al*. 2004). Digestion and ligation reactions for the *Pst*I/*Taq*I and *Pst*I/*BstN*I combinations were performed at 37°C for 2 hr and then 60°C for 2 hr while other combinations were performed at 37°C for 3 hr. 1 µl of digestion ligation product was used as a template in a 50 µl PCR reaction with 2 units of Red Taq Polymerase (Sigma) and 2µl 10µM *Pst*I+0 primer (5' - GAT GGA TCC AGT GC AG - 3'). After incubation at 94°C for 1 min, followed by 30 cycles of 94°C for 20 s, 58°C for 40 s, 72°C for 1 min, a final extension was performed at 72°C for 7 min (Wenzl *et al*. 2004). PCR products were analysed on a 1.2% agarose gel.

3.2.4. Creation of libraries/ arrays

Based on gel electrophoresis of PCR products showing homogeneous smear without distinct bands, the *Pst*I/*Taq*I and *Pst*I/*BstN*I methods were chosen for library development for both soybean and mungbean. Libraries were prepared from amplified fragments. 2 µl of PCR products of each genotype were pooled and cloned using "TOPO TA Cloning" kit (Invitrogen) according to the manufacturer's instructions. The transformed bacterial solution was plated on to a medium supplemented with ampicillin (100 µg/ml) and X-gal (40 µg/ml) and placed in an incubator at 37^{o}C overnight (no longer than 16 hr). Individual white colonies were selected against blue colonies and grown in 384-well plates containing LB medium supplemented with 100 mg/L ampicillin and kanamycin and a freezing mix [1 x LB containing 4.4% glycerol, 8.21 g/L K_2HPO_4, 1.8 g/L KH_2PO_4, 0.5 g/L Na_3-citrate, 0.1 g/L $MgSO_4$ x 7 H_2O, 0.9 g/L $(NH_4)_2SO_4$]. Aliquots of the cultures were used as templates to amplify inserts in 384-well plates containing insert amplification mix consisting of 1 x Possum Taq buffer (homemade), 200 µM dNTPs, 0.2 µM M13 forward and reverse primers (Invitrogen) and 0.1 – 2.0 µl of Possum Taq. The reactions were incubated for 4 min at 95^{o}C, 35 s at 57^{o}C, 1 min at 72^{o}C, followed by 35 cycles of 94^{o}C for 35 s, 52^{o}C for 35 s, and 72^{o}C for 1 min. The amplicons were dried, washed with 1 volume of ice cold 77% ethanol and dissolved in a new spotting buffer developed specifically for poly-L-lysine microarray slides.

3.2.5. Printing and processing

Amplicons suspended in the spotting buffer were arrayed with two replicates onto Poly-L-lysine-coated microscope slides using MicroGrid II arrayer (Genomics Solution; Lincoln, NEB.). At least 1 day after arraying, the DNA deposited onto slides was denatured by incubation in hot water (92°C) for 2 min, followed by dipping in MiliQ water supplemented with 0.1 mM DTT (Dithiothreitol) and 0.1 mM EDTA and then dried by centrifugation at 500 x g for 7 min.

3.2.6. Genotyping of DNA samples

3.2.6.1. Target preparation and labelling

Genomic representations with two replicates were generated using the same complexity reduction methods as for library construction (i.e. *Pst*I/*Taq*I and *Pst*I/*BstN*I). They were precipitated with one volume of isopropanol, washed with 77% ethanol and dried. Each genomic representation was combined with 5 µl of Labelling Mix containing 1 x NEB buffer 2, 50 µM random decamers and labelling dNTPs (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 0.2 mM dTTP), denatured in a Corbett PCR machine at 95^{o}C for 3 min and cooled to 25^{o}C before labelling with 5 µl of Cy-dye mix containing 1 x NEB buffer 2, 25 µM cy3-dUTP or cy5-dUTP (Amersham) and 2.5 units of Klenow exo- fragment of E. coli polymerase I (NEB). The labelling reaction was performed at 37^{o}C for 3 hr.

3.2.6.2. Hybridization

Cy3 and cy5 labelled representations, called targets were mixed and added with 5 µl of Inactivation Mix (1 x NEB buffer 2, 60 mM EDTA pH 8.0) and 50 µl of DArT hybridizer preheated to 65°C [FAM labelled polylinker of the pCR 2.1 vector, ExpressHyb (Clontech), 10 mg/ml herring sperm DNA (Promega), 2 mM EDTA pH 8.0]. The polylinker fragment was used as a reference to determine the amount of DNA spotted on the array. After denaturing at 95°C for 3 min followed by 56°C for 5 min, labelled targets were pippeted to the microarray surface, covered with a glass coverslip and incubated in a 65°C water bath overnight. Coverslips were removed, slides were placed into slide-racks and rinsed in 1 x SSC (Saline Sodium Citrate) with 0.1% SDS (Sodium Dodecyl Sulfate) for 5 min, 1 x SSC for 5 min, 0.2 x SSC for 2 min, 0.02 x SSC for 30 s and deionised water for 1.5 min, all with 0.5M DTT and at RT. Slides were quickly dried by centrifugation at 500 x g for 7 min and then in a dark desiccator under vacuum with silica gel for 30 min (Jaccoud *et al*. 2001; Wenzl *et al*. 2004).

3.2.6.3. Scanning, image analysis and data manipulation

Slides were scanned using the Affymetrix 428 (Santa Clara, CA) or Tecan LS300 (Grödig, Austria) confocal laser scanner. DArTsoft version 7.4 was used to automatically analyse TIF images derived from slide scanning, and to identify and score polymorphic clones as described in Wenzl *et al*. (2004) and Yang *et al*. (2006). The relative hybridisation intensity of each clone on each slide was determined by dividing the hybridisation signal in the target channel (genomic representation) by the hybridisation signal in the reference channel (polylinker) (= log[cy3target/cy5reference]). Clones with variable relative hybridisation intensity across slides were subjected to fuzzy k-means clustering to convert relative hybridisation intensities into binary scores (presence vs. absence). A combination of marker quality parameters automatically generated from DArT soft was used as quality thresholds for the identification of polymorphic clones: (a) Reproducibility of 100% (percentage of identical results for replicated spots) and (b) Call rate of 100% (percentage of effective scores). Sample dissimilarity matrixes were also generated by DArT soft.

DArTtoolbox was used for score merge analysis, which checked the quality of data from replicates and identified shared polymorphic clones between the two complexity reduction methods (*Pst*I/*Taq*I and *Pst*I/*BstN*I). Polymorphic clones were selected with Consensus of 100% and Call rate of 100%. Shared clones were defined as ones which, when hybridized with *Pst*I/*Taq*I and *Pst*I/*BstN*I genomic representations, produced the same binary score.

3.2.7. Development of full-size arrays

Based on an initial hybridization test generated from the five individual soybean genotypes (G2120, Valder, CPI 26671, OAC Morris and AC Colibri) or the four mungbean genotypes (Kiloga, Berken, ACC 1, ACC 87) against 3072 clones, the *Pst*I/*BstN*I method was selected for expansion of each species' library by an additional 4608 random clones. For the soybean library expansion, 3840 clones

from the five soybean genotypes and 768 clones from the three wild *Glycine* species were generated. The full soybean library was printed in duplicate on the poly-L-lysine-coated slides and then evaluated by hybridization using genomic representations generated from both the *Pst*I/*Taq*I and *Pst*I/*Bst*NI methods.

For the mungbean library expansion, 2304 clones from the pool of mungbean and wild mungbean, and 768 clones from each of the pool of 12 *V. lanceolata* accessions, the pool of *V. mungo*, *V. mungo* var. *sylvestris*, and *V. trilobata* and the pool of four *V. vexillata* accessions, were generated. The full mungbean library was printed in duplicate on the poly-L-lysine-coated slides and then evaluated by hybridization using genomic representations generated from only the *Pst*I/*Bst*NI method.

3.2.8. DArT marker transferability between species

Cross hybridization between soybean and mungbean libraries with genomic representations of mungbean and soybean DNA respectively was tested. Ten 384-well plates generated by *Pst*I/*Bst*NI method from the five soybean and the four mungbean samples were selected from each of the soybean and mungbean libraries and printed in duplicate. Genomic representations with two replicates were generated from three soybean samples (G2120, CPI 26671 and Valder) and four mungbean samples (Berken, Kiloga, ACC 1 and ACC 87) using only the *Pst*I/*Bst*NI method. In a separate assessment of DArT transferability among the different *Vigna* spp, full size mungbean libraries were printed in duplicate. Genomic representations with two replicates were generated from 11 selected *Vigna* samples above using the *Pst*I/*Bst*NI method (Table 3.1).

Evaluation of genetic diversity among Vigna species using the mungbean library

From the assessment of DArT transferability among *Vigna* spp, polymorphic DArT clones with Reproducibility of 100% and a Call Rate of 100% were selected for genetic diversity analysis using NTSYS pc2.1. Simple Matching coefficients were used to calculate genetic similarity. The dendrogram showing the relations between accessions was made using the SAHN (sequential, agglomerative, hierarchic, non-overlapping clustering) method with UPGMA (unweighted pair-group method using the arithmetic average) (Rohlf 2000).

3.3. Results and Discussion

3.3.1. Complexity reduction and development of small arrays

Genomic complexity reduction is a critical step in the DArT technique to prepare genomic representations. Results from genomic complexity reduction of several other plant genomes such as barley (Wenzl *et al*. 2004), pigeonpea (Yang *et al*. 2006) and oat (Tinker *et al*. 2009) suggested that digestion with the *Pst*I RE together with a more frequently cutting RE, combined with adapter ligation-based amplification of intact *Pst*I fragments, is an efficient method. Therefore, seven combinations of *Pst*I and a frequent cutting RE (*Taq*I, *Bst*NI, *Alu*I. *Ban*II, *Hae*III, *Mse*I and *Msp*I)

were tested using a pool of genomic DNA from the five soybean genotypes or the four mungbean genotypes. Gel electrophoresis of soybean PCR products showed homogenous smears without visible and distinct bands for all seven combination methods. Mungbean PCR products also showed homogenous smears, except for *Pst*I/*BstN*I, *Pst*I/*Hae*III and *Pst*I/*Mse*I which showed slightly visible bands (data not presented).

Based on the experience with library creation for other plant genomes, the combinations of *Pst*I/*BstN*I and *Pst*I/*Taq*I, which produced more polymorphic fragments than other RE combinations, were selected for both soybean and mungbean library development. There were differences in the percentage of polymorphic clones revealed by each complexity reduction method (Table 3.2). The *Pst*I/*BstN*I array had slightly fewer polymorphic clones than the *Pst*I/*Taq*I array in soybean (i.e. 10% and 12.3% compared with 10.5% and 13.4% respectively). However, more polymorphic clones were identified using the *Pst*I/*BstN*I targets (12.9% compared with 10.2%), even within the *Pst*I/*Taq*I array (13.4% compared with 10.5%). Thus, *BstN*I as a co-digesting enzyme was superior to *Taq*I in soybean. The polymorphism levels reported here in soybean are broadly similar to those in some other species e.g. the *Pst*I/*Taq*I and *Pst*I/*BstN*I methods respectively resulted in 10.4% and 10% polymorphism in barley (Wenzl *et al*. 2004), 14.6% and 17.2% in cassava (Xia *et al*. 2005), and 9.4% and 5.3% in wheat (Akbari *et al*. 2006).

Table 3.2. Polymorphic clones identified in (a) Soybean and (b) Mungbean libraries/ arrays from the initial hybridization tests

Library/Array		**DArT Clones**						
Method	**Sources of DNA samples**	**Total number of generated clones**	**Polymorphisms for *Pst*I/*Taq*I targets**		**Polymorphisms for *Pst*I/*BstN*I targets**		**Shared polymorphic clones**	
			No.	%	No.	%	No.	%
(a) Soybean								
*Pst*I/*Taq*I	G2120 CPI 26671 Valder	1536	161	10.5	206	13.4	33	2.1
*Pst*I/*BstN*I	AC Colibri OAC Morris	1536	154	10.0	189	12.3	35	2.3
Total		3072	315	10.2	395	12.9	68	2.2
(b) Mungbean								
*Pst*I/*Taq*I	Berken Kiloga	1536	389	25.3	331	21.5	197	12.8
*Pst*I/*BstN*I	ACC 1 ACC 87	1536	379	24.7	415	27.0	190	12.4
Total		3072	769	25.0	746	24.3	387	12.6

In contrast to soybean, the frequencies of polymorphic clones in mungbean (Table 3.2) were nearly the same for both methods (25% and 24.3% for *Pst*I/*Taq*I and *Pst*I/*BstN*I, respectively). Also in contrast to soybean, the number of polymorphic clones identified in the *Pst*I/*Taq*I array was higher for the *Pst*I/*Taq*I targets than for the *Pst*I/*BstN*I targets (i.e. 389 compared with 331). In addition, the numbers of polymorphic clones in the mungbean arrays were around twice those in the soybean arrays (769 and 746 in mungbean compared with 315 and 395 respectively in soybean). The inclusion of the wild mungbean progenitors, which are phenotypically very different from cultivated mungbean, is a likely reason for the higher polymorphism level in the mungbean library.

The percentage of shared polymorphic markers between the two methods was around six times higher in the mungbean libraries than in the soybean libraries (12.6% compared with 2.2%). Soybean is believed to be an ancient tetraploid with multiple homeologous regions and large numbers of duplicate loci detected throughout the genome (Shoemaker *et al*. 1996; Lee *et al*. 2001; Schmutz *et al*. 2010) while mungbean is more similar to the ancestral diploid genome (Boutin *et al*. 1995). Consistent with this, the soybean genome size is two times as large as mungbean (1,100 Mbp compared with 520 Mbp) (Choi *et al*. 2004). The number of shared markers in the soybean library may have been underestimated to the extent that same-sequence clones were not detected using the DArT approach. In total, there were 1128 (36.7%) and 642 (20.9%) polymorphic clones identified from 3072 generated DArT fragments in the mungbean and soybean libraries respectively. Those totals could be overestimates because of sequence redundancy, which was shown in oat (Tinker *et al*. 2009) and *Arabidopsis* (Witternberg *et al*. 2005).

3.3.2. Development of full size arrays

As the *Pst*I/*BstN*I method produced more polymorphic markers in soybean and a good level of polymorphism in mungbean in the initial testing, the *Pst*I/*BstN*I RE combination was selected for library expansion. A total of 7680 fragments were generated, of which 1536 were from the *Pst*I/*Taq*I method and 6144 were from the *Pst*I/*BstN*I method (Table 3.3). More polymorphic clones were identified using *Pst*I/*BstN*I than the *Pst*I/*Taq*I targets in soybean (i.e. 11.7%, 12.1%, and 9.6% compared with 11.1%, 10.8% and 7.3% respectively), as similarly occurred in the initial screening. In total, 907 (11.8%) and 804 (10.4%) polymorphic clones were identified using the *Pst*I/*BstN*I and *Pst*I/*Taq*I targets, respectively, a rate quite consistent with the results obtained with the initial smaller array (12.9% and 10.2%).

There were 256 shared polymorphic clones identified in the soybean libraries, which accounted for a small percentage (3.3%) of the total clones generated, very similar to the level in the smaller array test (2.2%). The wild *Glycine* genotypes produced the fewest polymorphic clones, with an average of only 7.3% to 9.6% being polymorphic. They also had the fewest shared polymorphic clones between the two methods with only three clones (0.4%) being polymorphic. This low polymorphism level could be due to cytogenetic differences between *G. falcata, G. tomentella* and *G. max* (Hymowitz *et al*.

1998). However, the inclusion of *G. soja* which belongs to the same gene pool as soybean (Singh and Hymowitz 1989) still did not produce more polymorphic clones. The *Pst*I/*Taq*I and *Pst*I/*BstN*I arrays had similar levels of shared clones (3.5% and 3.7% respectively – Table 3.3).

Table 3.3. Polymorphic DArT clones identified in full size arrays for (a) Soybean and (b) Mungbean

Library/Array		**DArT clones**						
Method	**Sources of DNA samples**	**Total number of generated clones**	**Polymorphism for *Pst*I/*Taq*I targets**		**Polymorphism for *Pst*I/*BstN*I targets**		**Shared polymorphic clones**	
			No.	%	No.	%	No.	%
(a) Soybean								
*Pst*I/*Taq*I	G2120 CPI 26671 Valder	1536	170	11.1	180	11.7	53	3.5
*Pst*I/*BstN*I	AC Colibri OAC Morris	5376	578	10.8	653	12.1	200	3.7
*Pst*I/*BstN*I	*G. falcata* *G. soja* *G. tomentella*	768	56	7.3	74	9.6	3	0.4
Total		7680	804	10.4	907	11.8	256	3.3
(b) Mungbean								
*Pst*I/*Taq*I	Berken Kiloga	1536	n.a.		401	26.1	n.a.	
*Pst*I/*BstN*I	ACC 1 ACC 87	3840	n.a.		1036	27.0	n.a.	
*Pst*I/*BstN*I	*Vigna* spp	2304	n.a.		320	13.9	n.a.	
Total		7680	n.a.		1757	22.9	n.a.	

n.a. = Not applicable

In the mungbean array, using only the *Pst*I/*BstN*I for generation of genomic representations, 1757 (22.9%) polymorphic clones were identified. The percentage of polymorphic clones identified from the distantly related *Vigna* spp array was about half that from the cultivated and wild mungbean arrays (13.9% compared with 26.1% and 27.0%). Similar to the results obtained with the initial smaller array, mungbean had around twice the number of polymorphic clones as soybean (1757 and 907 respectively). Thus, the *Vigna* samples used to generate the library were more diverse than the soybean samples. This was illustrated by the relatively smaller dissimilarity coefficients among the five soybean varieties which ranged from 0.26 to 0.76 (Table 3.4a), compared with 0.16 to 0.85 among the cultivated and wild mungbean samples (Table 3.4b). Even between the two wild mungbean accessions, ACC 1 and ACC 87, the results indicated dissimilarity of 0.39. These measures were consistent with the observed large differences in phenotypic characteristics among the four mungbean genotypes. While the two soybean samples from Canada, AC Colibri and OAC Morris, were somewhat more different from the other three soybean samples, Valder, CPI26671 and G2120, with

0.41 to 0.76 dissimilarity, the general similarity among the soybean genotypes was reflected in the fewer polymorphic markers identified in soybean. This finding was consistent with the reported narrow genetic base in soybean (Delannay *et al*. 1983; Skorupska *et al*; 1993; Gizlice *et al*. 1994, 1996; Zhou *et al*. 2000; Hwang *et al*. 2008). However, about a thousand polymorphic DArT clones were identified, which illustrated the potential application of DArT in soybean genetic studies.

Table 3.4. Dissimilarity coefficients among (a) Soybean and (b) Mungbean samples based on *Pst*I/*Bst*NI targets

(a) Soybean					**(b) Mungbean**			
	Valder	OAC Morris	CPI 26671	AC Colibri		ACC 87	Berken	ACC 1
OAC Morris	0.41				Berken	0.69		
CPI 26671	0.26	0.61			ACC 1	0.39	0.81	
AC Colibri	0.70	0.38	0.76		Kiloga	0.67	0.16	0.85
G2120	0.41	0.75	0.25	0.73				

3.3.3. DArT marker transferability between species

A subset of 10 random 384-well plates containing DArT clones was selected from each of the soybean and mungbean libraries to investigate transferability between species. The modified array thus comprised a total of 7680 clones representing equally the two genomes (3840 clones from soybean and 3840 from mungbean arrays). At 13.6%, the rate of soybean marker transferability to mungbean (Table 3.5) was nearly five times higher than that of mungbean to soybean (3.1%). The total number of clones on the modified array identified as polymorphic for soybean was only one third of that for mungbean (419 vs 1930, respectively), which again revealed the narrow diversity in the soybean genotypes evaluated. More polymorphic clones in the soybean array were detected for mungbean than for soybean (522 compared with 301). Again, the rate of soybean marker transferability may have been overestimated to the extent that it has a larger proportion of duplicate loci throughout its genome.

Among mungbean and the other *Vigna* species, the total number of polymorphic DArT clones was highest for mungbean (1757), followed by *V. vexillata* (1032) and *V. lanceolata* (300) (Table 3.5). The percentage of DArT marker transferability among mungbean and the other *Vigna* spp ranged from a low of 3.4% to a high of 20.2%, depending on the combination. Generally high DArT marker transferability rates (10.0 – 20.2%) were obtained across mungbean and *V. vexillata* (Table 3.5) while the lowest transferability rates were obtained across *V. lanceolata* (3.4% – 3.6%). Interestingly, *V. lanceolata* DArT marker transferability across mungbean and *V. vexillata* was high (10.0 and 14.5%, respectively). *V. mungo* and *V. trilobata* are taxonomically closer to mungbean than to *V. vexillata* (Lawn 1995) but their DArT transferability to mungbean (11.5%) was only half that of *V. vexillata*

(20.2%). The *V. vexillata* array produced polymorphism frequencies very similar to the mungbean array (20.2% compared with 26.7%). This finding was consistent with inheritance studies (James and Lawn 1991; James *et al*. 1999; Damayanti *et al*. 2010b), which have identified analogous expression and apparently similar genetic control of both qualitative and quantitative traits in *V. radiata* and *V. vexillata*.

Table 3.5. Polymorphic DArT clones identified in cross – species hybridization (a) between soybean and mungbean and (b) among mungbean and other *Vigna* species

Library/ Array	Number of tested clones	Polymorphic DArT clones♪							
		Soybean		Mungbean		*V. vexillata*		*V. lanceolata*	
		No.	%	No.	%	No.	%	No.	%
(a) Soybean and mungbean									
Soybean	3840	301	7.8	522	13.6	-	-	-	-
Mungbean	3840	118	3.1	1408	36.7	-	-	-	-
Total	7680	419	5.1	1930	25.1	-	-	-	-
(b) Mungbean and other *Vigna* species									
Mungbean	5376	-	-	1437	26.7	662	11.5	181	3.4
V. vexillata	768	-	-	155	20.2	149	19.4	26	3.4
V. lanceolata	768	-	-	77	10.0	111	14.5	65	8.5
V. mungo	768	-	-	88	11.5	110	14.3	28	3.6
V. mungo v. *sylvestris*									
V. trilobata									
Total	7680	-	-	1757	22.9	1032	13.4	300	3.9

♪: Underlined values indicate the rate of across-species transferability of DArT markers

Cross-species transferability of DArT markers were observed with limits across two *Eucalyptus* genera (Sansaloni *et al*. 2010) and applied to construct linkage maps of wheat or triticale using wheat, triticale and rye DArT arrays (Tyrka *et al*. 2011; Zhang *et al*. 2012). The transferability rates of DArT markers between soybean and mungbean and among mungbean and other *Vigna* species shown in Table 3.5 are the first cross-species transferability rates reported for DArT markers in legume, and were toward the lower end of the range reported for other markers in legumes. SSR markers exhibited moderately high cross-species transferability, ranging from 29.4% to 61.7% across the seven legumes, soybean, pigeon pea, berseem clover, black gram, field pea and barrel medic (Choudhary *et al*. 2009). Similarly, 30%, 42%, 65% and 75% of SSR markers from cowpea, common bean, adzuki bean and black gram respectively were transferable to mungbean (Zhao *et al*. 2010). Transferability rates for field pea Sequence Tagged Microsatellite Site (STMS) primers across vetch, lentil, and chickpea were likewise high, at 39%, 60%, and 62% respectively, although the transferability of chickpea STMS primers was low, at 5%, 3% and 18% for lentil, vetch and field pea respectively (Pandian *et al*. 2000).

The level marker transferability across species can be used as an indication of the level of sequence conservation and of genetic relationship between those species. As shown in Pandian *et al*. (2000), the high level of successful transferability of STMS primers across field pea, chickpea, vetch and lentil indicated a high level of sequence conservation among the flanking regions of microsatellites regions. In addition, since field pea, vetch and lentil are taxonomically closer and belong to the same tribe, *Vicieae* (Davidsion and Davidsion 1993), field pea STMS primers had the higher level of transferability rates across vetch and lentil than chickpea. Similarly, Choi *et al*. (2004) supported gene orthology among seven legumes (barrel medic, alfalfa, pea, soybean, mungbean, common bean and bird's foot trefoil) when they employed their gene-specific PCR primers to test for amplification and polymorphism and to construct phylogenetic relationships.

In this study, the transferability of DArT markers also indicated a level of sequence conservation among species as the DArT approach is hybridization based. Moreover, the relationship among soybean, mungbean and several *Vigna* spp included in the library development can be deduced from the higher rates of DArT transferability from mungbean to the other *Vigna* spp than to soybean and from *V. vexillata* to mungbean than to *V. lanceolata*.

The rates of DArT marker transferability between soybean and mungbean suggests their potential for comparative genomic studies which employ common sets of markers among species and genetic mappings. Several comparative genome mapping studies among legumes species including soybean and mungbean detected different levels of conservation in their genomes during evolution. Boutin *et al*. (1995) reported only short and scattered linkage blocks with two or three markers were conserved between mungbean and soybean whereas Lee *et al*. (2001) revealed that homeologous segments of soybean chromosomes showed a high degree of synteny with chromosomes of common bean and mungbean. However, the Lee *et al*. (2001) study failed to detect complete regions of putative ancestral homology due to the limited number of markers tested and low levels of marker polymorphism. Low levels of polymorphism, together with high level of gene duplication, can also constrain and complicate comparative analyses (Zhu *et al*. 2005), as in case between soybean and *Medicago truncatula* (Choi *et al*. 2004). The DArT technique therefore is potentially an effective method for comparative genomic studies, as thousands of markers can be tested in a single assay.

3.3.4. Analysis of genetic similarity among Vigna species

Given the observed level of marker transferability between mungbean and the other *Vigna* species (Table 3.5), the utility of the mungbean libraries for studying genetic similarities among this group of species was evaluated using a subset of 11 *Vigna* accessions. 1125 polymorphic DArT clones were selected for dendrogram construction. Cluster analysis of the 11 *Vigna* samples yielded a dendrogram comprising three main clusters, each of which corresponded with a different *Vigna* sub-genus (Figure 1). Cluster I comprised the four *V. vexillata* samples which exhibited generally high similarity coefficients (> 0.95). Taxonomically, *V. vexillata* is located within the subgenus *Plectrotopis*, a group

that is considered intermediate between the 'African' and 'Asian' *Vigna* species (Verdcourt 1970). Cluster II comprised the four mungbean accessions, which taxonomically are located within sub-genus *Ceratotropis* (the 'Asiatic' *Vigna* species – Lawn 1995). Cluster III comprised the three accessions of the endemic Australian species, *V. lanceolata*, which taxonomically is located within the sub-genus *Vigna* (Lawn and Watkinson 2002) (Figure 3.1).

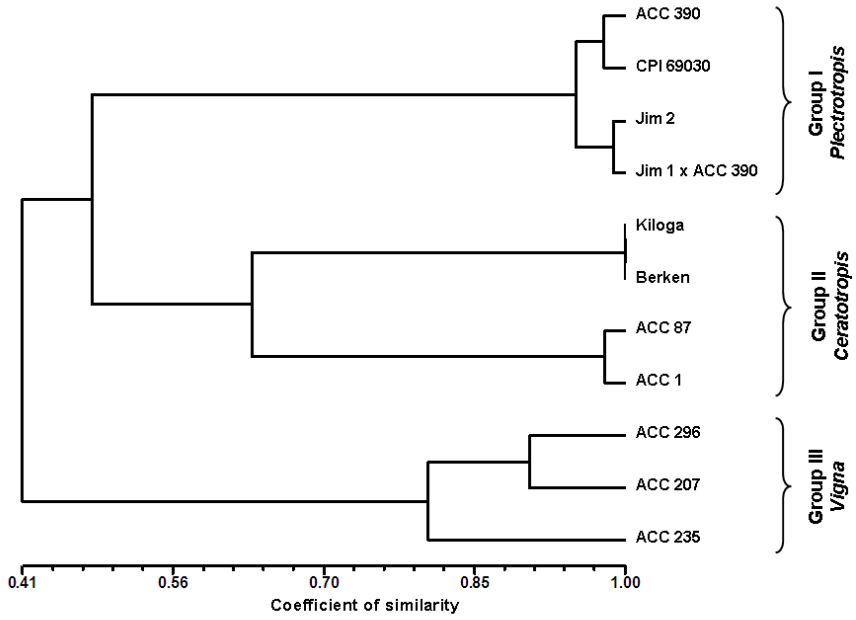


Figure 3.1. A dendrogram depicting the putative genetic relations among 11 *Vigna* spp accessions based on DArT markers. Taxonomically, the accessions in Groups I, II and III are located in sub-genus *Plectrotropis*, sub-genus *Ceratotropis* and sub-genus *Vigna*, respectively.

The four *V. vexillata* samples were sub-divided in turn into two sub-clusters (Figure 3.1), the first comprising the wild accession ACC 390 and *V. vexillata* v. *macrosperma* and the second comprising the cultivated Bali accession Jim 2 and a self-sterile F_1 hybrid between the cultivated Bali accession Jim 1A and the wild accession ACC 390. This sub-division matches a recent study (Damayanti *et al*. 2010a) which has shown that the cultivated Bali accessions exhibit genetic incompatibilities with both the wild Australian accession ACC 390 and *V. vexillata* v. *macrosperma*, which in turn are genetically quite compatible and so belong to the same primary genepool. The four mungbean samples were also sub-divided into two sub-clusters, respectively comprising the two cultivars, Berken and Kiloga, and the two wild accessions, ACC 1 and ACC 87, with 0.5 – 0.6 similarity between the cultivated and wild samples. The three *V. lanceolata* accessions within Cluster III, ACC 207, ACC 235 and ACC 296, showed relatively high similarity. These three accessions represent distinct morphotypes within

the *V. lanceolata* complex (Lawn and Holland 2003), and have been found to exhibit genetic incompatibilities in cross-pollination studies (A. Holland and R.J. Lawn, unpublished data).

The DArT library for mungbean therefore revealed genetic diversity relations among the 11 *Vigna* samples that were consistent with the taxonomic information available on these samples. The three Clusters (Figure 3.1) corresponded to three separate sub-genera, while the main sub-divisions within each Cluster corresponded to genetically distinct taxa based on available current knowledge. There was some inconsistency, however, in the apparent degree of relatedness between the different sub-divisions within the three Clusters and their genetic compatibility based on cross-pollination studies. The level of dissimilarity observed between the cultivated and wild mungbean accessions was greater than that observed within either the *V. vexillata* or *V. lanceolata* clusters (Figure 3.1). However, whereas the divisions within the two latter clusters differentiate taxa that are genetically incompatible, the cultivated and wild mungbean are strongly genetically compatible (James *et al*. 1999). In part, this apparent anomaly may reflect the fact that all the *V. vexillata* accessions (Cluster I) and the *V. lanceolata* morphotypes (Cluster III) were wild or, in the case of some *V. vexillata* accessions, only partially domesticated, whereas the mungbeans (Cluster II) comprised both cultivars and wild plants. There are large differences between cultivated and wild mungbean for many quantitative and qualitative traits (Lawn and Rebetzke 2006). Thus the phenotypic differences between the mungbean cultivars and their wild relatives (Cluster II) were large compared to those within Cluster I and within Cluster II, which in each case comprised only wild or weedy genotypes.

In conclusion, soybean and mungbean arrays of DArT markers were successfully developed from each of two complexity reduction methods. The soybean array comprised approximately 800 – 900 polymorphic DArT clones, while using the same methods the mungbean array comprised around double that number. The results show that the DArT technique is an effective tool for generating large numbers of markers quickly. Moderate levels of DArT marker transferability were demonstrated between soybean and mungbean, and among mungbean and other *Vigna* species, indicating the potential for using DArT markers as a tool for comparative genome studies in these legumes. The DArT approach also successfully discriminated between 11 diverse *Vigna* genotypes, suggesting that it is also useful for genetic diversity studies in these species. Research on other genomes such as wheat (Semagn *et al*. 2006c), barley (Hearnden *et al*. 2007) and sorghum (Mace *et al*. 2008), have shown that DArT markers produce broad coverage and medium to high density maps which are at worst equivalent to other marker-based maps and at best, provide much better coverage. Although there will be a level of redundancy in DArT marker identity, the DArT technique still provides quick and exhaustive fingerprinting as thousands of markers can be screened. The DArT markers generated for soybean and mungbean will be evaluated in linkage map construction and identification of QTLs associated with physiological traits in soybean and mungbean in the following chapters.

CHAPTER 4. PHENOTYPIC EVALUATION OF MUNGBEAN POPULATIONS

4.1. Introduction

In order to identify QTLs or DArT markers associated with traits in mungbean, the initial step was to undertake a phenotypic evaluation of plants differing in trait expression. This experiment used four F_5 recombinant inbred line (RIL) mungbean populations created by hybridising wild mungbean genotypes (known to possess traits of interest) with cultivated mungbean varieties.

In inheritance studies, Nguyen (2011), using the F_1, F_2 and backcross generations of cultivated x wild hybrid populations found that there were large trait differences between the cultivated and wild parental plants as well as segregation in the progenies. Further, many polymorphisms were detected in the parental plants when the DArT platform was developed for mungbean (Chapter 3). Given these findings, RILs developed from cultivated x wild hybrid populations were considered likely to offer strong potential for (i) revealing a wide range of trait differences; and (ii) the application of DArT markers to identify QTLs linked with the different traits.

Wild mungbean represents a potentially usefull additional source of germplasm for crop improvement (Lawn and Cottrell 1988; Lawn and Rebetzke 1991; Lambrides and Imrie 2000; Sangiri *et al*. 2007). While most wild traits are not desirable agronomically, some traits including tolerances to environmental stresses, late flowering and perenniality may be useful. For example, the late flowering trait may be useful in broadening latitudinal adaptation, extending the crop duration for higher yield potential or delaying pod maturity until unfavourable weather conditions have ceased (e.g. Yeates *et al.* 2000). As this trait is believed to be quantitatively inherited, QTL analysis may provide better understanding and identify markers associated with the trait. The inheritance of perenniality was reported to be controlled by two dominant genes with complementary action based on the phenotypic study in F_2 generation (Nguyen *et al*. 2012).

The main objectives of the research reported in this chapter were to describe the expression of a wide range of traits including qualitative and quantitative morphological traits, phenology and attributes of yield in the F_5 generation of cultivated x wild mungbean hybrids, including the late flowering and perenniality traits. Comparisons with the previous study of Nguyen (2011) on the same traits were also undertaken.

4.2. Materials and methods

The development of the four F_5 mungbean RIL populations and their phenotypic evaluation were conducted using potted plants grown on benches in glasshouses and in the field at the CSIRO Davies Laboratory, in Townsville, Queensland, Australia (19°32'S, 146°77'E; alt. 50 m) during March 2009 – September 2010.

4.2.1. Germplasm and population development

Four F_5 RIL populations were developed from crosses of two cultivated mungbean varieties (Berken and Kiloga) and two wild parents (ACC 1 and ACC 87). The two mungbean cultivars are characterised by erect stems, determinate habit, large entire leaflets and large shiny green seed (Table 4.1) and were early flowering when grown in SE Queensland (Lawn 1979a, 1979b). In contrast, the two indigenous wild mungbean accessions are twining, viny plants with indeterminate flowering habit, small and lobed leaflets and small, black seeds. The accession ACC 1 was very late flowering even when grown under short days (Rebetzke and Lawn 2006a). ACC 87 is typical of a tuberous rooted perennial form of ssp. *sublobata* that is endemic to the Townsville – Charters Towers region of north Queensland (Rebetzke and Lawn 2006c). ACC 87 has thicker stem, larger leaflets, flowers and seeds than ACC 1, but nonetheless these traits are still smaller than in the cultivars.

Table 4.1. Mungbean germplasm used to generate the genetic populations for DArT study (from Nguyen 2011)

Accession/ cultivar	Cultivated or wild	Main traits of interest
Kiloga	Cultivated variety from USA	Early maturing (68 – 84 d), annual (see Lawn 1979b). Erect stem, large shiny green seed.
Berken	Cultivated variety from USA	Early flowering (68 – 97 d), annual (see Lawn 1979a). Erect stem, large shiny green seed.
ACC 1	Wild accession from near Mackay, Australia	Prostrate vine, very late flowering (> 110 d), not affected by extended photoperiod (see Rebetzke and Lawn 2006a). Very small black seed.
ACC 87	Wild accession from near Townsville, Australia	Twining vine, relatively large flowers, and black seed. Sensitive to photoperiod. Tuberous rooted and perennial (see Rebetzke and Lawn 2006c).

Each cultivated parent had been crossed with each wild parent to create four hybrid populations (Kiloga x ACC 1; Kiloga x ACC 87; Berken x ACC 1; Berken x ACC 87). F_1 hybrid seeds had been created and were also allowed to self in prior years, so producing F_2 seeds (RJ Lawn, personal communication). F_3 seeds from 84 x F_2 plants of each population were available from the study on the expression and inheritance of qualitative and quantitative traits in segregating progeny generations by Nguyen (2011).

RILs were developed via the single seed-descent breeding method without selection via generation advancement in a glasshouse. F_2 seeds were planted by Nguyen (2011) in March 2009 and grown in pots located in the field. As soon as F_3 seeds were available, seeds were planted in small black pots (12 cm diameter, 14 cm depth) located in the glasshouse and using vermiculite (Grade 3, Peter Bacon Enterprise P/L, T/AS Australian Vermiculite) as the medium. As short days induced quick flowering and small sized plants, two generations were advanced in the period from April 2009 to March 2010. Initially, two F_3 seeds from each F_2 plant were scarified using a scalpel to overcome seedhardness. When the seeds germinated, one plant was selected at random and kept to obtain F_4 seeds. After five to ten pods were collected, the F_3 plants were discarded and some F_4 seeds were immediately sown. The same procedure of scarifying seeds and thinning plants was applied. Thus, the F_3 and F_4 seeds were sown at various different times depending on seed availability. Plants were watered daily and sprayed with pesticides and fungicides. Dimethoate (100 mg/mL) was sprayed at the seedling stage to control beanfly (*Ophiomyia phaseoli*). Methomyl (225 g/L) was sprayed to control aphids (*Aphis craccivora*). Wettable sulphur (2.745 g/L) and mancozeb (1.175 g/L) were used to control powdery mildew (*Podosphaera xanthii*). Harvested seeds were stored in a cold room (4^{o}C) for use in the phenotyping experiment in 2010.

While the initial number of F_2 plants (84) was the same across the four populations, slightly fewer RILs were developed in three of the four populations (Table 4.2). In these instances, some plants that died during generation advancement were unable to be replaced. In most cases where plants died, seeds were re-planted, but with a small number of very late flowering genotypes, there was not enough time left to grow replacement plants.

Table 4.2. Mungbean genetic RIL populations advanced to the F_5 generation

Crossed populations	Assigned name	Number of RILs
ACC 1 x Berken	1xB	83
ACC 1 x Kiloga	1xK	81
ACC 87 x Berken	87xB	83
ACC 87 x Kiloga	87xK	84
Total		**331**

4.2.2. Cultural details and experimental design of the phenotyping study

The cultural details for this study were broadly similar to those used reported in Nguyen *et al.* (2012). Briefly, the F_5 plants were grown for phenotyping in spaced PVC pots (200 mm in diameter and 190 mm deep) which were placed on wire mesh benches (0.95 m x 2.5 m) *c.* 1 m above the ground. Pots were painted silver to reflect radiation for temperature reduction inside the pots. The pots were arranged in a completely randomized design. There were 12 pots to a bench, with the benches located

in an outdoor field area, so that the plants were exposed to the natural environment. The pots were moved to a new location within a bench two times during the experiment to reduce possible local spatial effects (e.g. wind direction).

Two weeks prior to sowing, the experimental field area was cleaned and sprayed with the herbicide Glyphosate to minimize weed growth and hence the risk of insect and disease build-up. The pots were filled with equal volumes of a uniform high-grade commercial potting mix soil and watered before sowing to provide suitable moisture for seed germination. Seeds were scarified by removing 0.5 – 1 mm^2 of the testa using a scalpel to overcome seed hardness and to assist them in imbibing water and emerging uniformly and quickly. Two or three seeds were sown per pot at a depth of *c*. 1 cm and covered by vermiculite. After emergence, the seeding plants were dusted with Derris to avoid insect damage. In each pot, a single plant selected at random was kept and evaluated.

In order to enhance expression of the late-flowering trait from ACC 1, seeds of all four RIL populations and six plants of each original parent were sown on the same day in mid-autumn (12^{th} March, 2010). Short day conditions (in March) were chosen to promote rapid flowering in the photoperiod-sensitive plants and shorten the time plants were exposed to heavy wet season rains, but still leave adequate time for the expression of traits. Unseasonal cloudy and wet weather during the first five weeks after sowing resulted in less vigorous growth before flowering in some of the earliest flowering plants.

Osmocote Plus^R controlled-release fertilizer (7.5 g) was applied to each pot three weeks after sowing to provide adequate nutrients for plant growth. The soil surface was covered by vermiculite to *c*. 1 cm depth. Plants were tied to bamboo stakes to avoid lodging, minimize wind damage and to ensure that stems and branches from adjacent plants did not intertwine, as the wild parents and many of the hybrids had long twining branches. Plants were watered daily by hand. Watering was carefully adjusted to avoid either drought or waterlogging stresses: initially, pots were watered on daily basis and then twice daily. A water-filled saucer under each pot was used as a water reservoir when the plants had large leaf areas.

Insecticides and fungicides were applied during plant growth to minimize damage from insects and diseases. Dimethoate was sprayed twice per week at the seedling stage to control beanfly. Sprays for powdery mildew and thrips were only applied after susceptibility scores were recorded. Wettable sulphur and mancozeb were applied every two weeks to control powdery mildew. Methomyl was given to control thrips (*Megalurothrips usitatus*), bean podborer (*Maruca testulalis*) and aphids.

4.2.3. Data collection and measurement

Measurements were made on a wide range of traits including morphology, perenniality, seed traits, phenology and agronomic traits for each plant, based on the descriptions by Fery (1980), Lawn *et al.*

(1988b), James *et al*. (1999) and Rebetzke and Lawn (2006a, 2006b, 2006c). Young leaves were also collected for DNA extraction and subsequent DArT analysis.

4.2.3.1. Qualitatively inherited traits

4.2.3.1.1. Morphological traits and apparent reaction to powdery mildew and thrips

Observed morphological traits listed in Table 4.3 were described and categorized as qualitative traits based on the review by Fery (1980).

Table 4.3. Qualitatively inherited morphological traits observed in the parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Traits	Parental genotypes	Phenotypic score/ definition
Hypocotyl pigment	ACC 1, ACC 87	1. Some purple spotting
	Berken, Kiloga	2. Solid purple
Stem colour	Berken, Kiloga, ACC 1, ACC 87	1. Almost green, with some purple spots
		2. Some purple areas
		3. Purple
Leaf rachis	Berken, Kiloga, ACC 1, ACC 87	1. Slightly purple
		2. Solid purple
Leaf petiole colour	Berken, Kiloga, ACC 1, ACC 87	1. Slightly purple
		2. Solid purple
Plant hair density	Berken, Kiloga	1. Light (just a scattering of hairs)
	ACC 1, ACC 87	2. Dense
Plant hair colour	ACC 1, ACC 87	1. Light brown
	Berken, Kiloga	2. Brown
Growth habit	Berken, Kiloga	1. Erect
		2. Partly erect
		3. Partly prostrate
	ACC 1, ACC 87	4. Strongly prostrate
Twining	Berken, Kiloga	0. Non-twining
	ACC 1, ACC 87	1. Twining (1 to 3)
Leaflet lobing	Berken, Kiloga	0. Leaflet entire
	ACC 1, ACC 87	1. Leaflet scalloped (1 to 5)
Flower colour	ACC 1, ACC 87	1. Pale green yellow
	Berken, Kiloga	2. Yellow
Inflorescence structure	ACC 1and ACC 87	1. Simple
		2. Intermediate compound
	Berken, Kiloga	3. Compound
Dry pod colour	Berken, Kiloga	1. Brown
	ACC 1, ACC 87	2. Black
Pod dehiscence	Berken, Kiloga (no)	Score 1 to 4: none to completely dehiscent
	ACC 1and ACC 87 (yes)	
Powdery mildew	ACC 1, ACC 87	1. Slightly susceptible
	Berken, Kiloga	2. Severely susceptible
Thrips	ACC 1, ACC 87	1. Slightly susceptible
		2. Mild susceptible
	Berken, Kiloga	3. Severely susceptible

Hypocotyl pigment was observed five days after emergence. Other qualitative traits including stem colour, leaf rachis colour, petiole colour, plant hair density and colour, growth habit and leaflet lobing were recorded during plant growth.

There was also variable expression in inflorescence structure from simple to compound, and flower colour from pale green-yellow to yellow. For measurement of dehiscence and dry pod colour, the first ten mature pods from each plant were put in a paper envelope and stored at room temperature for two weeks. Reaction to powdery mildew and thrips was qualitatively scored from slightly susceptible to severely susceptible, 10 days after the first symptoms of powdery mildew and thrip damage were observed on any plants in the field. Powdery mildew scoring was based on fungal density on leaf surfaces. The score for thrip damage was opportunistic, and was based on the degree of leaf curling, number of leaves invaded by thrips and stunting of plant growth.

4.2.3.1.2. Perenniality trait

Perenniality is characterized by fleshy, tuberous and shallow root systems and the development of new shoots from the tuberous roots after winter. This trait was only recorded for the RIL populations involving ACC 87 as a parent (i.e. 87xB and 87xK).

In his study, Nguyen (2011) encountered difficulties when maintaining the roots of the perennial genotypes over the winter after the shoots had died back. Firstly, it was necessary to adjust the plant water supply in winter when conditions were cool to avoid waterlogging. Secondly, the evidence of tuberization was sometimes confounded by infection of charcoal root disease (*Macrophomina phaseoli*) or root rotting. In particular, there was evidence that when the above ground shoots were harvested in the late autumn/ winter period, some root systems became infected by fungi and/ or bacteria through the cut stem surface, and then died.

Therefore, the adjustment was made in this study that both roots and shoots were collected for evaluation in springtime, rather than the shoots being harvested as they died back. The expression of the perenniality trait was evaluated based on Rebetzke and Lawn (2006c), Nguyen (2011) and observations in this study. Since plants were smaller and grew less vigorously than reported in Nguyen (2011), the diameter criterion applied for thickened lateral roots was 1.5 mm instead of 2 mm as described in Rebetzke and Lawn (2006c). Attributes describing the degree of tuberization included new shoots from stems or basal nodes above ground, adventitious shoots developing or emerging from the thickened tap or lateral roots underground, measurement of main tap root diameter and number of thickened lateral roots. Using these attributes, the visual appearance of the roots was qualitatively scored from 1 (fibrous) to 4 (strongly tuberous) as follows: 1. roots fibrous; 2. slightly tuberous; 3. tuberous with new shoots from stems or basal nodes; 4. strongly tuberous with adventitious shoots developing underground or emerging from the thickened tap or lateral roots.

4.2.3.1.3. Visual seed traits

Visual seed traits (Table 4.4) were measured, using a dissecting binocular microscope, based on the descriptors used by Lawn *et al.* (1988b) and James *et al.* (1999). In addition, overall visual seed traits were categorized from 1 to 10 as described in Lawn *et al.* (1988b).

Table 4.4. Putative qualitative seed traits observed in parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Traits	Parental genotypes	Phenotypic score/ definition
Testa colour	Berken, Kiloga	1. Green
	ACC 1, ACC 87	2. Speckled black
Seed mottling	Berken, Kiloga	1. Absent
	ACC 1, ACC 87	2. Present
Seed coat ridging/ Texture layer	Berken, Kiloga	0. Absent
	ACC 1, ACC 87	1. Present
Lustre	Berken, Kiloga	0. Shiny
	ACC 1, ACC 87	1. Dull
Texture layer depth	Berken, Kiloga	0. No layer
	ACC 87	1. Shallow
	ACC 1	2. Deep
Hilum colour	Berken, Kiloga	1. White
	ACC 1, ACC 87	2. Tan
Texture layer colour	Berken, Kiloga	1. Clear
	ACC 1, ACC 87	2. Brown
Overall visual seed traits (Lawn *et al*. 1988b)	Berken, Kiloga	1. Shiny green
		2. Dull green
		3. Waxy dull green
		4. Shiny khaki
		5. Dull khaki
		6. Shiny speckled black
		7. Dull speckled black
		8. Waxy dull speckled black
	ACC 87	9. Shiny khaki speckled black
	ACC 1	10. Dull black

4.2.3.2. Quantitatively inherited traits

4.2.3.2.1. Phenological traits

Dates were recorded for germination, flowering (defined as the first completely open flower on the plant), end of flowering (when major flowering flushes had ceased and only sporadic flowering persisted) and physiological maturity (recorded when > 95% of the pods had ripened). For pod development, two flowers per plant were tagged, and records made of dates when the flowers fully opened and when the pods were mature (i.e. the pod lost chlorophyll and darkened).

4.2.3.2.2. Morphological and yield related traits

Traits for which variation was apparent in the hybrid progenies and for which data were recorded are shown in Table 4.5. Various vegetative traits were recorded such as leaflet length (mm) and width (mm), stem diameter (mm), internode length (mm), flower size (mm) and number of leaves on the main stem (Table 4.5). Leaf size (mm), which was determined using the size of the terminal leaflet, and petiole length (mm) were average values for the 4^{th} and 5^{th} trifoliolate leaves. Stem diameter (mm) was measured on the 4^{th} and 5^{th} nodes using a Vernier calliper. To determine internode length (mm), the length between the 4^{th} and 7^{th} nodes was measured and the average length per node was calculated. These traits were measured on week 8 after sowing. Other traits including stem length (mm), branch length (mm), peduncle length (mm), numbers of main branches per plant, total numbers of leaves on the stems, number of nodes on the stem, and total numbers of pod clusters (on primary branches and main stem) were recorded at physiological maturity.

Table 4.5. Quantitative traits observed in the parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Traits	Definition/ Measurement
Leaflet size	mm; length & width of terminal leaflet of 4^{th} and 5^{th} trifoliolate leaves
Leaflet ratio	Leaflet length : leaflet width ratio, calculated from the 4^{th} and 5^{th} trifoliolate leaves
Petiole length	mm; petiole length of the 4^{th} and 5^{th} leaves
Stem diameter	mm; diameter; average of measures below the node 4^{th} and 5^{th} trifoliolate leaves
Internode length	mm; the average node length between the 4^{th} and 7^{th} leaves
Floral standard width	mm; width of floral standard; average of 3 flowers/plant
Stem length	cm; from the ground to the furthest point of the main stem
Branch length	cm; average of 2 branches/plant
Peduncle length	cm; average of the 3 very first peduncles/plant
No. of branches	Number of all branches/plant
No. of leaves on stem	Number of all leaves on main stem
No. of nodes on stem	Number of nodes on main stem
No. of nodes on branches	Number of nodes on all primary branches
Node of 1^{st} pod	Node where the first pod formed
No. of pods per peduncle	Average number of pods for the 3 very first peduncles/plant
Total pod clusters	Number of peduncles/plant
Pod traits	
- No. of seeds per pod	- 10 first collected pods for each plant
- Pod size	- Length and width (mm) of 5 pods/plant
- Seed size	- Weight of 100 seeds (g)
Hardseededness	- %; 100 seed samples were placed on moistened filter paper. Number of germinating seeds were counted after 3 days
Pod dry mass (including seed)	g/plant
Seed yield	g/plant
Standing dry biomass	g/plant; the standing biomass was removed, dried in a fan forced dehydrator at 60^{o}C for 72 hours and weighted
Harvest index (HI)	HI = Seed yield/(Pod dry mass + Standing dry biomass)

Pod and seed traits included the number of seeds per pod, length and width (mm) of pods and seed size (g/100 seed). The first 10 collected pods were counted and values averaged for number of seeds per pod. 100 seeds were then weighed for measurement of seed size (g). For those plants which had fewer than 100 seeds, the weight of all harvested seeds were determined and converted into 100 seed size (g). Hardseededness (%) was determined by placing 100 seeds (from the seed size measurement) on moistened filter paper in petri dishes at room temperate. The number of germinating seeds was counted after 3 days.

Yield related traits consisted of pod dry mass (g), seed yield (g), standing dry biomass (g) and harvest index (HI). After pod dry mass was weighed and recorded, the pods were shattered by hand and the seeds were cleaned and weighed for seed yield. Standing dry biomass (g/plant) was estimated after plant maturity. The standing biomass was removed and dried in a fan forced dehydrator at 60^{o}C for 72 hours. HI was calculated as the ratio of seed yield to total dry biomass.

4.2.4. Statistical analyses

ANOVA was performed using SAS 9.0 to evaluate the differences in the various trait means between the parents and RILs in the four mungbean populations. Frequency distributions for each trait were used to separate qualitative traits with discrete distributions (i.e. those apparently under simple genetic control) from quantitative traits with continuous distributions (i.e. those apparently under multi-gene control). Phenotypic correlations between traits were also evaluated.

4.2.4.1. Qualitative trait analysis

The standard Chi-square (χ^2) test was used to test the goodness of fit of the observed categorical data for each putative qualitative trait in the F_5 generation to possible models of single and two gene control for that trait.

$$\chi^2 = \frac{\Sigma(f_o - f_e)^2}{f_e}$$

Where:

Null Hypothesis (H_o): the hypothesis to be disapproved (i.e. H_o: the trait is controlled by single or two genes); If probability value $P > 0.05$, H_o is accepted

f_o = Observed sample frequency

f_e = Expected frequency

The expected ratios in the F_5 were tested for the most common single and digenic Mendelian inheritance models (Table 4.6) (Hossain *et al.* 2010).

Table 4.6. Gene action and expected Mendelian segregation ratios for monogenic and digenic traits in the F_2 and F_5 generations

Number of genes	Gene action	Expected ratio in F_2	Expected ratio in F_5
Single gene		1: 3	1: 1
Two genes	Independent segregation	1: 1: 1: 1	1: 1: 1:1
	Dominant epistasis	12: 3: 1	2: 1: 1
	Recessive epistasis	9: 3 : 4	1: 1: 2
	Two dominant genes with additive effect	9: 6: 1	1: 2: 1
	Duplicate dominant genes	15: 1	3: 1
	Duplicate recessive genes	9: 7	1: 3
	Dominant and recessive interaction	13: 3	3: 1

The results were compared with the findings by Nguyen (2011), who studied the expression and inheritance of qualitative and quantitative traits in segregating F_2 and backcross progenies of these same mungbean populations.

4.2.4.2. Quantitative trait analysis

The average values were calculated for the traits that had more than two measurements per plant such as leaflet size, stem diameter, flower size, branch and peduncle length, number of pods per peduncle, pod size and number of seeds per pod. ANOVA was then used to test for differences in mean expression of traits in the parental and progeny generations. The genetic parameters calculated were coefficient of variation (CV, %) and broad sense heritability (H_b^2, %) (Falconer 2000; Acquaah 2007). Standard error of broad sense heritabiltiy estimates was calculated by the method of Osborne and Patterson (1952).

$$CV\% = \frac{S.D \times 100}{\overline{X}}$$

$$Ve = \frac{VP1 + VP2}{2}$$

$$H_b^2 = \frac{VF_5 - Ve}{VF_5} \times 100$$

Where:

CV%: Coefficient of variability

S.D: Phenotypic standard deviation,

$\overline{X}$: mean value of the trait

VP1 and VP2: Phenotypic variance of parent 1 and parent 2 respectively

Ve: Environmental variance

VF_5: Phenotypic variance in F_5

H_B^2: Broad sense heritability

The correlation coefficient formula as given by Acquaah (2007) was used to calculate phenotypic correlation (r). If there is no association, covariance will be zero or close to zero. The magnitude of covariance is often related to the size of the variables and also depends on the scale of measurement.

$$r = \frac{N * \Sigma(X * Y) - (\Sigma X) * (\Sigma Y)}{\sqrt{N * \Sigma X^2 - (\Sigma X)^2} * \sqrt{N * \Sigma Y^2 - (\Sigma Y)^2}}$$

where:

N: population size

X, Y: measured values of the traits X and Y, respectively

4.3. Results and discussion

Unseasonal unfavourable conditions, including persistent rain for the first five weeks after seedling emergence, and a thrip invasion during the early stage of plant growth, resulted in some stunting and generally less vigorous initial growth. A small number of plants also died before all traits were expressed e.g. before pods were formed. Therefore, the total numbers of plants for each evaluated population were slightly fewer than the number of RILs developed (Table 4.2) and varied slightly among the qualitatively inherited traits.

4.3.1. Qualitatively inherited traits

4.3.1.1. Morphological traits

Parental plants and RILs exhibited variation in all morphological traits. Figure 4.1 illustrates this variation for some representative traits such as twining, lobed leaflet, flower and dry pod colour and thrip damage.

The numbers of plants in the parental and F_5 generations in each phenotypic category for 14 morphological traits, and the apparent responses to powdery mildew and thrips, are presented in Table 4.7. Also presented are the χ^2 values for appropriate models of gene action based on the observed frequency distributions of the different phenotypes in the F_5 RILs. Gene action models were also compared with those reported by Nguyen (2011).

(a) Twining habit

Score 0
No twining present

Score 1
Slightly twining

Score 2
Intermediate twining

Score 3
Strongly twining

(b) Leaflet lobing

Entire

Slightly lobed

Strongly lobed

(c) Flower colour

Score 1 – Pale green yellow

Score 2 – Yellow

(d) Dry pod colour

Score 1 – Brown

Score 2 – Black

(e) Thrip damage

Slightly susceptible

Severly susceptible (stunted growth)

Figure 4.1. Representative examples illustrating the degree of expression in mungbean for (a) Twining habit; (b) Leaflet lobing; (c) Flower colour; (d) Dry pod colour; (e) Thrip damage

Table 4.7. Probable qualitative traits for which differences were observed in the parental genotypes and/or among the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87), the observed category frequencies, the chi-square (χ^2) values and probabilities for the goodness-of-fit to putative ratios

a: Trait categories with the same superscript letter were combined into a single phenotype for that trait
♪: Assigned RIL populations' names were explained in Table 4.2.

RIL♪	Observed frequencies for rating categories				Putative ratio	χ^2	*P*
(a) Hypocotyl pigment (Berken, Kiloga solid; ACC 1, ACC 87 slight spotting)							
	Slightly purple spotted		Purple				
1xB	63		20		3:1	0.04	0.84
1xK	62		18		3:1	0.27	0.60
87xB	75		5		-	-	-
87xK	79		5		-	-	-
(b) Stem colour (all parents slightly purple, with hybrids ranging almost green to completely purple)							
	Almost green, some purple spots	Slightly purple	Purple				
1xB	16	42	25		1:2:1	1.09	0.58
1xK	18	41	21		1:2:1	0.28	0.87
87xB	14	42	23		1:2:1	3.02	0.22
87xK	7	52	17		-	-	-
(c) Leaf rachis colour							
	Slight purple		Purple				
1xB	46		37		1:1	0.98	0.30
1xK	42		38		1:1	0.20	0.65
87xB	17		58		1:3	0.22	0.64
87xK	18		56		1:3	0.02	0.89
(d) Leaf petiole colour							
	Slight purple		Purple				
1xB	34		49		1:1	2.71	0.10
1xK	22		55		1:3	1.67	0.19
87xB	48		27		-	-	-
87xK	50		24		-	-	-
(e) Plant hair density (cultivated light, wild dense)							
	Light		Dense				
1xB	35		48		1:1	2.04	0.15
1xK	46		34		1:1	1.80	0.18
87xB	46		34		1:1	1.80	0.18
87xK	44		40		1:1	0.19	0.66
(f) Hair colour (cultivated brown, wild light brown)							
	Light brown		Brown				
1xB	46		37		1:1	0.98	0.32
1xK	41		39		1:1	0.05	0.82
87xB	65		15		3:1	1.67	0.20
87xK	63		21		3:1	0.00	1.00
(g) Growth habit (cultivated erect, wild prostrate, some hybrids intermediate)							
	Erect	Prostrate					
	1	2[a]	3[a]	4[a]			
1xB	15	24	41	3	1:3	2.69	0.10
1xK	18	27	31	4	1:3	0.28	0.60
87xB	14	16	30	20	1:1:1:1	7.60	0.06
87xK	13	15	31	23	-	-	-
Nguyen (2011): ACC 1 crosses: duplicate dominant epistasis, prostrate dominant over erect ACC 87 crosses: digenic, incomplete dominance, prostrate dominant over erect							
(h) Twining (cultivated none, wild strongly twining, some hybrids intermediate)							
	None	Twining					
		1[a]	2[a]	3[a]			
1xB	20	33	13	17	1:3	0.04	0.84
1xK	24	15	20	21	1:3	1.06	0.30
87xB	38	27	8	7	1:1	0.20	0.65
87xK	49	14	9	10	1:1	3.12	0.07
Nguyen (2011): Twining dominant over non-twining; ACC 1 crosses: two genes with dominant-recessive epistasis; ACC 87 crosses: single gene							

Table 4.7. Continued…

RIL	Observed frequencies for rating categories					Putative ratio	χ^2	*P*
(i) Leaflet lobing (cultivated entire, wild lobed, with ACC strongly so)								
	Entire	Lobed						
		1[a]	2[a]	3[a]	4[a]			
1xB	36	20	14	13		1:1	1.46	0.23
1xK	29	17	18	13	3	-	-	-
87xB	31	20	19	5	5	-	-	-
87xK	30	31	10	7	5	-	-	-
Nguyen (2011): single gene, lobed dominant over entire								
(j) Flower colour (cultivated yellow, wild lemon)								
	Pale green yellow			Yellow				
1xB	34			49		1:1	2.71	0.10
1xK	33			47		1:1	2.45	0.12
87xB	25			56		1:3	1.49	0.22
87xK	22			56		1:3	0.43	0.51
(k) Inflorescence structure (cultivated compound, wild simple)								
	Simple	Compound						
	1	2[a]		3[a]				
1xB	33	28		23		2:1:1	4.95	0.08
1xK	44	18		18		2:1:1	0.80	0.67
87xB	38	33		8		1:1	0.11	0.74
87xK	32	43		8		-	-	-
(l) Dry pod colour (cultivated brown, wild black)								
	Brown			Black				
1xB	18			65		1:3	0.49	0.48
1xK	17			63		1:3	0.60	0.44
87xB	37			42		1:1	0.32	0.85
87xK	33			50		1:1	3.48	0.06
(m) Pod dehiscence (cultivated none, wild strong, hybrids intermediate)								
	None	Present						
	0	1[a]	2[a]	3[a]				
1xB	16	26	24	17		1:1:1:1	3.60	0.30
1xK	18	24	23	15		1:1:1:1	2.70	0.40
87xB	19	20	18	22		1:1:1:1	0.44	0.93
87xK	22	13	18	27		1:1:1:1	5.30	0.15
Nguyen (2011): ACC 1 crosses: dehiscence conditioned by two dominant genes of additive effect ACC 87 crosses: dehiscence conditioned single dominant gene, incomplete dominance?								
(n) Powdery mildew (cultivated severe, wild very slight)								
	Slightly susceptible			Severely susceptible				
1xB	16			67		1:3	1.45	0.23
1xK	14			66		1:3	2.40	0.12
87xB	12			54		1:3	1.60	0.21
87xK	16			52		1:3	0.08	0.78
Nguyen (2011): Susceptibility dominant over resistance; ACC 1 crosses: Single gene; ACC 87 crosses: two genes, dominant-recessive epistasis								
(o) Thrips (cultivated severe, wild slight, some hybrids intermediate)								
	Slight	Mild		Severe				
1xB	29	22		32		1:1:2	5.53	0.06
1xK	19	29		32		1:1:2	5.70	0.06
87xB	26	25		29		1:3	2.40	0.12
87xK	24	24		35		1:3	0.68	0.41

For many traits, including growth habit, twining, leaflet lobing, pod dehiscence and powdery mildew reactions (Table 4.7g – i, m, n), the segregation ratios in the F_5 RILs were broadly consistent with the inheritance models reported by Nguyen (2011) based on F_2 data. Exceptions were lobed leaflet inheritance in three populations, 1xK, 87xB and 87xK, and pod dehiscence inheritance in the ACC 87

crosses (Table 4.7i, m). Because of strong skewness toward the wild type of leaflet lobing, no models were proposed for this trait in those three populations at the F_5 generation. For pod dehiscence in the ACC 87 crosses, the segregation ratios in the F_5 generation indicated digenic action (Table 4.7m), whereas Nguyen (2011) suggested a single dominant gene with incomplete dominance. The degree of pod dehiscence ranged from slightly to completely shattering, as was observed by Nguyen (2011).

Additional qualitative traits not measured by Nguyen (2011) included pigmentation (colour of hypocotyl, stem, leaf rachis and petiole), plant hair density and colour, flower colour, inflorescence structure, dry pod colour and thrip reaction. In the ACC 1 crosses, a single gene with incomplete dominance was indicated for leaf rachis colour, plant hair density and colour, and flower colour (Table 4.7c, e, f , j) while a digenic model was indicated for the rest of these traits. However, the expression of leaf petiole colour indicated two different gene models within the ACC 1 crosses, i.e. a single gene for 1xB and two genes for 1xK (Table 4.7d). Similarly, in the ACC 87 crosses, hair density and inflorescence structure (Table 4.7e, k) were conditioned by a single gene and the other traits by two genes. In some cases, such as hypocotyl pigment, stem and leaf petiole colour or infloresence structure in 87xK (Table 4.7a, b, d, k), no gene models were proposed because of strong phenotypic skewness to either the cultivated or wild parents.

4.3.1.2. Perenniality trait

The degree of perenniality expression observed in this study varied considerably between plants. Figure 4.2 shows the different levels of expression and the scoring system used for the trait. However, the expression was not as strong as that in the F_2 generation (Nguyen 2011), perhaps because of stunting and less vigorous growth. The numbers of plants observed at the various levels of expression, in the two RIL populations involving ACC 87, are shown in Table 4.8.

Score 1 Roots fibrous Annual habit	Score 2 Roots slightly tuberised (> 1.5 mm diameter)	Score 3 Roots tuberised, shoots emerging from basal stem nodes	Score 4 Roots tuberised adventitious shoots from roots

Figure 4.2. Degree of expression of the perenniality trait and the four scoring levels used in the study.
(The cultivated parent plants scored 1 (i.e. annual, fibrous rooted), while ACC 87 scored 4 (i.e. perennial, roots tuberised, with adventitious shoots developing underground or emerging from roots). Arrows indicate tuberous roots and/ or adventitious shoots).

Consistent with previous experience (Rebetzke and Lawn 2006c, Nguyen 2011), both cultivated parents Berken and Kiloga exhibited fibrous roots and a typical annual growth cycle while ACC 87 exhibited the perennial habit (Table 4.8), with tuberised roots and shoots emerging from the roots (Figure 4.2). In both populations, more plants expressed fibrous than tuberised roots. Within the tuberised group, most plants were scored at level 2 and only two plants expressed the trait as strongly as ACC 87.

Table 4.8. Observed frequencies of different levels of expression of the perennial trait in the parental genotypes and the two F_5 RIL populations, 87xB and 87xK

Genotype/ Populations	Rating♪				Perenniality concluded		Total lines
	1	2	3	4	No	Yes	
Berken	6	0	0	0	6	0	6
Kiloga	6	0	0	0	6	0	6
ACC 87	0	0	2	4	0	6	6
87xB	44	26	8	0	44	34	78
87xK	54	19	5	2	54	26	80

♪: Refer Figure 4.2 for details

Consistent with Nguyen (2011), the data were aggregated across the different perenniality scores to create two contrasting groups: i) annual fibrous rooted type which had no evidence of tuberisation; and ii) perennial, tuberous rooted type which did not necessarily produce either basal stem shoots or adventitious shoots from the roots within the timeframe of the evaluations. The segregation ratios of the pooled F_5 data were not consistent between the two populations (Table 4.9).

Table 4.9. Hypothesised models used to test segregation ratios for the perennial trait in the two F_5 RIL populations, 87xB and 87xK

a: Trait categories with the same superscript letter were combined into a single phenotype for that trait

Populations	Expected segregation ratio (no : yes)	Observed frequency (Perenniality rating)				No. of lines	χ^2	df	*P*	Nguyen (2011)
		1	2[a]	3[a]	4[a]					
87xB	1:1	44	26	8	0	78	1.28	1	0.26 *ns*	two complementary dominant genes
		44	34							
87xK	3:1	54	19	5	2	80	2.40	1	0.12 *ns*	
		54	26							

a: categories combined

ns = Not significant

The segregation model that provided an acceptable fit to the F_5 87xB data was 1:1 while that for the 87xK data was 3:1. The 1:1 model suggested a major single gene control while the 3:1 model was consistent

with the result found by Nguyen (2011) that the expression and inheritance of the trait was controlled by two genes.

The reason behind the difference in the trait ratios between the two populations is unknown. In both populations, there were more tuberous rooted plants than would be expected given the findings of Nguyen (2011), especially in the 87xB cross. It is possible that there were errors when roots were scored either 1 or 2. While the stunted growth of some of the plants limited the expression of the tuberised roots, the use of the smaller diameter criterion (1.5 mm compared to 2 mm as used by Nguyen (2011)) for distinguishing between score 1 and 2 may have grouped more lines into score 2 than 1. Possible genetic reasons for various degrees of perenniality expression even at F_5 can be that there may be also gene modifiers with minor effects influencing the trait. Studies on the inheritance of root characters such as tuber dry weight and tuber HI, which are related to the expression of perenniality in *V. vexillata* indicated the trait may be multi-genic or quantitatively inherited in that species (James and Lawn 1991; Damayanti *et al*. 2010c).

4.3.1.3. Visual seed traits

Differences in seed visual appearance attributes are illustrated in Figure 4.3 while the phenotypic ratios for six seed appearance attributes are shown in Table 4.10. Two seed traits, the depth and colour of the texture layer when present, were not measured by Nguyen (2011).

Green, shiny, white hilum, no texture layer	Green, shiny, ridged, tan hilum	Speckled black, shiny, white hilum	Speckled black, dull, ridged (shallow), tan hilum	Speckled black, dull, ridged (deep), tan hilum

Figure 4.3. Representative examples illustrating the expression of visual seed traits in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Consistent with Nguyen (2011), testa and hilum colour were conditioned by two and one gene respectively in all four RIL populations (Table 4.10a, e). However, for seed coat ridging and lustre, in the ACC 1 crosses, the segregations were heavily biased to the wild type, with only one (1xK) and two (1xB) lines expressing no layer or two lines expressing shiny (Table 4.10b, c). Considering only the shallow and deep layer phenotypes, the segregation ratio of 1:3 in both the 1xB and 1xK crosses suggested the depth was controlled by two genes, with deep dominant over shallow (Table 4.10d).

Table 4.10. Qualitative seed traits for which differences were observed in the parental genotypes and/ or among the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87), the observed category frequencies, the Chi-square (χ^2) values and probabilities for the goodness-of-fit to putative ratios

Trait categories with the same superscript letter were combined into a single phenotype for that trait.

♪: Assigned RIL populations' names were explained in Table 4.2.

RIL♪	Observed frequencies for rating categories		Putative ratio	χ^2	*P*
(a) Testa colour/ Seed mottling (cultivated green, wild speckled black)					
	Green	Speckled black			
1xB	25	58	1:3	1.16	0.28
1xK	27	53	1:3	3.27	0.07
87xB	24	55	1:3	1.22	0.27
87xK	26	57	1:3	1.77	0.18
Nguyen (2011): all crosses digenic; ACC 1 crosses duplicate dominant epistasis; ACC 87 crosses dominant recessive epistasis					
(b) Seed coat ridging/ Texture layer (cultivated absent, wild present)					
	Absent	Present			
1xB	2	81	-	-	-
1xK	1	79	-	-	-
87xB	22	57	1:3	0.34	0.56
87xK	36	47	1:1	1.46	0.23
Nguyen (2011): ACC 1 crosses digenic (duplicate recessive & dominant-recessive epistasis); ACC 87 crosses single gene, presence dominant over absence					
(c) Lustre (cultivated shiny, wild dull)					
	Shiny	Dull			
1xB	2	81	-	-	-
1xK	2	78	-	-	-
87xB	35	44	1:1	1.03	0.31
87xK	21	62	1:3	0.00	0.95
Nguyen (2011): two complementary dominant genes in 1xB, 1xK and 87xB; single dominant gene in 87xK					
(d) Texture layer depth when present (cultivated unknown, ACC 1 deep, ACC 87 shallow)					
	Shallow[a]	Deep[a]			
1xB	14	67	1:3	2.57	0.10
1xK	16	63	1:3	0.95	0.33
87xB	26	31	1:1	0.44	0.51
87xK	35	12	3:1	0.01	0.92
(e) Hilum colour (cultivated white, wild tan)					
	White	Tan			
1xB	41	42	1:1	0.12	0.91
1xK	41	39	1:1	0.50	0.82
87xB	33	46	1:1	2.14	0.14
87xK	48	35	1:1	2.04	0.15
Nguyen (2011): all crosses, single gene, tan dominant over white					
(f) Texture layer colour when present (cultivated unknown, but presumably clear; wild brown)					
	Clear	Brown			
1xB	39	42	1:1	0.11	0.74
1xK	38	41	1:1	0.11	0.74
87xB	18	39	1:3	1.32	0.25
87xK	14	33	1:3	0.57	0.45

(g) Overall visual seed traits	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1xB	2	6	10	-	7	-	8	15	-	35
1xK	-	6	9	1	11	1	9	14	-	29
87xB	11	3	3	5	2	11	10	2	8	24
87xK	12	11	4	7	2	24	8	1	19	5

In the ACC 87 crosses, different segregation models were observed in 87xB and 87xK for seed coat ridging, lustre and texture layer depth (Table 4.10b – d). In the 87xB population, the fitted model of 1:3 suggested that seed coat ridging was controlled by two genes with presence dominant over absence. In contrast, the fitted model of 1:1 in the 87xK population suggested single gene control which was also consistent with Nguyen (2011). For lustre, the segregation ratios suggested either single gene (87xB) or two genes (87xK) with dull was dominant over shiny. Nguyen (2011) also suggested single and digenic models but his were the reverse of those observed here. Similarly for texture layer depth, single gene and digenic models were suggested for 87xB and 87xK, respectively.

Nguyen (2011) found texture layer colour to be linked with hilum colour and controlled by a single dominant gene. However, different segregation ratios of 1:1 for the ACC 1 crosses and 1:3 for the ACC 87 crosses were observed at the F_5 generation, which suggested a single and two genes controlling the trait, respectively (Table 4.10f).

Only a few F_5 generation lines expressed overall visual seed traits similar to the cultivated types (score 1) (Table 4.10g) indicating strong skewness toward the wild types.

4.3.2. Quantitatively inherited traits

4.3.2.1. Phenological traits

4.3.2.1.1. Time to flowering

The combined analysis of variance for all genotypes for time to flowering showed significant differences among the means for the parental plants and the RIL populations (Table 4.11), indicating large genetic variability among the genotypes.

Table 4.11. ANOVA among the four parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87) for time to flowering

Sources of variation	df	S.S.	M.S.	F	*P*
Populations and parents	7	9014	1288	18.47	0.00
Error	349	24333	69.72		
Total	356	33347			

df = Degree of freedom, S.S. = Sum of squares, M.S. = Mean square; F = F-value, *P* = probability

Consistent with previous experience, the parental plants exhibited extremes of response with the cultivars Kiloga and Berken being early flowering (< 5 weeks), and the wild accession ACC 1 being very late flowering (~ 11 weeks). ACC 87 also flowered earlier than ACC 1 (6 – 7 weeks after emergence) (Table 4.12a).

Table 4.12. Means and ranges for phenological phases (days, d) in the four parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Means followed by the same letter are not statistically different ($P > 0.05$)

	Berken	Kiloga	ACC 1	ACC 87	1xB	1xK	87xB	87xK
a. Time to flowering (d)								
Mean	32.7a	33.2a	73.7d	47.2c	43.7bc	44.3bc	40.5bc	38.6ab
Range	32 - 34	32 - 35	70 - 77	45 - 49	30 - 81	30 - 71	31 - 74	28 - 54
b. Duration of flowering (d)								
Mean	6.7a	7.2a	72.8d	33.8c	25.1b	23.5b	18.0b	17.8b
Range	6 - 9	7 - 9	70 - 77	27 - 37	8 - 64	8 - 69	6 - 34	2 - 48
c. Pod growth duration (d)								
Mean	18.5a	18.2a	21.8b	18.7a	17.8a	18.1a	17.4a	16.9a
Range	17.5 - 19.5	17.0 - 19.5	20.5 - 24.0	17.5 - 20.0	14.5 - 27.0	15.5 - 25.0	15.0 - 26.5	15.0 - 21.0
d. Growth duration (d)								
Mean	126a	130ab	184d	166cd	151bc	153bc	116a	116a
Range	112 - 147	122 - 148	178 - 187	166	90 - 180	95 - 185	53 - 180	47 - 173

Mean time to flowering of ACC 1 (74 days) was significantly different from all other groups (Table 4.12a). The mean time to flowering of both crosses 1xB and 1xK (43.7 and 44.3 days respectively) were in the range between the cultivated parents (~ 33 days) and ACC 1 (74 days), with only a small number of RILs expressing the late flowering trait. In the 1xB population, eight late flowering lines were recovered, but apart from one line that flowered one week later than ACC 1 (81 days), most of these still flowered >10 d before ACC 1 (Figure 4.3). In the 1xK population, 11 lines expressed the late flowering trait. Compared to Nguyen (2011), more late flowering plants were recovered in the F_5 than in the F_2 progenies. Four lines in the 87xB population were also late flowering, with the latest at 74 days (Table 4.12a; Figure 4.4).

The frequency distributions indicated that the variation in time to flowering in each of the four RIL populations was essentially continuous (Figure 4.4). The distributions were skewed, with most of the lines flowering closer to the cultivated parents. On average, the mean time to flowering was similar for the four RIL populations (Table 4.12a), although the populations involving ACC 1 had greater CV (%) values reflecting wider dispersion among RILs in days to flowering (Table 4.13a). Since at the F_5 generation, the RILs were approaching genetic homozygosity (~97%), dominance genetic variance can be essentially disregarded, and the observed genetic variance would have comprised mainly additive effects. Thus, the generally high broad-sense heritability (97%) for the time to flowering trait in the ACC 1 crosses suggested that this trait is mainly under additive genetic control. A similar broad sense heritability range was also found by Nguyen 2011 (95 – 96%).

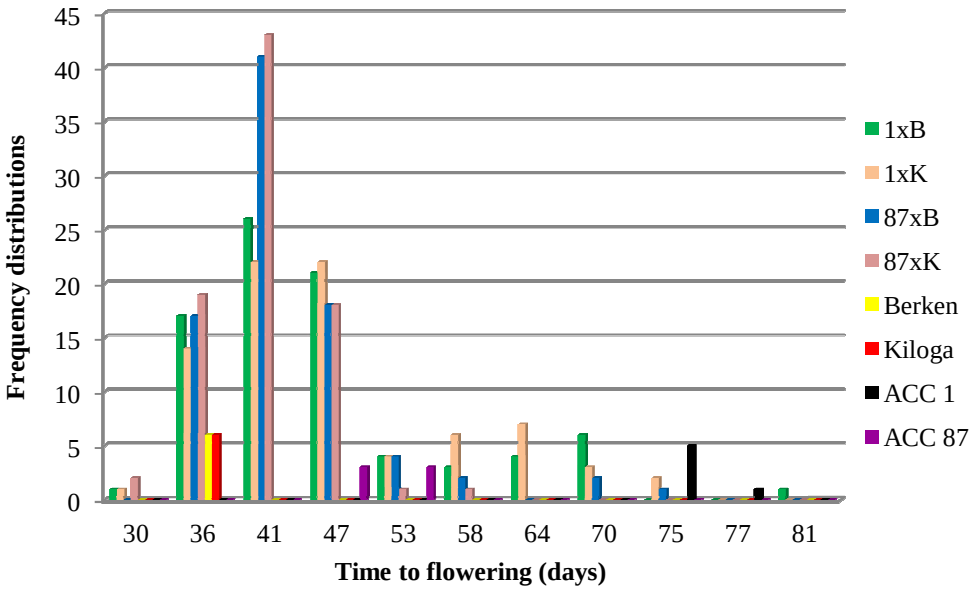


Figure 4.4. Variation in time to flowering in the four parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Table 4.13. Coefficients of variation (CV%) and broad sense heritability (H^2_b ± standard error) for phenological phases in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

	1xB	**1xK**	**87xB**	**87xK**
a. Time to flowering (d)				
CV%	24.3	23.7	18.0	11.9
H^2_b ± standard error	97.1 ± 1.0	96.9 ± 1.1	97.3 ± 0.9	92.6 ± 2.3
b. Duration of flowering (d)				
CV%	40.0	44.2	33.0	37.7
H^2_b ± standard error	95.6 ± 1.5	95.8 ± 1.4	77.2 ± 8.1	82.0 ± 6.3
c. Pod growth duration (d)				
CV%	5.2	5.8	3.1	1.3
H^2_b ± standard error	79.2 ± 6.6	81.5 ± 6.0	68.1 ± 10.1	23.3 ± 24.4
d. Growth duration (d)				
CV%	12.7	11.5	31.2	30.9
H^2_b ± standard error	77.0 ± 8.1	80.0 ± 7.0	94.2 ± 2.2	95.9 ± 1.5

4.3.2.1.2. Durations of flowering, pod growth and total growth

Analysis of variance revealed significant differences in the mean durations of flowering, pod growth and growth duration among the parental plants and the four RIL populations (Table 4.12b – d). Apart from total growth duration, however, most of the differences reflected large differences between the parents, rather than mean differences between the RIL populations. The wild parental plants exhibited longer

durations of flowering and of total growth, reflecting their indeterminate habit (Table 4.12b, d). Within each RIL population, while there was wide dispersion among individual RILs which spanned the full parental range, the population means were intermediate between the parents. For example, in the 1xB cross, the discrepancy in growth duration between parents ACC 1 and Berken was 58 d and the growth duration range of RILs was 90 – 180 d (Table 4.12d). However, on average, most lines were closer to the cultivated than the wild parents.

Generally, 2 – 3 weeks were required for the growth to maturity of individual pods (Table 4.12c). There was not much difference in pod growth duration among the parents or the four RIL populations, except for a marginally longer time in ACC 1 (21.8 d), indicating the trait was generally stable. However, caution is needed as only two flowers were measured on each plant. Similar differences among parents and RILs were observed by Nguyen (2011).

The dispersion from the mean (CV%) was greater for the duration of flowering (range of 33.0 – 44.2%), and to a lesser extent, the total growth duration (11.5 – 31.2%), than the time to flowering (11.9 – 24.3%) (Table 4.13b – d). This indicated greater variation among lines within a population for the duration of flowering than time to flowering. CV% was higher in the ACC 1 than in the ACC 87 RILs for the duration of flowering which perhaps reflected the influence of the late flowering recovery in some ACC 1 lines. However, the converse was observed for the total growth duration, which was possibly a result of expression of perenniality in some ACC 87 lines. CV% was low for the duration of individual pod growth (1.3 – 5.8%), again reflecting its stability as a trait.

Broad sense heritabilities were generally moderately high (77% in 1xB) to high (95.9% in 87xK) which suggested large genetic effects among the RILs for most traits. An exception was pod growth duration for 87xB RILs with low heritability of 23.3%.

4.3.2.2. Vegetative and morphological traits

Analysis of variance revealed significant differences between the parental plants and in some instances, the means for the four F_5 RIL populations, for vegetative and morphological traits (Table 4.14). Size related traits such as leaflet length and width, petiole length, stem diameter, internode length, floral standard width and peduncle length were greater in magnitude in the cultivated than in the wild parents and in ACC 87 than in ACC 1. However, the converse was true for overall plant growth, as indicated by stem length, branch length, number of branches, and numbers of nodes and leaves on stems and branches and node of first pod.

Table 4.14. Means and ranges for vegetative and morphological traits in the four parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Means followed by the same letter are not statistically different ($P > 0.05$)

	Berken	Kiloga	ACC 1	ACC 87	1xB	1xK	87xB	87xK
a. Leaf length (mm)								
Mean	76.5ab	80.7b	64.2a	78.2b	84.5b	85.3b	74.3ab	76.5ab
Range	60 - 88	60 - 89	57 - 69	57 - 101	60 - 128	52 - 125	38 - 118	42 – 102
b. Leaf width (mm)								
Mean	70.0c	68.6c	40.0a	56.2b	69.4c	67.6c	60.7bc	61.7bc
Range	60 - 85	50 - 80	35 - 46	46 - 70	45 - 99	45 - 91	32 - 90	33 – 91
c. Leaflet ratio								
Mean	1.1a	1.2ab	1.6d	1.4c	1.2ab	1.3bc	1.2ab	1.2ab
Range	1.0 - 1.3	1.0 - 1.3	1.4 - 1.8	1.2 - 1.5	0.9 - 1.9	1.0 - 1.7	0.9 - 1.8	0.9 - 1.8
d. Petiole length (mm)								
Mean	81.7b	82.3b	64.0a	68.0a	85.9b	83.7b	63.8a	60.0a
Range	53 – 107	64 - 114	57 - 70	63 - 72	56 - 134	49 - 146	29 - 115	34 – 87
e. Stem diameter (mm)								
Mean	6.9b	7.8b	5.7a	5.2a	6.8b	6.8b	4.9a	5.0a
Range	5.4 - 9.1	6.1 - 8.8	4.9 - 6.3	4.8 - 6.1	3.8 - 10.9	3.9 - 9.1	2.5 - 10.9	2.1 - 8.1
f. Internode length (mm)								
Mean	23.2b	23.8b	8.1a	15.1ab	23.2b	22.8b	22.7b	24.7b
Range	18.0 - 28.3	21.3 - 26.0	6.0 - 12.3	12.0 - 21.7	7.3 - 61.0	9.3 - 64.0	10.7 - 65.7	9.3 - 68.7
g. Floral standard width (mm)								
Mean	17.4bc	18.4c	13.8a	16.4b	16.4b	16.6b	17.8c	18.0c
Range	16.6 - 19.3	17.3 - 19.4	13.3 - 14.9	15.9 - 17.1	13.3 - 19.3	13.3 - 19.2	13.3 - 20.4	15.3 - 20.1
h. Stem length (cm)								
Mean	21.0a	24.0a	78.2de	83.6e	52.0bc	57.6cd	34.3ab	30.6ab
Range	18 - 24	22 - 26	52 - 126	68 - 94	12 - 150	18 - 141	8 - 110	12 - 86
i. Branch length (cm)								
Mean	3.9a	2.3a	127.4d	95.2c	51.9b	53.5b	28.1ab	27.4ab
Range	1 - 11	2 - 3	110 - 149	80 - 102	3 - 156	6 - 128	3 - 105	2 – 89
j. Peduncle length (cm)								
Mean	5.0a	6.7ab	5.2a	7.1abc	9.1c	8.4bc	6.7ab	6.6ab
Range	4.0 - 6.5	5.5 - 8.1	4.5 - 5.7	5.8 - 8.8	4.1 - 15.3	2.4 - 18.5	2.3 - 11.6	2.7 - 11.4
k. No.of branches								
Mean	2.0a	2.5a	10.8e	5.3bcd	5.9d	5.7cd	3.9abc	3.6ab
Range	0 - 3	0 - 4	10 - 11	4 - 7	2 - 13	1 - 11	0 - 11	0 – 9
l. No.of leaves on stem								
Mean	5.2a	5.3a	15.3c	8.0b	8.4b	8.3b	6.2a	6.0a
Range	4 - 6	5 - 7	15 - 16	7 - 9	4 - 16	4 - 14	4 - 12	3 – 17
m. No. of nodes on stem								
Mean	9.2a	9.5a	18.3c	12.7b	13.4b	13.3b	10.4a	9.9a
Range	7 - 10	8 - 10	17 - 19	11 - 13	9 - 20	7 - 19	7 - 17	6 – 15
n. No. of nodes on branches								
Mean	4.2a	6.0a	131.2c	40.0b	41.8b	39.1b	19.1a	16.0a
Range	0 - 8	0 - 13	124 - 139	31 - 49	4 - 127	3 - 98	0 - 98	0 – 59
o. Node of 1st pod								
Mean	4.8a	4.7a	17.3c	8.2b	8.3b	8.3b	6.4ab	6.0a
Range	4 - 5	4 - 6	16 - 18	7 - 9	3 - 17	4 - 15	4 - 14	3 – 9

Table 4.15. Coefficients of variation (CV%) and broad sense heritability (H^2_b ± standard error) for vegetative morphological traits in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Heritability estimates indicated (*ns*) are not significantly different from zero

	1xB	**1xK**	**87xB**	**87xK**
a. Leaflet length (mm)				
CV%	16.2	15.8	20.3	18.9
H^2_b ± standard error	60.2 ± 13.9	59.2 ± 14.5	22.3 ± 24.4 *ns*	9.7 ± 28.2 *ns*
b. Leaflet width (mm)				
CV%	15.4	15.1	19.5	21.5
H^2_b ± standard error	50.9 ± 6.2	29.0 ± 24.4	39.0 ± 18.8	41.3 ± 18.2
c. Leaflet ratio				
CV%	13.6	11.3	14.6	12.9
H^2_b ± standard error	23.5 ± 24.5 *ns*	11.7 ± 30.3 *ns*	70.7 ± 9.3	74.1 ± 7.9
d. Petiole length (mm)				
CV%	18.0	20.6	26.2	22.2
H^2_b ± standard error	30.1 ± 25.2	43.8 ± 20.5	40.6 ± 21.6	6.6 ± 33.7 *ns*
e. Stem diameter (mm)				
CV%	20.9	18.2	26.7	25.5
H^2_b ± standard error	41.7 ± 20.2	54.7 ± 15.1	34.1 ± 23.4	59.2 ± 13.8
f. Internode length (mm)				
CV%	50.1	51.7	40.7	37.9
H^2_b ± standard error	90.3 ± 3.3	96.4 ± 1.1	79.3 ± 6.5	88.9 ± 3.6
g. Floral standard width (mm)				
CV%	7.4	7.4	7.1	6.2
H^2_b ± standard error	54.0 ± 14.6	66.4 ± 10.5	63.0 ± 12.0	66.2 ± 10.6
h. Stem length (cm)				
CV%	58.4	57.4	64.7	58.9
H^2_b ± standard error	44.6 ± 20.9	53.3 ± 17.8	91.3 ± 3.2	87.4 ± 4.7
i. Branch length (cm)				
CV%	63.2	60.9	80.0	76.7
H^2_b ± standard error	86.0 ± 5.1	86.8 ± 5.0	91.6 ± 2.9	92.7 ± 2.9
j. Peduncle length (cm)				
CV%	30.2	33.1	30.1	31.0
H^2_b ± standard error	92.1 ± 2.6	93.9 ± 2.0	73.2 ± 8.4	76.7 ± 7.4
k. No.of branches				
CV%	40.3	40.0	56.4	58.4
H^2_b ± standard error	77.1 ± 8.3	79.8 ± 7.4	59.8 ± 12.6	61.5 ± 11.9
l. No.of leaves on stem				
CV%	30.3	29.5	26.9	32.0
H^2_b ± standard error	93.7 ± 2.0	92.3 ± 2.5	75.5 ± 7.7	79.9 ± 6.2
m. No.of nodes on stem				
CV%	15.2	16.8	19.6	17.2
H^2_b ± standard error	75.6 ± 7.8	86.4 ± 4.3	75.5 ± 7.9	76.1 ± 7.4
n. No.of nodes on branches				
CV%	60.8	58.9	94.9	70.6
H^2_b ± standard error	97.1 ± 0.9	95.7 ± 1.4	91.1 ± 3.0	73.9 ± 8.4
o. Node of 1st pod				
CV%	35.2	36.5	30.4	24.6
H^2_b ± standard error	92.7 ± 2.5	90.6 ± 3.0	90.3 ± 3.2	71.7 ± 8.7

Within the four populations, there was a wide range among individual RILs for most traits (Table 4.14). While most RILs were intermediate between the parents, transgressive segregation occurred in all observed traits with some individual RILs outside the parental ranges. Mean size-related traits in the RILs were generally closer to the cultivated than to the wild parents, exceptions being petiole length and stem diameter in the ACC 87 crosses (Table 4.14d, e) and floral standard width in the ACC 1 crosses (Table 4.14 g). For example, ACC 1 had the shortest internode length of 8.1 mm, ACC 87 was intermediate and the cultivated plants had the longest (about 23 mm). The mean internode lengths of all crosses were closer to the cultivated parents with range of 22.7 – 24.7 mm (Table 4.14f). The converse where the RILs were closer to the wild types was true for expression of most of the overall plant growth traits (Table 4.14h – o). Exceptions included stem length, and numbers of stem leaves, stem nodes and branches in the ACC 87 crosses (Table 4.14h, l – n).

Some traits such as leaflet ratio and floral standard width were stable with small CV% (ranges of 11.3 – 14.6% and 6.2 – 7.4% respectively) (Table 4.15c, g). Other size traits (e.g. leaflet length and width, stem diameter, petiole and peduncle length) exhibited intermediate CV% (range of 15.8% for leaflet length and 33.1% for peduncle length, both in 1xK) (Table 4.15a, b, d, e, j). In contrast, CV% values for some overall plant growth traits were high such as 57.4 – 80.0% for stem and branch lengths and 58.9 – 94.9% for number of nodes on branches. The remaining plant growth traits expressed intermediate CV% values. Overall, the CV% data suggested that variation among the RILs was relatively greater for the plant growth traits than for the organ size-related traits.

Broad sense heritability values generally ranged from moderate to very high (40 – 96.4%), suggesting that there were genetic effects on the traits (Table 4.15). However, low broad sense heritability estimates with high standard errors, indicating heritability estimates were not significantly different from zero, were observed in some cases such leaflet length in the ACC 87 crosses and leaflet ratio in the ACC 1 crosses (Table 4.15a, c).

4.3.2.3. Pod and seed traits

The relative differences between the cultivated and wild parents in means for pod and seed traits were largest for number of pod clusters per plant (Table 4.16b), reflecting the large differences in total number of nodes on stem (Table 4.14m). Differences in numbers of pods per peduncle and numbers of seeds per pod were relatively small (Table 4.16a, c). There were also differences in seed size and hardseededness and to a lesser extent, pod length and width (Table 4.16d – g).

The means for the RILs were intermediate between the parents with some evidence of transgressive segregation at the upper end of the range, such as for number of pods per peduncle, total pod clusters, number of seeds per pod and pod length. However, no transgressive segregation occurred in pod width, seed size and hardseededness.

Table 4.16. Means and ranges for pod and seed traits in the four parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Means followed by the same letter are not statistically different ($P > 0.05$)

	Berken	Kiloga	ACC 1	ACC 87	1xB	1xK	87xB	87xK
a. No.of pods per peduncle								
Mean	3.8b	3.9b	2.4a	5.7c	4.9bc	4.8bc	4.0b	4.1b
Range	3.0 - 5.3	3.3 - 4.3	2.0 - 2.7	5.3 - 6.7	2.3 - 9.0	2.3 - 9.3	1.3 - 6.3	1.7 - 6.7
b. Total pod clusters								
Mean	7.8a	10.2a	119.5c	44.3b	46.3b	41.2b	21.8a	19.0a
Range	6 - 12	6 - 12	102 - 146	39 - 52	7 - 130	11 - 124	3 - 104	2 - 61
c. No. of seeds per pod								
Mean	9.6ab	10.4b	9.7ab	8.4a	10.4b	10.6b	9.8ab	9.8ab
Range	8.3 - 10.5	9.1 - 11.6	8.2 - 10.6	7.6 - 9.0	4.9 - 14.1	5.2 - 13.9	4.0 - 13.6	2.9 - 13.6
d. Pod length (mm)								
Mean	91.6c	92.6c	49.5a	54.4a	71.1b	70.6b	69.3b	68.0b
Range	86 - 95	86 - 97	47 - 54	52 - 56	51 - 101	51 - 100	47 - 93	46 - 89
e. Pod width (mm)								
Mean	5.7d	5.5d	3.0a	3.7b	4.3c	4.3c	4.3c	4.2c
Range	5.2 - 6.0	5.2 - 6.0	3.0	3.2 - 4.0	3.2 - 5.1	3.2 - 5.1	3.6 - 5.1	3.7 - 5.6
f. Seed size (g/100)								
Mean	6.6c	6.6c	1.2a	1.5a	3.0b	3.0b	2.9b	2.7b
Range	5.6 - 7.4	6.0 - 7.4	1.1 - 1.3	1.4 - 1.6	1.9 - 4.8	1.8 - 5.0	1.7 - 5.2	1.4 - 5.4
g. Hardseededness (%)								
Mean	0.0a	0.0a	93.5c	99.8c	36.2b	42.0b	57.7b	51.3b
Range	0	0	87 - 98	99 - 100	0 - 100	0 - 100	0 - 100	0 - 100

Variation among the RILs as indicated by CV% was generally highest for total pod clusters (60.3 – 81.5%) and hardseededness (61.6 – 92.5%), and intermediate for most other traits except pod width and length where CV% was low (7.8 – 14.5%) (Table 4.17).

Broad sense heritability was generally intermediate to very high for most traits in most RIL populations. The exceptions were low hertitabiliy estimates for pod width in the ACC 87 crosses with high standard errors, indicating that heritability esitmates were not significantly different from zero (Table 4.17e). Broad sense heritability was particularly high for hardseededness (99.3 – 100%), indicating strong additive genetic effects for that trait. Sriphadet *et al*. (2010) reported high heritability for pod length, pod width, hardseededness (89.9 – 98.9%) using a RIL population from crossing Berken and a wild mungbean accession.

Table 4.17. Coefficients of variation (CV%) and broad sense heritability (H^2_b ± standard error) for pod and seed traits in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Heritability estimates indicated (*ns*) are not significantly different from zero

	1xB	**1xK**	**87xB**	**87xK**
a. No. of pods per peduncle				
CV%	28.7	27.1	26.4	30.4
H^2_b ± standard error	80.3 ± 7.1	93.4 ± 2.2	54.9 ± 14.6	85.8 ± 4.5
b. Total pod clusters				
CV%	61.5	60.3	81.5	63.9
H^2_b ± standard error	81.6 ± 7.0	75.8 ± 9.2	96.1 ± 1.3	91.4 ± 2.9
c. No. of seeds per pod				
CV%	16.6	16.0	22.6	21.2
H^2_b ± standard error	74.4 ± 7.9	70.7 ± 9.2	88.8 ± 4.6	85.4 ± 4.8
d. Pod length (mm)				
CV%	14.4	14.5	13.6	13.3
H^2_b ± standard error	88.7 ± 3.6	86.9 ± 4.3	89.6 ± 3.7	86.3 ± 5.0
e. Pod width (mm)				
CV%	10.3	9.5	8.6	7.8
H^2_b ± standard error	67.4 ± 12.3	73.3 ± 10.2	13.7 ± 26.8 *ns*	3.2 ± 30.7 *ns*
f. Seed size (g/100)				
CV%	21.9	22.5	23.6	22.6
H^2_b ± standard error	41.1 ± 22.1	72.4 ± 10.4	45.7 ± 20.4	66.3 ± 12.6
g. Hardseededness (%)				
CV%	92.5	82.9	61.6	72.8
H^2_b ± standard error	99.3 ± 0.3	99.4 ± 0.2	100	100

4.3.2.4. Yield related traits

Analysis of variance showed that there were significant differences between parental means for all yield related traits, and among the four RIL populations for all yield related traits except HI (Table 4.18). In general, pod dry mass, seed yield, standing dry biomass were greater in the wild accessions than in the cultivars, reflecting the fact that that the wild types had longer growth duration (Table 4.12d). Only HI was smaller in the wild accession (Table 4.18d). The low standing dry biomass of the parents (1.6 – 1.8g) partly reflected the fact that the initial prolonged wet period represented a relatively greater proportion of their total growth period.

The means of the RIL populations were mostly intermediate although the seed yields of the ACC 87 crosses were similar to cultivated parents, as was average HI. Transgressive segregation where the values of some individual lines were higher than the higher parent occurred in most traits except HI.

Table 4.18. Means and ranges for yield related traits in the four parental genotypes and the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Means followed by the same letter are not statistically different ($P > 0.05$)

	Berken	Kiloga	ACC 1	ACC 87	1xB	1xK	87xB	87xK
a. Pod dry mass (g)								
Mean	15.9a	17.2a	50.0b	37.4ab	57.6b	48.9b	22.5a	18.9a
Range	10 - 28	13 - 23	30 - 60	31 - 45	10 - 126	10 - 115	1 - 138	1 – 57
b. Seed yield (g)								
Mean	12.1a	13.3a	24.6ab	17.5a	36.1b	30.7b	14.3a	11.8a
Range	7.7 - 20.9	10.1 - 17.7	14.7 - 28.6	14.4 - 21.3	6.0 - 82.8	5.8 - 71.9	0.4 - 88.6	0.2 - 35.0
c. Standing dry biomass (g)								
Mean	1.6a	1.8a	58.2d	12.6c	11.4c	9.4bc	4.1ab	2.6ab
Range	1.0 - 3.2	1.5 - 2.1	45.5 - 66.1	9.9 - 15.2	1.4 - 56.3	1.0 - 44.3	0.1 - 51.6	0.2 - 12.9
d. Harvest index (HI)								
Mean	0.7a	0.7a	0.2b	0.3b	0.5c	0.5c	0.6c	0.5c
Range	0.7	0.7	0.2 - 0.3	0.3 - 0.4	0.1 - 0.7	0.3 - 0.6	0.4 - 0.7	0.3 - 0.7

CV% were moderate to very high for most of the yield related traits (47.9 – 198.1%), indicating moderate to very large variation of these traits among lines in a population (Table 4.19). The exception was HI where the CV% range was 11.5 – 15.4%.

In general, broad sense heritability for yield related traits was high to very high (80.2 – 97.1%), indicating large genetic effects for these traits in most crosses. The exception was standing dry biomass in Kiloga crosses, where H^2_b was intermediate (60.7% for 1xK and 63.3% for 1xB).

Table 4.19. Coefficients of variation (CV%) and broad sense heritability (H^2_b ± standard error) for yield related traits in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

	1xB	1xK	87xB	87xK
a. Pod dry mass (g)				
CV%	48.8	50.8	115.7	77.3
H^2_b ± standard error	89.0 ± 3.5	88.7 ± 4.0	94.3 ± 1.8	89.6 ± 3.3
b. Seed yield (g)				
CV%	47.9	50.6	115.0	75.8
H^2_b ± standard error	91.0 ± 2.8	92.8 ± 2.4	93.6 ± 2.1	90.1 ± 3.1
c. Standing dry biomass (g)				
CV%	104.8	89.4	198.1	80.7
H^2_b ± standard error	80.2 ± 7.4	60.7 ± 15.0	97.1 ± 1.0	63.3 ± 13.7
d. Harvest index (HI)				
CV%	15.4	12.5	11.5	11.9
H^2_b ± standard error	94.8 ± 1.6	95.5 ±1.6	93.2 ± 2.2	97.1 ± 1.0

4.3.3. Phenotypic correlations between quantitative traits in the mungbean F_5 RIL populations

There was a large number of statistically significant phenotypic correlations between pairs of quantitative traits in the F_5 plants in each RIL population. The pairwise interrelations between a subset of key traits for each of the four RIL populations are presented in Table 4.20a – d. The interrelations between the full set of traits recorded in the study are listed in Appendix 4.1. Some of the correlations appeared to reflect underlying physiological processes associated with plant growth and development.

There were significant positive correlations ($P \leq 0.05$) between the key phenological traits, time to flowering and total growth duration in all four populations (Table 4.20a – d), indicating that those RILs which took longer to flower also had longer growth duration, presumably as a direct consequence. A positive correlation between time to flowering and the total growth cycle among wild mungbean accessions was similarly observed by Lawn and Rebetzke (2006). In turn, both of these phenological traits were positively correlated with other morphological and agronomic traits that in some way reflected plant size. For example, in all four populations, both time to flowering and total growth duration were significantly positively correlated with the morphological traits, number of nodes on stem and total pod clusters, as well as with the agronomic traits such as seed yield and standing dry biomass plant. These positive correlations presumably reflected the fact that the later flowering RILs grew longer, developed more stem nodes and pod clusters, and so produced greater biomass and more seed yield per plant.

Others have also reported positive correlations between longer growth cycle and total biomass (e.g. Makeen *et al*. 2007) and time to flowering and seed yield (e.g. Arshad *et al*. 2009). The interrelations between these growth-related traits may have implications for the subsequent marker studies, to the extent that the individual traits may not be truly independent in a physiological sense, but rather, be manifestations of an overriding effect of phenology, especially time to flowering, on the potential for subsequent plant growth.

That said, the correlations between time to flowering and total growth duration were somewhat greater in the ACC 1 crosses ($r = 0.48^{**}$ and 0.52^{**}, respectively) than in the two ACC 87 crosses ($r = 0.30^{**}$ and 0.25^{*}, respectively). In the ACC 1 populations, there were more late flowering RILs, which necessarily grew for longer and so grew larger. In contrast, in the ACC 87 populations, there were more early flowering plants, providing greater opportunity for dispersion in total growth duration, depending on how long different RILs flowered. In all four populations, there was a significant negative correlation between time to flowering and HI, which indicated that earlier flowering RILs tended to produce a higher proportion of seed per unit biomass than the later flowering plants. The cultivated parents were both earlier flowering and had higher HI than the wild parents (Table 4.12a, 4.18d). These finding were consistent with Nguyen (2011).

Table 4.20. Pairwise phenotypic correlations (*r*) among a subset of quantitative traits observed in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87).

Entries are the linear correlation co-efficients (*r*) between the respective trait pairs

		NoS	PP	PC	SP	PL	PW	SS	Flo	Mat	SY	DM
a. ACC 1 x Berken	PP	.04										
	TC	.58**	-.23*									
	SP	.07	-.38**	.14								
	PL	.04	-.02	-.16	.30**							
	PW	.03	.25*	-.38**	-.14	.65**						
	SS	.03	.22*	-.35**	-.23*	.67**	.81**					
	Flo	.67**	-.19	.62**	.27*	.03	-.11	-.19				
	Mat	.48**	-.10	.52**	.26*	.09	-.10	-.18	.48**			
	SY	.64**	.07	.60**	.28**	.18	-.02	-.03	.44**	.61**		
	DM	.64**	-.18	.73**	.22*	-.02	-.21	-.24*	.86**	.46**	.42**	
	HI	-.04**	.28**	-.47**	-.19	.17	.33**	.43**	-.75**	-.35**	-.10	-.81**
b. ACC 1 x Kiloga	PP	.13										
	TC	.74**	-.03									
	SP	-.03	-.25*	.07								
	PL	-.01	-.11	-.16	.36**							
	PW	-.16	.05	-.31**	.07	.68**						
	SS	-.14	.03	-.28**	.07	.67**	.85**					
	Flo	.68**	-.12	.81**	.10	-.15	-.32**	-.23*				
	Mat	.48**	.02	.55**	.15	.06	-.11	-.09	.52**			
	SY	.65**	.05	.78**	.13	.00	-.07	.03	.63**	.56**		
	DM	.68**	-.12	.88**	.06	-.13	-.28**	-.22*	.84**	.53**	.66**	
	HI	-.39**	.16	-.49**	.14	.37**	.50**	.53**	-.59**	-.33**	-.14	-.65**
c. ACC 87 x Berken	PP	.38**										
	TC	.79**	.42**									
	SP	.42**	.34**	.31**								
	PL	.34**	.23*	.24*	.59**							
	PW	.06	-.27**	-.03	-.07	.39**						
	SS	.03	-.15	.06	-.25*	.40**	.75**					
	Flo	.63**	.19*	.66**	.35**	.27**	-.01	-.002				
	Mat	.63**	.47**	.71**	.41**	.40**	.05	.13	.30**			
	SY	.78**	.40**	.93**	.34**	.35**	.13	.21*	.68**	.67**		
	DM	.71**	.26**	.87**	.22*	.17	.07	.13	.76**	.44**	.89**	
	HI	-.14	.04	-.17	-.05	-.05	.21*	.21*	-.50**	-.05	-.14	-.26**
d. ACC 87 x Kiloga	PP	.54**										
	TC	.65**	.37**									
	SP	.29**	.34**	.37**								
	PL	.39**	.32**	.16	.61**							
	PW	.18	.12	-.17	-.07	.43**						
	SS	.04	-.08	-.31**	-.32**	.32**	.69**					
	Flo	.43**	.22*	.26**	.35**	.43**	.16	-.09				
	Mat	.46**	.44**	.67**	.50**	.29**	-.02	-.30**	.25*			
	SY	.65**	.51**	.79**	.50**	.41**	.09	-.16	.37**	.75**		
	DM	.66**	.39**	.61**	.49**	.44**	.14	-.14	.62**	.61**	.79**	
	HI	.18	.21*	.18	.05	.18	.06	.28**	-.29**	.02	.14	-.17

*, ** Indicate significant correlations at $P < 0.05$, $P < 0.01$ respectively

NoS = No. of nodes on stem; PP = No. of pods per peduncle; PC = Total pod clusters; SP =No. of seeds per pod; PL= Pod length; PW = Pod width; SS = Seed size; Flo = Time to flowering; Mat = Growth duration; SY = Seed yield; DM = Standing dry biomass; HI = Harvest index

Another example of correlations reflecting underlying physiological processes was the fact that in all four populations, pod length was significantly positively correlated with seeds per pod (Table 4.20a –d). These correlations probably reflected the fact that, to the extent that seed size was similar among RILs, the pods with more seeds were necessarily longer. Interestingly, these relations were somewhat stronger in the ACC 87 crosses than in the ACC 1 crosses. This would be expected given that the parental range in seed size was greater in the ACC 1 than in the ACC 87 crosses. In all four crosses, pod width was strongly positively correlated with seed size (100-seed weight), which again would be expected, as wider pods are needed to accommodate larger seeds.

In other instances, the correlations appeared to reflect differences between parental extremes for the traits, and may in turn reflect underlying genetic associations or possibly even linkages between traits. For example, seed size was significantly positively correlated with HI in all four populations (Table 4.20a – d). While it is possible there is some underlying physiological basis to this relation, it is more likely to simply reflect the strong parental differences in these traits. The cultivated parents were characterized by high HI and large seed size, while the wild parents were small-seeded with small HI (Tables 4.16f, 4.18d).

4.3.4. General conclusions

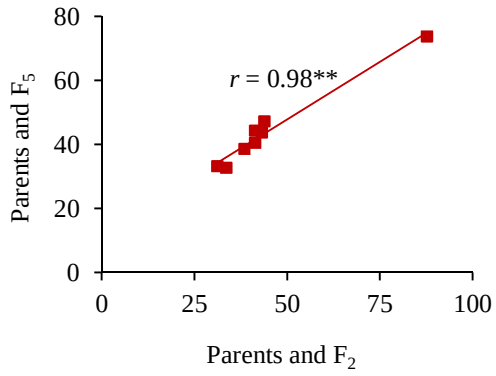
There was clear evidence from this mungbean phenotypic evaluation study of large differences between the cultivated and wild parents and in the four RIL populations for a wide range of phenological, vegetative morphological, pod and seed, and yield-related traits. In broad terms, these large differences were strongly consistent with previous observations based on these same four parents and the same four crosses in the F_2 generation (Nguyen 2011). In terms of qualitative traits, with few exceptions, similar parental and hybrid phenotypes were observed in the present study as were observed by Nguyen (2011). Further, with some exceptions such as pod dehisence, perenniality and seed lustre, the inheritance models suggested by the segregation ratios in the F_5 generation RILs in this study were broadly consistent with those observed in the earlier generations of Nguyen (2011).

In terms of quantitative traits, the consistency between this study and that of Nguyen (2011) is illustrated by comparing a representative trait from each of the four trait categories evaluated in this study with the results obtained by Nguyen (2011). Mean values for time to flowering, leaflet length, hardseededness and seed yield for the four parents parents and four F_2 populations in Nguyen (2011) were plotted against the mean parental and F_5 generation values in this study (Figure 4.5a – d). For all four traits, there was a high and statistically significant positive correlation (r = 0.67 – 0.98) between the results of the two studies.

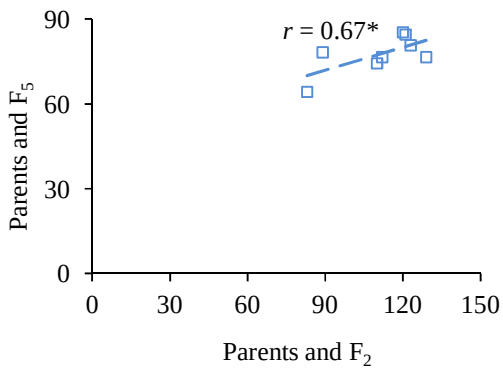
For most traits, the means of the RIL populations were intermeditate between the parents although transgressive segregation occurred in some traits. In most instances where there was transgressive segregation, especially for growth-related traits that revealed vegetative vigour, some lines exceeded the more vigorous wild parents. Transgressive segregation at the lower end of the range was observed in a few mainly size-related traits.

For a large number of traits, there were wide ranges between the smallest and largest RILs and large CV%, indicating the existence for most traits and in most crosses of considerable variation among lines. In addition, the moderate to high broad sense heritability estimates for most traits suggested genetic effects contributed to the observed variation. As noted previously, the high level of homozygosity that would be expected in the F_5 generation should mean that dominance effects would be small, and much of the observed genetic variance would be additive.

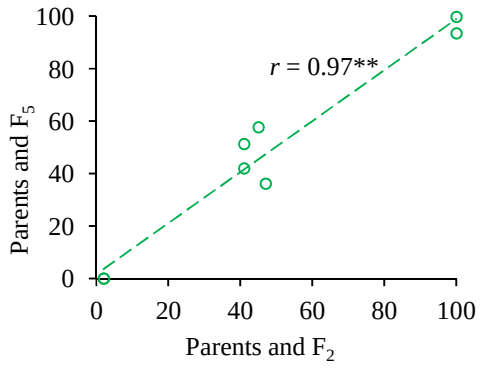
(a) Time to flowering (days)



(b) Leaflet length (mm)



(c) Hardseededness (%)



(d) Seed yield (g/plant)

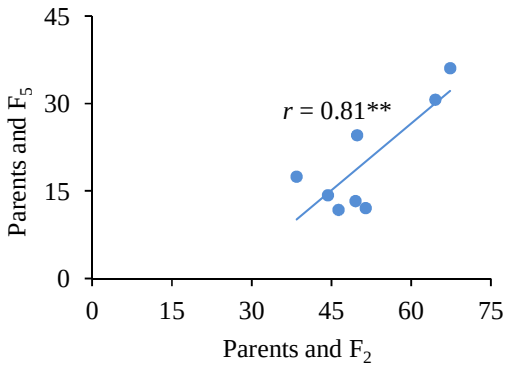


Figure 4.5. Correlations between trait means (a) Time to flowering; (b) Leaflet length; (c) Hardseededness; (d) Seed yield for the four mungbean parents and the four F_2 hybrid populations in Nguyen (2011) with those for the same four parents and the four F_5 RIL populations in the present study

Thus, the four mungbean RIL populations arising from the crosses of cultivated and wild parents exhibited a rich pool of genotypic variation and high heritability for a wide range of traits relating to phenology, mophology, pods and seeds, and yield as well as a number of significant phenotypic correlations between pairs of traits. In addition, high levels of polymorphism were detected in the development of the DArT mungbean libraries (Chapter 3). Given the richness of diversity in this

germplasm, it was considered a useful plant model for evaluating the application of DArT for manipulating QTLs in mungbean. In turn, it was anticipated that DArT would be informative in suggesting inheritance models in both qualitative and quantitative traits, explaining observed differences between models in the F_2 and F_5 generations, clarifying which correlated traits are genetically linked, and which traits might be exploited for crop development in the future. These issues were explored in the research reported in the next chapter.

CHAPTER 5. USE OF DArT MARKERS TO IDENTIFY QUANTITATIVE TRAIT LOCI (QTLs) IN MUNGBEAN

5.1. Introduction

This chapter reports on research in which four F_5 mungbean RIL populations were genotyped against previously developed mungbean and soybean DArT arrays (Chapter 3). QTL analysis was applied to detect QTLs linked with the 54 traits evaluated in Chapter 4.

The classical Mendelian approach for studies of qualitative trait inheritance based on phenotypic segregation in different generations such as the F_1, F_2, backcrosses or RILs has been widely used until the present day. However, the process whereby models are fitted to progeny segregation ratios requires that some caution is exercised in interpreting the models of inheritance, as was argued by Nguyen *et al.* (2012). In some cases, distortion of segregation ratios can occur in phenotypic observations, which limits interpretation (e.g. Lambrides *et al*. 2004).

In addition, there are many complex traits known as quantitative traits, in which inheritance is polygenic, and cannot be unravelled easily using the simple Mendelian approach. In these situations, molecular markers have provided an alternative strategy to reveal insights into inheritance. Molecular markers can be useful, regardless of whether the trait is qualitatively or quantitatively inherited.

As noted in Chapter 2, to date, DArT markers have been applied to a number of species such as barley (Wenzl *et al*. 2004), cassava (Xia *et al*. 2005) and pigeon pea (Yang *et al*. 2006) to investigate genetic diversity; to the model plant *Arabidopsis* (Wittenberg *et al*. 2005), wheat (Semagn *et al*. 2006c), barley (Wenzl *et al*. 2006), Triticale (Alheit *et al*. 2011) and rapeseed (Raman *et al*. 2012) for high-density DArT consensus maps; and to barley (Rheault *et al*. 2007) for QTL mapping. Incorporation of DArT markers with other marker types has been shown to provide reliable linkage maps and to increase map resolution as well as QTL detection rates. By applying the DArT marker protocols that were developed for mungbean (Chapter 3), this research investigated: (i) the application of polymorphic DArT markers to construct linkage maps in each individual mungbean RIL population; and (ii) the detection of QTLs linked to the 54 traits described in Chapter 4.

5.2. Materials and methods

5.2.1. Plant materials and phenotypic evaluation

The four F_5 RIL populations obtained from crosses of the two mungbean cultivars, Berken and Kiloga and the two wild accessions, ACC 1 and ACC 87 were used. The creation and advancement of these populations were described previously in Chapter 4. The phenotypic information on 54 qualitative and quantitative traits, developed previously during the phenotypic evaluation described in Chapter 4, was used for the QTL analyses using DArT markers. Traits were assigned to six categories (Table 5.1).

Table 5.1. Assigned categories and abbreviations for 54 traits used for QTL analysis in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Colour♪	Trait categories	Abbreviations
	Qualitative morphological traits	**HPc**: Hypocotyl colour; **STc**: Stem colour; **LRc**: Leaf rachis colour; **LPc**: Leaf petiole colour; **PHd**: plant hair density; **PHc**: Plant hair colour; **GH**: Growth habit; **TW**: Twining; **LLb**: Leaflet lobing; **FLc**: Flower colour; **FSt**: Inflorescence structure; **DPc**: Dry pod colour; **PD**: Pod dehiscence; **PM**: Powdery mildew reaction; **Thr**: Thrip reaction; **TuB**: Perenniality
	Qualitative seed traits	**Tc**: Testa colour; S**M**: seed mottling; **SCr**: Seed coat ridging; **Lus**: Lustre; **TL**: Texture layer depth; **HLc**: Hilum colour; **TLc**: Texture layer colour; **Ost**: Overall visual seed traits
	Phenological traits	**Flo**: Time to flowering; **DFlo**: Duration of flowering; **PG**: Pod growth duration; **Mat**: Growth duration
	Quantitative morphological traits	**LL**: Leaflet length; **LW**: Leaflet width; **LR**: Leaflet ratio; **PeL**: Petiole length; **STd**: Stem diameter; **IL**: Internode length; **FloS**: Floral standard width; **SL**: Stem length; **BL**: Branch length; **PdL**: Peduncle length; **BpP**: No. of branches per plant; **LoS**: No. of leaves on stem; **NoS**: No. of nodes on stem; **NoB**: No. of nodes on branches; **N1P**: Node of 1st pod
	Pod and seed traits	**PP**: No. of pods per peduncle **PC**: Total pod clusters; **SP**: No. of seeds per pod; **PL**: Pod length; **PW**: Pod width; **SS**: Seed size; **HS**: Hardseededness;
	Yield related traits	**DPM**: Dry pod mass; **SY**: Seed yield; **DM**: Standing dry biomass; **HI**: Harvest index

♪: Colours of trait categories are used later in Figures 5.2 and 5.7

While the plants were being grown for the phenotypic assessment at the CSIRO Davies Laboratory in Townsville, young fully expanded leaves from each parental plant and each line of the four F_5 RIL populations were collected, lyophilised at the Davies Laboratory and then taken to the Queensland

Biosciences Precinct in Brisbane for DNA extraction in 2011. The DArT genotyping analyses were conducted at the DArT Pty Ltd laboratory in Canberra during 2011 – 2012.

5.2.2. Genetic analysis

5.2.2.1. DNA extraction and genotyping

DNA extraction followed the standard DArT protocol which was described in Chapter 3. The only modification was the use of beads instead of liquid nitrogen for grinding the lyophilised leaf materials into fine powder. After DNA extraction, DNA was repurified using a DNA repurification kit [ZR – 96 215 DNA clean & concentrator 216TM – 5 (Zymo Research)].

Two RE combinations, comprising the rare cutter *Pst*I and a frequent cutter (either *Taq*I or *BstN*I), which were chosen for creation of the mungbean and soybean DArT libraries (Chapter 3) were used for genome complexity reduction. Digestion and ligation reactions were performed at 37 ^{o}C for 2 h and then 60 ^{o}C for 2 h. Subsets of DNA samples from each RIL population and parents were randomly chosen for replicates. After PCR and electrophoresis gel performance, PCR products were precipitated and labelled with Cy3 and Cy5 dye (targets) (see details in Chapter 3).

Since a significant number of DArT markers were transferable between soybean and mungbean (Chapter 3), the mungbean DNA samples were genotyped using both mungbean and soybean arrays. Hence, all amplicons (15,360 amplicons) in forty 384-well plates (20 plates each from the soybean and mungbean libraries) were arrayed onto Poly-L-lysine-coated microscope slides using a MicroGrid II arrayer. The DNA deposited on the slides was denatured by incubation in hot water (92 ^{o}C) for 2 min.

Labelled targets were mixed and added with Inactivation mix and DArT hybridizer, then denatured at 95 ^{o}C for 3 min. Hybridization was done by pipetting labelled targets onto the microarray surface, covering with a glass coverslip and incubating in a 65 ^{o}C water bath overnight. Slide washing and drying were undertaken as described in Chapter 3, after which the slides were scanned using a confocal laser scanner.

5.2.2.2. Polymorphic DArT marker selection

TIF images derived from slide scanning were analysed by DArTsoft 7.4 (Diversity Arrays Technology P/L, Canberra, Australia) to identify and score polymorphic clones. Marker names were generated by DArTsoft including abbreviations for species (mb for mungbean; so for soybean), method (Pb for *Pst*I/*BstN*I and Pt for *Pst*I/*Taq*I) and the clone identification number (indicating the position – row and column – of the clone on a specific library plate). Markers were scored '1' for the presence in the genomic representation of the sample, '0' for absence, and 'x' when the clustering algorithm deployed in DArTsoft was unable to score the sample with sufficient confidence. Then, thresholds of P > 0, Callrate > 80 and Reproducibility > 90 (P – percentage of effective clustering; Callrate – percentage of effective scores; Reproducibility – percentage of identical results) were applied for DArT Standard Analysis (Diversity Arrays Technology P/L, Canberra, Australia).

Initially, marker selections were based on a number of criteria: Consensus - the consensus of five different statistical methods in marker scoring, Average Reproducibility, Average P, Discordance (overall variation of scores within the replicates) and PIC (informativeness of a genetic marker). The selection was conducted in Excel as follows: markers were first selected for Consensus > 97%, then Average Reproducibility > 97%; markers in the range of 96.5 – 97% average reproducibility were only selected for Average P > 80%. Then markers of discordance < 1%, PIC > 0.2 and Average P > 65% were selected. Although mungbean is inbreeding, there were still cases where DArT markers were scored differently across the replicated parental plants (6 replicate plants for each parent). Hence, after initial selection, only markers which consistently scored across replicated parental samples and differentiated the parents in a cross were selected.

5.2.2.3. Genetic linkage map construction

Map construction was performed with JoinMap 3.0 (van Ooijen and Voorips 2001) and involved three steps: grouping, ordering and rippling. For genotyping input, DArT marker polymorphic scores (0; 1) were transformed into Joinmap genotype codes according to the score of the parents. Chi-square values for the marker segregation ratio of 1:1 were calculated with "Set χ^2 – Test classification for selected loci" in Joinmap.

Markers were placed into linkage groups (LGs) with the "LOD groupings" and "Create groups for mapping" commands using the Kosambi map function. By convention, a LOD score greater than 3.0, indicating that linkage is 1000 times more likely (i.e. 1000:1) than no linkage, is considered evidence for linkage (Morton 1955). Initially, a LOD range of 2 to 10 was used to test for linkage groups. In cases where many markers (> 200) were in a group, LOD scores were modified to a higher cutoff value.

LGs with more than five markers were selected for the ordering and rippling steps. These steps were initiated by the "Calculate map" command using the default settings of mapping parameters. The process was carried out by simulated annealing, excluding markers that contributed to unstable marker orders in the first two ordering rounds, to yield a high likelihood support framework map. Markers with mean χ^2 contribution > 5 were excluded. Presentation of linkage groups then was drawn using Mapchart 2.2 (Voorips 2002).

Genetic dissimilarities among the four RIL populations were calculated by NTSYS 2.1 (Rohlf 2000) based on Nei's distance (Nei 1972) and using the total number of DArT markers that mapped in the four individual maps.

5.2.2.4. QTL statistical analysis

Linkage map construction and QTL analyses using linkage maps are sometimes computing intensive, especially when several populations are studied, a large number of polymorphic markers are utilized and various numbers of traits are analysed. This was the case in this mungbean study where four RIL mungbean populations were genotyped with thousands of polymorphic DArT markers and phenotyped for

54 traits. In addition, there are still linkage map errors which could affect QTL analyses (Kearsey and Farquhar 1998; Collard *et al.* 2005; Cheema and Dicks 2009; Li *et al.* 2012c). Therefore, the Statistical Machine Learning method (SML), which is more robust to genotyping and linkage mapping errors, and which identifies markers linked to QTLs in the absence of a genetic map (Bedo *et al.* 2008), was applied.

However, an alternative QTL analysis method using linkage maps – Inclusive Composite Interval Mapping (ICIM) – was also applied. Since ICIM has a fast convergence speed, it is less computing intensive than other QTL analysis methods such as Composite Interval Mapping (CIM). ICIM also retains all the advantages of several methods for additive mapping such as CIM and interval mapping (IM). It was also shown to increase detection power, reduce false detection rates and produce less biased estimates of QTL effects (Wang *et al.* 2011a). Therefore the ICIM method was used for QTL analysis in this study.

5.2.2.4.1. QTL statistical analysis without linkage maps – Statistical Machine Learning (SML) method

SML detects QTLs by determining the contribution of each marker to the model performance during the recursive feature elimination (RFE) procedure (Bedo *et al*. 2008). Initially, a linear model based on every marker is fitted to the phenotype. RFE then eliminates the least useful marker. The model is then refitted and the process repeated, until only a single marker is left. The change in variance explained after each elimination is assigned to the marker that was removed and interpreted as the percentage of the phenotypic variation explained by that QTL. QTL analysis by SML was done by "Standard marker analysis" software (Diversity Arrays Technology P/L, Canberra, Australia).

Significant QTLs were defined as those with a significance probability level $P \leq 0.01$.

5.2.2.4.2. QTL statistical analysis with linkage maps – Inclusive Composite Interval Mapping (ICIM) method

Although the number of linkage groups yielded was more than the mungbean haploid number (11), all linkage groups were used for ICIM QTL analysis, which was performed by QTL IciMapping 3.2 (Wang *et al.* 2011a). The ICIM with additive effects (abbreviated as ICIM – ADD) method was applied to identify putative DArT markers/ QTL regions for traits. This method is based on the property that, under the assumption of additivity of QTL effects on the phenotype of a trait of interest, the additive effect of a QTL can be completely absorbed by the two flanking marker variables. Stepwise regression is applied to identify the most significant regression variables/ markers with different probability levels of entering and removing the variables. The step in scanning was 1 cM and the LOD score threshold was determined by a permutation test with 1000 replications (Churchill and Doerge 1994). The default probability in stepwise regression (PIN – the largest *P*-value for entering variables in the stepwise regression of phenotype on marker variables) was 0.001. Where no QTLs linked to the trait were detected with a PIN of 0.001, a relaxed PIN of 0.01 was applied. QTLs of LOD > 3.0 were selected. Li *et al.* (2007, 2008) also argued that the phenotypic variation explanation (PVE) after fitting all marker variables and marker pairs should approximate the proportion of phenotypic variation explained by additive QTLs; that is, the broad sense

heritability. Therefore, in order to enhance the reliability and to not overestimate effects of QTLs reported here for quantitative traits, only QTLs for which PVE ≤ broad sense heritability estimates (H_b^2) (Chapter 4) were selected, regardless of the PIN values.

5.2.2.4.3. Overlapped, common and co-localized QTLs

SML provided a marker link to a QTL while ICIM provided a marker interval conditioning that QTL. Pearson correlations (*r*) among markers linked to QTLs and with markers mapping on the individual linkage map were calculated, using marker genotype (scoring pattern). Due to the relatively small population size and a high number of markers, there is a substantial redundancy in the marker set (i.e. same pattern of segregation among the progenies). In addition, as the cloning step in DArT library construction selects occasionally a particular DNA sequence more than once, there is a technical component to marker redundancy. Finally, closely linked markers are very likely to represent the same linked QTL. Therefore marker pairs with correlation $r \geq 0.84$ were considered to map on the same LG and target the same QTL.

Although SML is non-map based, linkage groups and marker positions are included for comparison wherever those QTLs mapped. For QTLs detected by the SML method and not located on the linkage map, their positions were assigned to LG and position of mapped markers wherever correlations *r* were ≥ 0.84.

An *overlapped or a common QTL between the two methods* was accounted for when the markers/QTL detected by SML were highly correlated ($r \geq 0.84$) or shared a common marker with the QTL detected by ICIM – ADD, regardless of whether that marker flanked the left or right end of the QTL region.

A *common QTL among the traits* in a population was defined as the QTL located by the same or high correlated ($r \geq 0.84$) markers in the case of the SML method or by the same marker interval in the case of the ICIM method.

Co-localized QTLs were the QTLs that mapped on the same LG in a population.

QTLs were congruent across populations for a trait if those QTLs share the same or highly correlated markers ($r \geq 0.84$) in the case of the SML method or shared at least a common marker regardless of its flanking position in the case of the ICIM method.

5.3. Results and discussion

5.3.1. Polymorphic DArT markers

Based on the selection criteria, there were different numbers of polymorphic DArT markers identified among the four RIL populations (Table 5.2).

Table 5.2. Number of selected polymorphic DArT markers and levels of redundancy in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

Populations		No. of selected DArT markers	Redundancy♪	
Cross	Notation		No.	(%)
ACC 1 x Berken	1xB	2013	345	17.1
ACC 1 x Kiloga	1xK	1995	299	15.0
ACC 87 x Berken	87xB	1062	37	3.5
ACC 87 x Kiloga	87xK	1887	107	5.7

♪: Redundancy obtained from the "Similarity of loci" calculation in Joinmap 3.0

Although the number of polymorphic markers was highest in 1xB, followed by 1xK, these two populations had the highest percentage of redundancy (17% and 15% respectively). These rates were in the range of DArT redundancy in other species such as *Eucalyptus* (17%) (Sansaloni *et al*. 2010). In contrast, the redundancy levels of 87xB and 87xK were much smaller (3.5% and 5.7% respectively). If the segregation pattern redundancy was accounted for, the numbers of markers in the four populations would be in the range of 1025 – 1785. The reported redundancy referred to markers which gave more than 99% similar or identical scores. Since most markers with identical/highly similar sequence would have 99% identity of scoring, this probably reflected a level of sequence redundancy, as shown in *Arabidopsis* (Wittenberg *et al*. 2005), oat (Tinker *et al*. 2009) and *Eucalyptus* (33.8%) (Petroli *et al.* 2012). Therefore, as was concluded in the development the DArT protocols for mungbean (Chapter 3), the number of unique polymorphic DArT markers selected in these four mungbean RIL populations may be overestimated.

5.3.2. Linkage maps for the individual mungbean F_5 RIL populations

Data sets with 2013, 1995, 1062 and 1887 selected DArT markers, for the 1xB, 1xK, 87xB and 87xK populations, respectively (Table 5.2), were subjected to linkage map analysis. The grouping analysis with LOD > 4 resulted in 39.3% to 63.3% of markers being grouped into 15 to 19 groups, depending on the RIL population (Table 5.3).

The number of DArT markers grouped on LGs was highest in the 1xB population (981), followed by 1xK (885), 87xK (741) and 87xB (672). Segregation distortion ($P < 0.05$) occurred in all four RIL populations with a range of 33.7% to 47.8%. These levels were in the range of levels of distortion detected for other markers in mungbean mapping projects such 36.7% for RFLP markers (Humphry *et al.* 2005), 38.3% for SSR, RAPD and STS markers (Zhao *et al.* 2010), or for DArT markers in Triticale mapping (8.3 – 34.3%) (Alheit *et al*. 2011) and for RFLP and SSR markers in *Brassica* (22% – 49%) (Wang *et al.* 2011b). In many cases, the markers segregating with distortion occurred in linked blocks and may simply reflect a lack of recombination in the distorted regions of the genome for these populations. However, these levels also could be overestimated because redundant markers were not accounted for. Generally,

more DArT markers segregated with distortion in favour of alleles from the cultivated parents in all crosses. For instance, 336 of 981 DArT markers (34.3%) mapped on the 1xB linkage map with aberrant segregation ratios in favour of alleles from Berken compared with only 80 markers (8.2%) segregating in favour of alleles from ACC 1 (Table 5.3). Berken crosses exhibited slightly greater segregation distortion in favour of the cultivar (34.3% – 31.4%) than the Kiloga crosses (27.8% – 30.1%). Meanwhile, the ACC 87 crosses exhibited more segregation distortion favourable to the wild than the ACC 1 crosses (14.0% – 16.4% compared to 5.9% – 8.2%). In the ACC 1 crosses, segregation distortion seemed to be random because it occurred in all LGs in both populations, except for LG6 and LG8 of the 1xK population. No aberrant segregation occurred on LG4, LG5 and LG6 of the 87xB, and LG7 of the 87xK.

Table 5.3. Mapping statistics of the DArT markers and individual maps of the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

		1xB	1xK	87xB	87xK
Total mapped markers	No.	981	885	672	741
	%	48.7	44.4	63.3	39.3
Segregation distortion markers	No.	416	298	321	327
	%	42.4	33.7	47.8	44.1
Distortion in favour of cultivated alleles	No.	336	246	211	223
	%	34.3	27.8	31.4	30.1
Distortion in favour of wild alleles	No.	80	52	110	104
	%	8.2	5.9	16.4	14.0
Linkage map					
LGs	No.	15	19	17	17
Number of markers on a LG	Min	9	12	9	6
	Max	184	114	95	87
	Average	65.4	46.6	39.5	43.6
LG size (cM)	Min	19.2	7.1	12.2	16.6
	Max	121.2	93.8	131.2	91
	Average	55.2	42.8	39.6	37.4
Inter-marker distance (cM)	Min	0.01	0.01	0.01	0.01
	Max	14.6	22.0	32.4	16.4
	Average	1.1	1.2	1.1	0.9
Aggregate size (cM)		883.5	812.7	634.3	629.7

The DArT markers spanned the linkage maps (Table 5.3), with the largest map size in 1xB (883.5 cM) and the smallest map sizes in 87xB (634.3 cM) and 87xK (629.7 cM). Figure 5.1 illustrates the 15 LGs constructed from the F_5 RIL population of 1xB while the LGs of the other three RIL populations, 1xK, 87xB and 87xK, are presented in Appendix 5.1. The individual linkage maps contained on average similar numbers of markers per LG (in the range of 39.5 – 46.6), except 1xB with 65.4. Markers with inter-distance < 0.01 cM were considered as co-segregated loci and on average, markers were positioned at inter-marker distances of 0.9 – 1.2 cM (Table 5.3). DArT markers were shown to provide high-density

consensus maps in several other species, including barley (Wenzl *et al*. 2004, 2006), *Arabidopsis* (Wittenberg *et al.* 2005), wheat (Akbari *et al.* 2006), sorghum (Mace *et al.* 2008) and oat (Tinker *et al*. 2009). However, with large ranges in the number of markers per LG (e.g. 9 – 184 in 1xB), in LG sizes (e.g. 12.2 – 131.2 cM in 87xB) and in inter-marker distances (e.g. 0.01 – 22 cM in 1xK), it is clear that the DArT markers were not evenly distributed along the chromosomes. Indeed, there was evidence of cluster formation of markers in different regions of the chromosomes (Figure 5.1; Appendix 5.1). These patterns were also observed in Triticale linkage maps when only DArT markers were applied (Alheit *et al*. 2011).

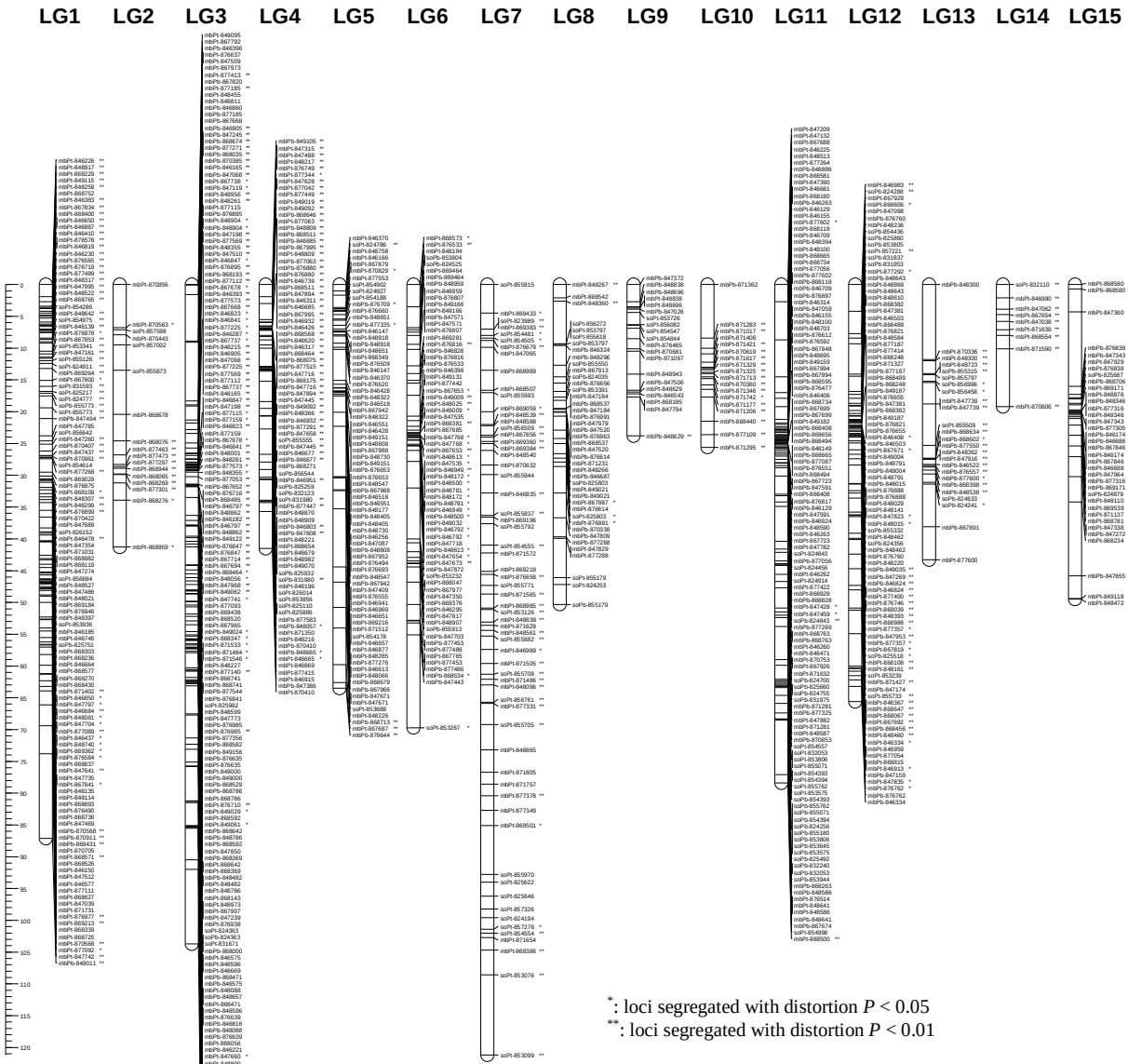


Figure 5.1. A genetic linkage map of mungbean derived from F_5 RILs of the cross of ACC 1 x Berken. The map includes 981 DArT markers. A centiMorgan scale is on the left.

Only nine molecular linkage maps, variously using RAPD, RFLP, SSR, STS and EST-SSR markers, have been published so far for mungbean (Menancio-Hautea *et al.* 1993; Boutin *et al.* 1995; Lambrides *et al.* 2000; Humphry *et al.* 2002; Zhao *et al.* 2010; Isemura *et al*. 2012; Kajonphol *et al.* 2012). The sizes of the present DArT linkage maps for populations 1xB and 1xK were in the range of published maps (691.7 cM – 1831.8 cM) while those of the 87xB and 87xK were shorter. The fewer DArT markers selected at the same quality thresholds for the ACC 87 RIL populations possibly caused this difference. The average distances between adjacent markers in published maps were at least three times greater than in the present DArT marker maps (3 – 10.2 cM compared to 0.9 – 1.2 cM respectively), except for the linkage map constructed by Isemura *et al*. (2012) (1.78 cM). Most linkage maps have resolved 12 – 14 LGs (i.e. more than 11, the haploid chromosome number of mungbean), the exceptions being the maps by Kajonphol *et al.* (2012) and Isemura *et al.* (2012), composed of, respectively, 150 and 430 markers in 11 LGs. However, the recommended interval length for genome-wide QTL scanning is less than 10 cM (Doerge 2002), and the DArT markers as applied here were thus very suitable for QTL scanning.

Overall, 1883 DArT markers mapped on the four individual linkage maps, and of these, 1308 (69.5%) were unique, although this did not take into account redundant sequencing markers. Between 292 (1xK and 87xB) to 545 markers (1xB and 1xK) were in common (Table 5.4). The higher number of common markers and lower value of Nei's genetic distance reflected greater similarity between populations. For example, 1xB was more similar to 1xK than to either 87xB or 87xK, with a higher number of common markers (545 compared to 294 and 341) and lower Nei's genetic distance (0.54 compared to 1.00 and 0.92). Generally, the genetic distances revealed that 1xB and 1xK had the lowest degree of genetic dissimilarity (0.54), followed by 87xB and 87xK (0.81) whereas populations 1xB and 87xB were more distant (1.00) (Table 5.4). A high negative correlation (r = -0.94) between the number of common markers and Nei's genetic distance was observed. This was consistent with previous observations on the genetic distance between parents in Chapter 3, which revealed that dissimilarity between Berken and Kiloga was less than that between ACC 1 and ACC 87 (dissimilarity of 0.19 for Berken – Kiloga and of 0.39 for ACC 1 – ACC 87).

Table 5.4. Pairwise common markers and genetic dissimilarity among the four mungbean mapping populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

The number of markers common between individual maps is shown above the diagonal.
Pairwise Nei population genetic distances are shown below the diagonal

	1xB	**1xK**	**87xB**	**87xK**
1xB		545	294	341
1xK	0.54		292	345
87xB	1.00	0.97		314
87xK	0.92	0.85	0.81	

5.3.3. Overview of QTL detection in the four mungbean F_5 RIL populations using the SML and ICIM-ADD methods

All detected putative QTLs (at a significance level of $P \leq 0.01$ for SML and at PIN of 0.001 and 0.01 for ICIM) are listed in Appendix 5.2. Overlapped and common QTLs between the two methods (hereafter referred to as overlapped QTLs) are indicated in that Appendix. Pairwise correlations between markers were consistent with linkage map construction in which markers mapped at close positions with average of 2.5 cM in distance on the same linkage group exhibited significant correlations ($r \geq 0.84$). This indicated that $r \geq 0.84$ was suitable to identify overlapped QTLs.

Taken together, the SML and ICIM – ADD methods identified QTL locations for the vast majority of the 54 evaluated traits. SML was superior to ICIM. While SML revealed many QTLs for most of the traits at significant level of $P \leq 0.01$ (51 – 53 traits), ICIM did not detect QTLs for several traits in each population even at relaxed PIN of 0.01 (Table 5.5; Appendix 5.2).

Table 5.5. Summary of QTLs detected in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87) by the SML and ICIM – ADD methods

Populations	1xB	1xK	87xB	87xK
Number of traits for which QTLs were detected				
SML method	51	51	52	53
ICIM – ADD method	29	41	32	36
Overlapped and common QTLs between two methods				
No. of traits	18	36	23	33
No. of QTLs	34	57	27	42
%♪	57.6	66.3	64.3	64.6
Number of QTLs detected ‡				
SML QTLs¶	91	101	61	98
ICIM QTLs	48	60	28	40
Total	***122***	***137***	***77***	***122***
No. of SML QTLs not mapped on LGs	38	63	30	63
QTLs with segregation distortion (no.)	57	67	23	53
QTLs with segregation distortion (%)	46.0	47.9	28.8	44.0

♪: Percentage was based on relative number of overlapped and common QTLs to total number of ICIM QTLs (Appendix 5.2)

‡: Common QTLs among the traits and overlapped QTLs were excluded (Count in Appendix 5.9)

¶: Only SML QTLs detected at *P*-value ≤ 0.001 were included. Markers link to SML QTLs which had $r \geq 0.84$ or mapped at the same position were considered to target the same QTL and therefore counted as one QTL (Appendix 5.9).

Overlapped QTLs between the two methods occurred in most of the traits that the ICIM – ADD method detected QTLs for (18 to 36 traits). Most QTLs detected by ICIM – ADD (hereafter referred to as ICIM QTLs) were also identified by the SML method (57.6 to 66.3%). In particular, many overlapped QTLs were observed in qualitative traits such as growth habit, leaflet lobing and qualitative seed traits

(Appendix 5.2). It was also noticed that many ICIM QTLs were overlapped with the most significant QTLs detected by SML (hereafter referred to as SML QTLs) ($P \leq 0.001$) (Appendix 5.2). This indicates broad consistency in QTL analysis by both methods.

At a significance level of $P \leq 0.001$, the number of SML QTLs (61 – 101) was still higher than that of ICIM QTLs (Table 5.5). In general, the number of SML QTLs seemed more consistent across the four populations, except 87xB with 61 QTLs. However, the numbers of ICIM QTLs were quite similar in the pairwise populations. For example, 87xB and 87xK (ACC 87 crosses) had similar numbers of detected ICIM QTLs (28 and 40 respectively). The total numbers of detected QTLs were fewer in the ACC 87 crosses than in the ACC 1 crosses. It is possible that the higher number of selected DArT markers and lower levels of marker segregation distortion observed in the ACC 1 crosses may have contributed to these differences (Table 5.3).

Many SML QTLs did not map on the linkage groups (LGs) (30 – 63). Generally, most detected QTLs showed normal segregation. It was also noticed that there were more aberrantly segregated QTLs in the ACC 1 than in the ACC 87 crosses. This possibly indicates that the inheritance of traits in crosses involving ACC 87 may be less complex than that in the crosses involving ACC 1. Nguyen *et al.* (2012) concluded that more qualitative traits appeared to be under monogenic control in the crosses involving ACC 87 whereas digenic control was more common with the ACC 1 crosses.

5.3.4. Resolution of congruent QTLs in the four mungbean F_5 RIL populations

QTL detection based on a single population usually results in only a limited number of QTLs and the results are often not conclusive. Therefore, research on QTL effects in more than one genetic background can provide more authentic and reliable QTL information. A number of QTL studies have used this approach. For instance, five independent and two related mapping populations were involved in comprehensive QTL scanning in rice (Uga *et al.* 2010; Li *et al.* 2012b). In mungbean, this is the first study to apply DArT markers, with four related populations derived from crosses between cultivars and wild accessions used to identify QTLs for 54 traits. Based on common markers observed among the individual populations, congruent QTLs i.e. QTLs which are common across at least two populations, were identified (Table 5.6; Appendix 5.3).

At a significant level of $P \leq 0.01$ for SML QTLs, congruent QTLs occurred in nearly all the traits except stem colour, leaf petiole colour and petiole length (Appendix 5.3). In all, 73 markers which were congruent between at least two populations were identified. Numbers of pairwise congruent QTLs were highest in the ACC 1 crosses (72), followed by the ACC 87 crosses (53) (Table 5.6). Qualitative traits including qualitative morphological and seed traits contained high numbers of common QTLs across populations.

Table 5.6. Number of pairwise congruent QTLs resolved in the four mungbean F_5 RIL populations derived from crosses between cultivars (Berken and Kiloga) and wild accessions (ACC 1 and ACC 87)

Trait categories	1xB - 1xK	1xB - 87xB	1xB - 87xK	1xK - 87xB	1xK - 87xK	87xB - 87xK
Qualitative morphological traits	10	12	9	8	12	9
Qualitative seed traits	11	16	18	10	15	23
Phenological traits	5	4	3	1	3	5
Quantitative morphological traits	29	12	9	9	7	13
Pod and seed straits	11	6	3	4	4	2
Yield-related traits	6	0	4	1	4	1
Total	**72**	**50**	**46**	**33**	**45**	**53**

Table 5.7 presents 21 significant congruent QTLs of which SML QTLs were selected at $P \leq 0.001$ for 20 traits. Most of these QTLs showed positive additive effect and quite similar effects (in terms of PVE% range), except for a few QTLs with large PVE ranges, such as the QTLs for leaflet lobing and hilum colour.

Most of these highly significant congruent QTLs (8 QTLs) governed seed appearance traits, such as testa colour and seed mottling, texture and hilum colour and overall visual seed traits. All of these QTLs came from the wild and had positive additive effects. The QTL mbPb-868147, which linked to testa colour, seed mottling and overall seed appearance, was common to all populations except 87xK. The QTL mbPb-868763 for hilum and texture layer colour was common for all. Other QTLs were common between pairwise populations such as mbPb-847032 in the Kiloga crosses and mbPb-868032 in the ACC 87 crosses, for testa colour and seed mottling.

The QTL mbPb-868706, linked to both perenniality and growth duration, was present in both 87xB and 87xK and had the effect of increasing the tuberisation of mungbean roots. The common QTL mbPb-870338, conditioning late flowering in the ACC 1 crosses, had the effect of delaying flowering, even under short day lengths and prolonging flowering duration. This QTL also conditioned some quantitative morphological traits such as number of leaves on stem and nodes on branches and node of the first pod formed.

Many QTL analysis studies have revealed the phenomenon where different QTLs could be detected for traits, especially quantitative traits, regardless of whether genetically related background populations were used, or sometimes when the traits were evaluated on the same population but in different environments (Hossain *et al*. 2010; Negeri *et al*. 2011; Ding *et al*. 2011; Li *et al*. 2012b). This phenomenon was also observed in this study where four related mungbean populations were assessed. For example, no common QTLs were detected for stem colour, leaf petiole colour and petiole length across populations. This may perhaps relate to the genetic distance between the populations (Table 5.3) or simply indicate that minor additive QTLs also accounted for trait expression.

Table 5.7. Congruent QTLs (*P*-value ≤ 0.001) resolved in the four mungbean F_5 RIL populations derived from crosses between cultivars (Berken and Kiloga) and wild accessions (ACC 1 and ACC 87)

Traits	Common marker♪	Populations				PVE % range
		1xB	1xK	87xB	87xK	
Hypocotyl pigment	mbPb-868147	✓/+	✓/+			1.1 – 5.8
Leaflet lobing	mbPb-848586 or mbPt-848586	✓/+	✓/+	✓/-		1.1 - 66.2
	mbPb-848641 or mbPt-848641	✓/-		✓/+	✓/-	1.4 – 15.5
Inflorescence structure	mbPt-868384	✓/+			✓/-	1.3 – 21.8
Perenniality	mbPb-868706			✓/+	✓/+	5.8 – 17.5
Testa colour	mbPb-847032		✓/+		✓/+	24.2 – 29.1
	mbPb-868032			✓/+	✓/+	1.9 – 3.6
	mbPb-868147	✓/+	✓/+	✓/+		40.8 – 79.6
Seed mottling	mbPb-847032		✓/+		✓/+	22.4 – 33.6
	mbPb-868032			✓/+	✓/+	4.3 – 5.9
	mbPb-868147	✓/+	✓/+	✓/+		55.9 – 76.8
	mbPt-868147	✓/+			✓/+	7.1 – 9.2
Texture layer depth	mbPb-868763		✓/+	✓/+		2.1 – 13.5
	mbPb-877325	✓/+	✓/+		✓/+	0.9 – 22.5
Hilum colour	mbPb-868763 or	✓/+	✓/+	✓/+	✓/+	3.9 – 53.7
	mbPt-868763	✓/+	✓/+	✓/+		7.1 – 14.5
	mbPb-877269	✓/+	✓/+		✓/+	2.7 – 55.5
Texture layer colour	mbPb-868763 or	✓/+	✓/+	✓/+		50.6 – 99.0
	mbPt-868763	✓/+		✓/+		10.8 – 14.9
	mbPb-877269	✓/+			✓/+	54.9 – 67.9
	mbPb-877325			✓/+	✓/+	13.2 – 21.9
	mbPt-868828		✓/+	✓/+	✓/+	0.1 – 37.3
Overall visual seed traits	mbPb-868147	✓/+	✓/+	✓/+		30.8 – 55.3
	mbPb-868763 or mbPt-868763	✓/+	✓/+	✓/+		13.8 – 20.5
	mbPb-877269	✓/+	✓/+			12.8 – 13.1
	mbPt-868147	✓/+			✓/+	3.5 – 10.0
Time to flowering	mbPb-870338	✓/+	✓/+			15.7 – 15.8
	mbPb-877288	✓/+			✓/+	0.9 – 2.5
	soPb-825660		✓/-	✓/+		3.0 – 21.0
Duration of flowering	mbPb-870338	✓/+	✓/+			1.7 – 6.6
Growth duration	mbPb-868706			✓/+	✓/+	3.3 – 18.2
Stem length	mbPb-848781		✓/+	✓/+		2.0 – 2.1
No. of leaves on stem	mbPb-870338	✓/+	✓/+			1.8 – 2.0
No. of nodes on branches	mbPb-870338	✓/+	✓/+			0.6 – 7.3
	mbPt-877288	✓/+	✓/+			1.9 – 2.6
Node of 1st pod	mbPb-870338	✓/+	✓/+			4.4 – 11.0
	mbPt-867926		✓/+		✓/+	35.3 – 43.2
	mbPt-877288	✓/+			✓/+	1.3 – 22.5
Pod length	mbPt-848177	✓/+		✓/+		3.2 – 4.5
	soPt-853267	✓/-	✓/+-			4.1 – 4.4
Pod dry mass	mbPb-847829		✓/+		✓/+	1.7 – 3.2
	soPb-824730	✓/-	✓/+			3.8 – 21.9
Seed yield	soPb-824730	✓/-	✓/+			2.2 – 23.5

♪: loci nearby the corresponding putative additive QTL are common among populations. Only SML QTLs detected at $P \leq 0.001$ were taken into account. Common markers across traits are highlighted in the same colour.

✓: indicating the loci are present in the population; + and - : indicating the additive effect direction

In addition, different sets of polymorphic DArT markers selected for each RIL population which were used for SML and ICIM – ADD QTL analysis obviously would result in different QTLs being detected for the same traits. The inability to identify sequencing redundancy of DArT markers may also have resulted in an underestimate of the presence of common markers among populations and traits. However, in this study, the level of congruence across populations was reasonable strong. The identification of overlapped markers based on correlation ($r \geq 0.84$) also increased the number of congruent QTLs.

In order to have detailed views for significant QTLs associated with traits and the QTL distribution on the linkage maps, highly significant SML QTLs selected at $P \leq 0.001$ (hereafter simply referred to as SML QTLs) and ICIM QTLs associated with six trait categories (qualitative morphological traits, qualitative seed traits, phenology, quantitative morphological traits, pod and seed traits, and yield related traits) in the four RIL populations were summarized in Tables 5.9 – 5.10 and Appendices 5.4 – 5.9 in the following manner: significant SML QTLs are subscripted with (a) and their LGs and marker positions are included; ICIM QTLs detected at relaxed PIN of 0.01 are marked (†); Overlapped QTLs are highlighted; Positive or negative signs of phenotypic variation explanation (PVE %) indicated the effect direction of that QTL on the corresponding trait (i.e. positive for increase and negative for decrease in the value of the corresponding trait). However, the PVE % values are not strictly comparable between the two methods since different statistical approaches are applied. The sequence of QTLs entered in the table is the sequence of the QTLs on the linkage maps. Tables 5.9 – 5.10 illustrate QTLs for representative traits or traits of interests while Appendices 5.4 – 5.9 represent selected QTLs for all 54 traits.

Mapped QTLs are labelled as follows: SML QTLs are in bold and red; ICIM QTLs are in bold and green; QTLs detected by both methods are in bold, underlined and in blue; traits are indicated to QTL regions with assigned abbreviations (Table 5.1). Distortedly segregated DArT markers linked to QTLs are indicated with (*) ($P < 0.05$) and (**) ($P < 0.01$) (Figure 5.2 – 5.5; Appendix 5.10).

5.3.5. QTLs associated with qualitatively inherited traits

When common QTLs across traits were not accounted for, in total, 219 and 76 QTLs were identified for 16 qualitative morphological traits and eight qualitative seed traits across the four RIL populations with PVE ranges of 0.4 – 74.4 % and 0.7 – 99.0% respectively (Figure 5.2; Appendices 5.4, 5.5). The respective numbers of major QTLs were 12 and 30. While the suggested single and double gene action models, based on phenotypic segregation data, were broadly consistent between the F_2 and F_5 generations for most traits (Tables 4.7, 4.9, 4.10), the numbers of associated QTLs that were identified for individual traits were variable. Several traits were identified with one or two major QTLs, models which supported the phenotypic observations. These traits included leaflet lobing (single gene in 1xK), dry pod colour (single gene in ACC 87 crosses), seed mottling (two genes in ACC 1 crosses), depth of texture layer (single gene in 87xB), hilum colour (single gene in 1xB, 1xK and 87xB) and texture layer colour (single gene in 1xB and 1xK and two genes in 87xK) (Table 5.8).

Table 5.8. Summary and comparison of gene action models for qualitative traits suggested by phenotypic observations at F_2 (Nguyen 2011) and F_5 generations (Chapter 4) with models suggested by QTL detection

(Numbers in -/-/-/- respectively denotes number of genes operating in 1xB/1xK/87xB/87xK crosses)

Traits	Nguyen 2011 model♪	F_5 phenotypic model♪	Number of detected QTLs‡	Suggested QTL models
	1xB/1xK/87xB/87xK		1xB/1xK/87xB/87xK	
Qualitatively inherited traits				
Hypocotyl pigment	-	2/2/-/-	3/6/-/-	Generally, additive QTLs; except a major QTL in 1xK for leaf rachis colour, in 87xK for plant hair density and in 1xB for plant hair colour
Stem colour	-	2/2/2/-	5/4/2/3	
Leaf rachis colour	-	1/1/2/2	3/**8**/1/3	
Leaf petiole colour	-	1/2/-/-	6/7/2/3	
Plant hair density	-	1	7/3/2/**4**	
Plant hair colour	-	1/1/2/2	**6**/4/2/4	
Growth habit	2	2/2/2/-	5/4/3/2	
Twining	2/2/1/1	2/2/1/1	**5**/4/3/5	A major QTL in 1xB; additive QTLs in other crosses
Leaflet lobing	1	1/-/-/-	3/**2**/**3**/3	A major QTL in 1xK; two major QTLs in 87xB
Flower colour	-	1/1/2/2	1/4/**3**/3	A major QTL in 87xB; minor QTLs in other crosses
Inflorescence structure	-	2/2/1/-	**4**/4/2/3	A major QTL in 1xB; minor QTLs in other crosses
Dry pod colour	-	2/2/1/1	4/3/**2**/**7**	A major QTL in 87xB and 87xK;
Pod dehiscence	2/2/1/1	2	6/3/2/3	Additive QTLs
Powdery mildew	1/1/2/2	2	7/4/3/4	
Thrips	-	2	2/**4**/3/2	A major QTL in 1xK; minor QTLs in other crosses
Perenniality	-/-/2/2	-/-/1/2	-/-/3/3	Additive QTLs
Qualitative seed traits				
Testa colour	2	2	**3**/**3**/**1**/**4**	Two major QTLs in 1xB; a major QTL in 1xK, 87xB and 87xK
Seed mottling	-	2	**4**/**3**/**1**/**3**	Two major QTLs in 1xB and 1xK; A major QTL in 87xB and 87xK
Seed coat ridging	2/2/1/1	-/-/1/2	-/-/3/4	Additive QTLs
Lustre	2/2/2/1	-/-/1/2	-/-/-/3	Minor QTL
Texture layer depth	-	2/2/1/2	3/3/**3**/4	A major QTL in 87xB;
Hilum colour	1	1	**1**/**2**/**3**/**2**	A major QTL in 1xB, 1xK and 87xB; two major QTLs in 87xK
Texture layer colour	-	1/1/2/2	**1**/**1**/**4**/**4**	
Overall visual seed traits	-	-	**4**/**3**/**3**/**3**	Two major QTL in 1xB and 87xK; one major QTLs in 1xK; three major QTLs in 87xB

♪: 1 = Single gene model; 2 = Two genes model; - = No suggested model;

‡: Bold numbers indicate where major QTLs were detected for corresponding traits;

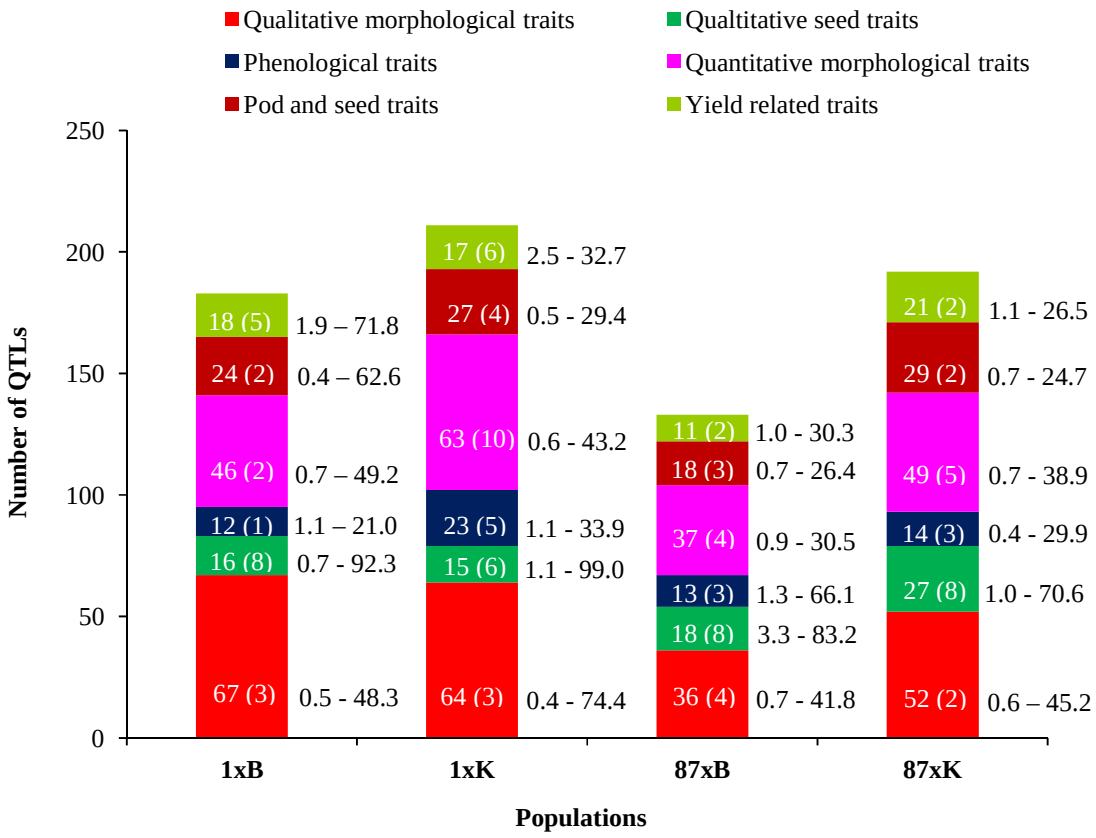


Figure 5.2. Number of QTLs detected for the six trait categories in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87). The first number in each category indicates the number of QTLs while the number in ( ) is the major QTLs. The PVE range (%) is labelled next to each category.

QTLs associated with qualitative morphological traits

Individual 1xB, 1xK, 87xB and 87xK RIL populations were detected with 67, 64, 36 and 52 QTLs linked to qualitative morphological traits, of which 3, 3, 4 and 2 were major QTLs respectively (Figure 5.2). In general, additive QTL models were suggested for most traits (e.g. Table 5.9a – d; Appendix 5.4). For example, although one and two major ICIM QTLs with PVE ≥ 30% were identified for leaflet lobing in the 1xK and 87xB populations respectively, another eight QTLs detected across the four populations indicated minor loci also additively contributed to the degree of expression (e.g. from slightly to strongly lobed) (Table 5.9a). This interpretation was consistent with the conclusions of James *et al*. (1999) based on classical analyses and of Humphry *et al*. (2005) based on QTL analyses of other populations involving mungbean cultivars and wild accessions.

A total of 18 and 11 additive QTLs were detected for plant reaction to powdery mildew and thrips across the four populations, with PVE 0.9 – 74.4% (Table 5.9b, c). Fourteen of those QTLs had the effect of increasing plant resistance (negative PVE) and as expected, most of them were donated by the wild parents. Different models of gene action were suggested based on field data: either a single gene (Nguyen

et al. 2012) or two genes, based on the F_5 RILs in both the ACC 1 and ACC 87 crosses for powdery mildew (Table 4.7n) and two genes for thrips (Table 4.7m). The presence of one normally segregating QTL in each of the 1xB, 87xB and 87xK for powdery mildew and one for thrips in the 87xB and 87xK RIL populations (Figures 5.3, 5.5, 5.6), given by the wild parent, suggests it may be possible to transfer and accumulate/pyramid resistance genes from the wild to the cultigen.

For the novel perenniality trait in mungbean, three QTLs linked to tuberisation were detected in each of 87xB and 87xK by both methods with various PVE from 1.5 to 22.6% (Table 5.9d). In 87xB, two SML QTLs on LG8 and LG15 which had the effect of increasing tuberisation were inherited as expected from ACC 87 and showed normal segregation (Figure 5.5). Although an additional ICIM QTL on LG17 accounted for 22.6% of the variation, it originated with Berken and had the effect of decreasing trait expression. In 87xK, two QTLs of close position on LG18 equally contributed around 17% to the total variation in tuberisation. The marker mbPb-868706 was also common with the QTL on LG15 of 87xB.

ACC 87 contributed alleles for tuberisation while Kiloga and Berken provided alleles for fibrous roots. The classical Mendelian analysis of tuberisation expression in the F_2 and BC progeny (Nguyen 2011) and the 87xK F_5 RIL population (Table 4.9), suggested two complementary dominant genes. The QTL results here supported that conclusion to the extent that QTLs from both parental sides were present in both the 87xB and 87xK F_5 RILs. However, many other minor QTLs detected by SML (Appendix 5.2) indicates minor additive genes contributing to the degree of expression of tuberisation from weak to strong in the ACC 87 crosses.

Table 5.9. Locations and effects of QTLs associated with qualitatively inherited morphological and seed traits detected by the SML (*P*-value ≤ 0.001) and ICIM - ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on LG (in cM); LOD = Logarithm of odds score; PVE = Phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect);

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. Leaflet lobing	11	63.0	soPb-854393/ soPb-855762	6.1	14.8	11	9	soPb-853216/ mbPb-848586	17.0	**66.2**	9	11.0	soPb-853806/ soPt-853944	8.3	**33.6**	11	13.0	mbPb-849000/ mbPb-868592 †	7.4	14.3
	11	68.0	mbPb-848586/ mbPt-876514	8.9	23.4	11	10.6	mbPb-848586 a		-5.1	12	0.0	soPb-831551/ soPb-857372	3.1	11.0	15	9.7	mbPb-867674 a		4.9
						11	10.8	mbPt-848586 a		-6.6						15	9.7	mbPb-848641 a		-4.5
	11	68.3	mbPt-848586 a		-1.4	11	14.3	mbPb-868263 a		-3.3	-	-	mbPb-848641 a		15.5	15	9.7	mbPt-848641 a		-5.6
	11	68.3	mbPt-848641 a		-1.1						-	-	mbPb-848586 a		-8.7	15	14.0	soPb-853645/ mbPb-870853 †	7.4	15.4
	-	-	mbPt-849022 a		1.1															
b. Powdery mildew	5	46.0	mbPb-867966/ mbPb-847671	4.3	10.7	4	29.2	mbPt-848599 a		0.9	3	106.0	mbPt-848435/ mbPt-848441	3.8	15.7					
	5	46.8	mbPb-847671 a		-2.4	7	40	mbPt-846324/ mbPt-868542	4.3	-19.0	7	10.9	mbPt-871507 a		-4.0	13	22.0	mbPb-848100/ mbPb-846991	3.8	23.2
	6	13.0	mbPt-869281/ mbPt-876816	6.3	-16.6	-	-	mbPt-877076 a		-1.0	7	12.0	mbPt-871507/ soPt-825810	6.3	-24.0	-	-	soPt-824890 a		4.8
						-	-	mbPt-871365 a		0.9						-	-	mbPb-869240 a		-4.6
	8	32.0	mbPt-876991/ mbPb-870338	5.9	-17.4						17	23.0	mbPt-846869/ mbPt-848216	3.7	14.5					
	11	0.0	mbPt-847209/ mbPt-847132	4.1	-9.5						17	23.4	mbPt-848216 a		3.5					
	11	42.0	mbPb-877269/ mbPt-868763	3.5	-8.9															
	-	-	mbPt-848368 a		2.5															
	-	-	mbPt-876596 a		2.2															
c. Thrips	11	22.6	mbPt-846155 a		2.5	4	5	mbPb-867668/ mbPb-877185 †	5.1	16.9	12	1.0	soPb-857372/ mbPb-868823	3.8	-16.4	3	34.0	mbPb-848400/ mbPt-847829	3.4	-18.0
	11	22.6	mbPt-868656 a		-22.3															
	11	22.6	mbPt-876612 a			4	11.7	mbPt-876847 a		1.4	-	-	mbPb-848630 a		3.1	13	24.7	mbPb-877602 a		2.4
	11	23.0	mbPt-877602/ mbPt-868118	4.9	3.1	15	19.9	mbPb-867920 a		1.1	-	-	mbPt-868763 a		-2.4	13	24.8	mbPb-876477 a		1.1
					1.5	15	20	mbPb-867920/ soPb-855725 †	17.6	**74.4**						13	24.8	mbPb-876817 a		1.1
						-	-	mbPt-868952 a		1.5										

♪: Underlined LG and Pos are position of SML QTLs which are assigned based on the genotype correlation ($r \geq 0.84$) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Markers highlighted in the same colour are overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold number indicated major QTL with PVE % ≥ 20% for SML and ≥ 30% for ICIM methods respectively

a: QTLs detected by SML method at significant *P* – value ≤ 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for a QTL region

Table 5.9. Continued…

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
d. Perenniality											8	28.6	mbPb-868592 a		1.5	3	32.9	mbPt-848400 a		1.9
											15	19.0	mbPb-868706 a		5.8	3	33.0	mbPb-848400 a		1.9
											17	18.0	mbPt-870410/ mbPt-877415 †	5.3	-22.6	8	15.0	mbPb-868706 a		1.8
																8	18.0	mbPb-868706/ mbPb-871035	5.3	17.5
																8	19.0	mbPb-871035/ mbPb-847338	4.0	17.4
e. Testa colour	11	14.0	mbPt-867688/ mbPt-846225	8.0	**45.6**	11	42.3	mbPb-847032 a		-4.1	-	-	mbPb-868147 a		**79.6**	13	6.0	mbPb-847032/ mbPt-848579	4.7	24.2
	11	14.5	mbPt-846225 a		5.5	11	43	mbPb-847032/ mbPb-847470	6.1	29.1	-	-	mbPb-868032 a		3.6	-	-	mbPt-868032 a		**70.6**
	-	-	mbPb-868147 a		**40.8**	-	-	mbPb-868147 a		**59.6**						-	-	soPb-854233 a		-1.9
	-	-	mbPt-868147 a		14.5	-	-	mbPt-868810 a		-1.7						-	-	mbPb-868032 a		1.9
f. Overall visual seed traits	11	12.0	mbPt-867688/ mbPt-846225	23.1	**67.7**	11	36.4	mbPb-868763 a	3.8	18.3	9	28.0	mbPt-868828/ soPt-824812	8.5	**38.0**	13	25.0	mbPb-876897/ mbPb-876592	9.0	**41.7**
	11	14.5	mbPt-846225 a		10.2	11	38	mbPb-877269/ mbPt-847428		12.1	-	-	mbPb-868147 a		**39.2**	-	-	mbPt-868032 a		**33.7**
	11	42.0	mbPb-877269/ mbPt-868763	4.5	13.8	11	42.3	mbPb-847032 a		-2.0	-	-	mbPb-868763 a		**20.5**	-	-	mbPt-868147 a		3.5
	-	-	mbPb-868147 a		**30.8**	11	43	mbPb-847032/ mbPb-847470	6.8	23.2						-	-	mbPb-868032 a		4.0
	-	-	mbPt-868147 a		10.0	-	-	mbPb-868147 a		**55.3**										

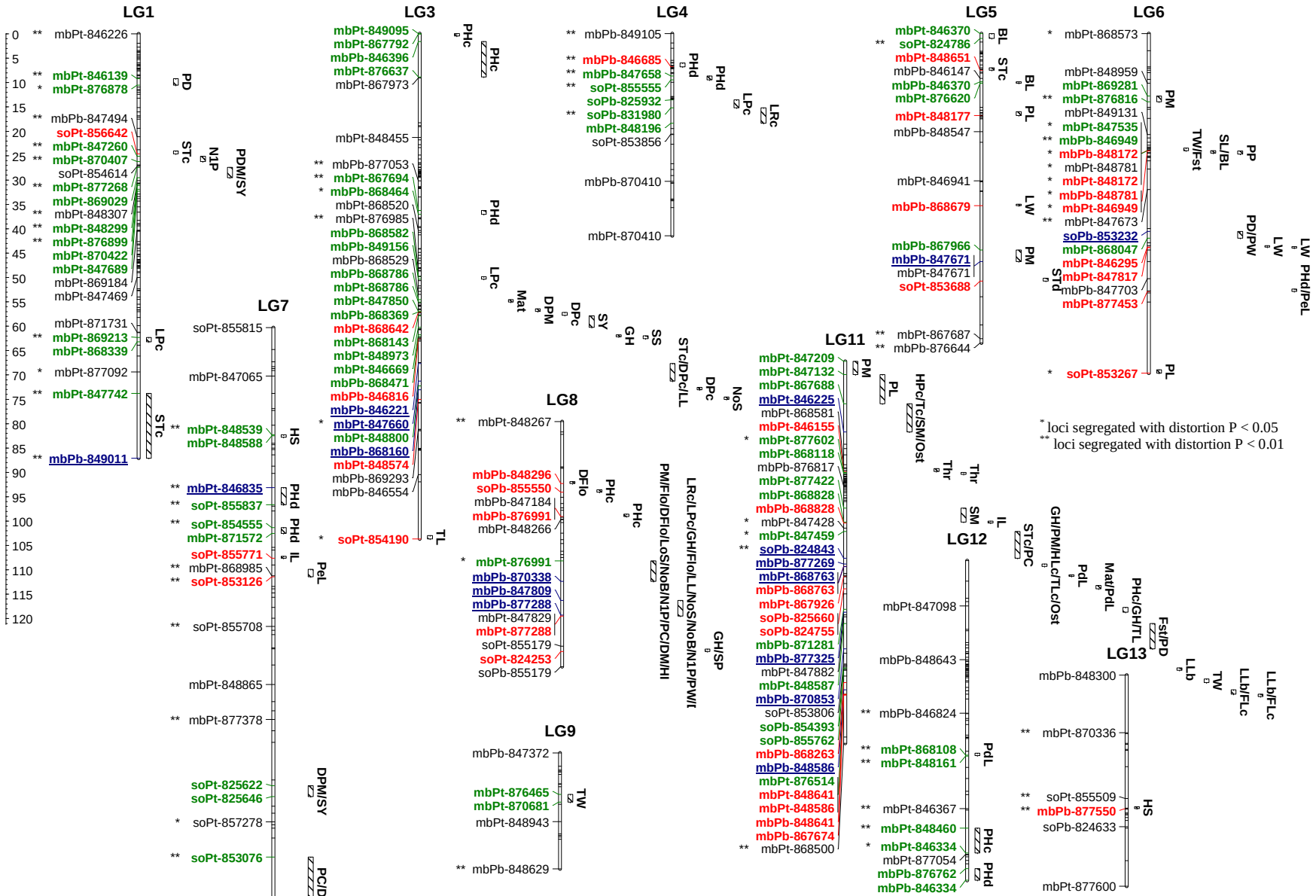


Figure 5.3. Partial DArT linkage map showing QTLs detected in the mungbean F_5 RILs of the cross ACC 1 x Berken.

A subset of 11 LGs containing QTLs is shown. QTLs detected by SML ($P \leq 0.001$) are shown in bold and red. QTLs detected by ICIM – ADD are shown in bold and green. Overlapped QTLs are underlined and in bold and blue. Traits are indicated next to QTLs (see Table 5.1 for trait abbreviation).

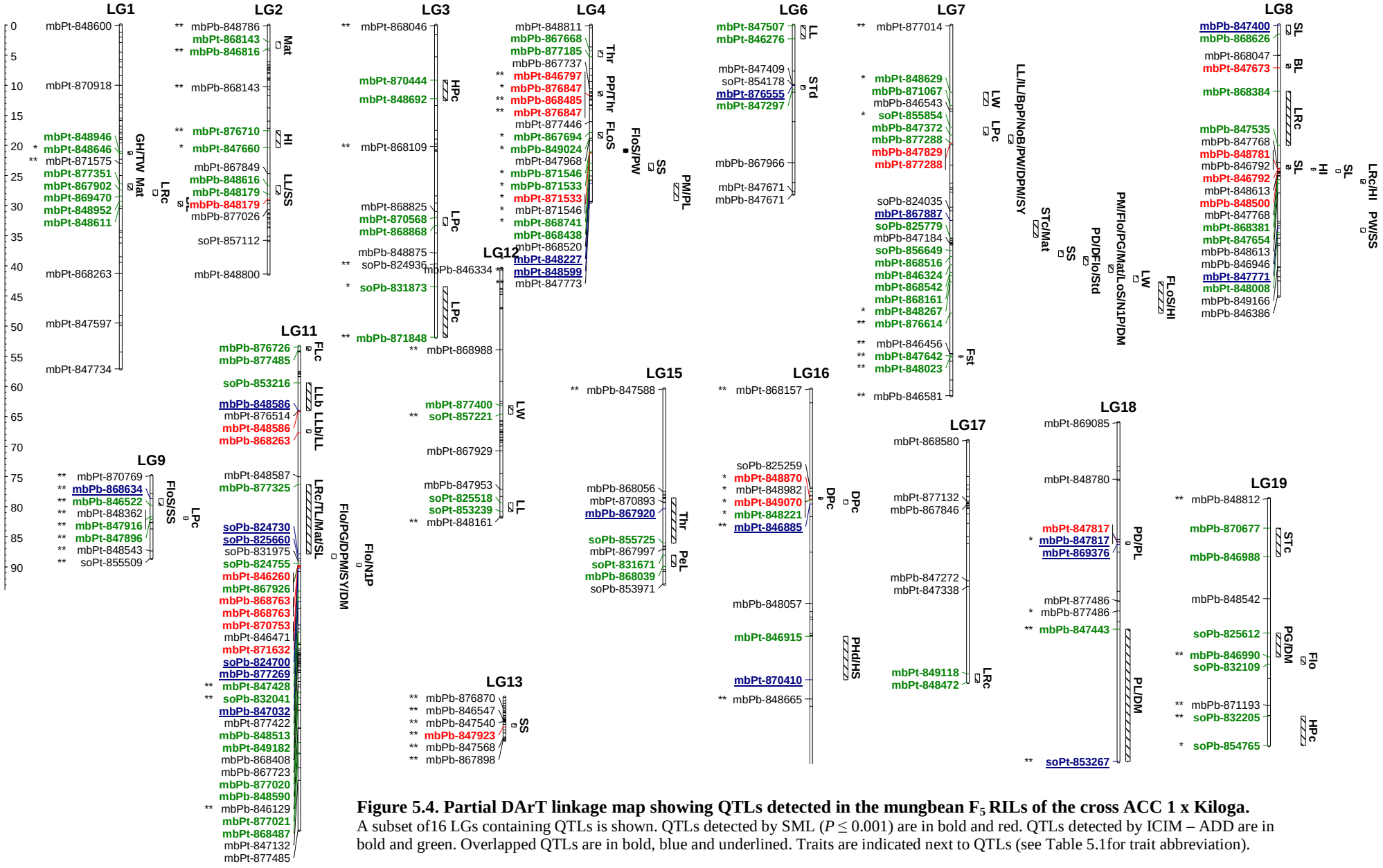


Figure 5.4. Partial DArT linkage map showing QTLs detected in the mungbean F_5 RILs of the cross ACC 1 x Kiloga.
A subset of16 LGs containing QTLs is shown. QTLs detected by SML ($P \leq 0.001$) are in bold and red. QTLs detected by ICIM – ADD are in bold and green. Overlapped QTLs are in bold, blue and underlined. Traits are indicated next to QTLs (see Table 5.1for trait abbreviation).

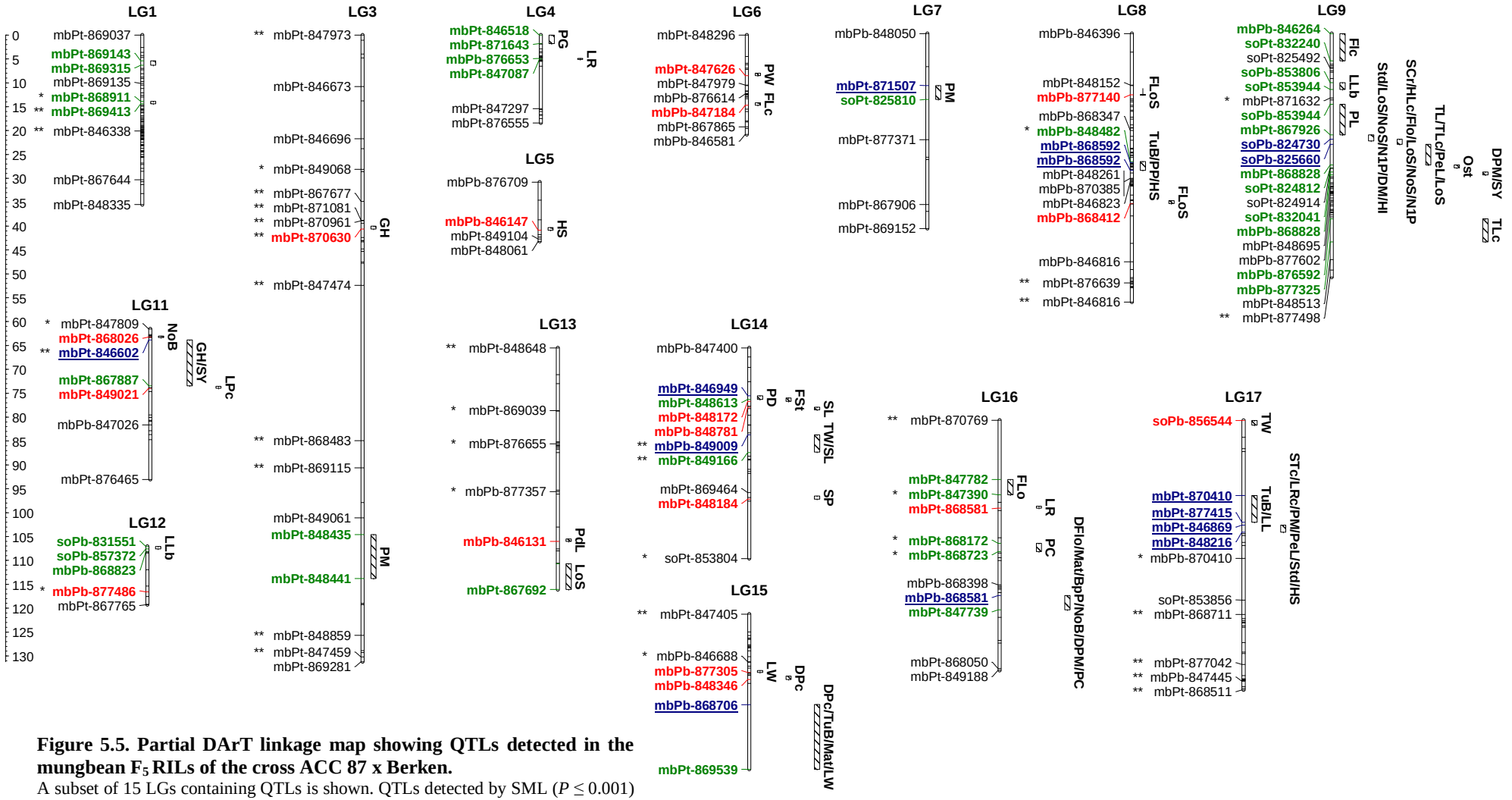


Figure 5.5. Partial DArT linkage map showing QTLs detected in the mungbean F_5 RILs of the cross ACC 87 x Berken.
A subset of 15 LGs containing QTLs is shown. QTLs detected by SML ($P \leq 0.001$) are in bold and red. QTLs detected by ICIM – ADD are in bold and green.

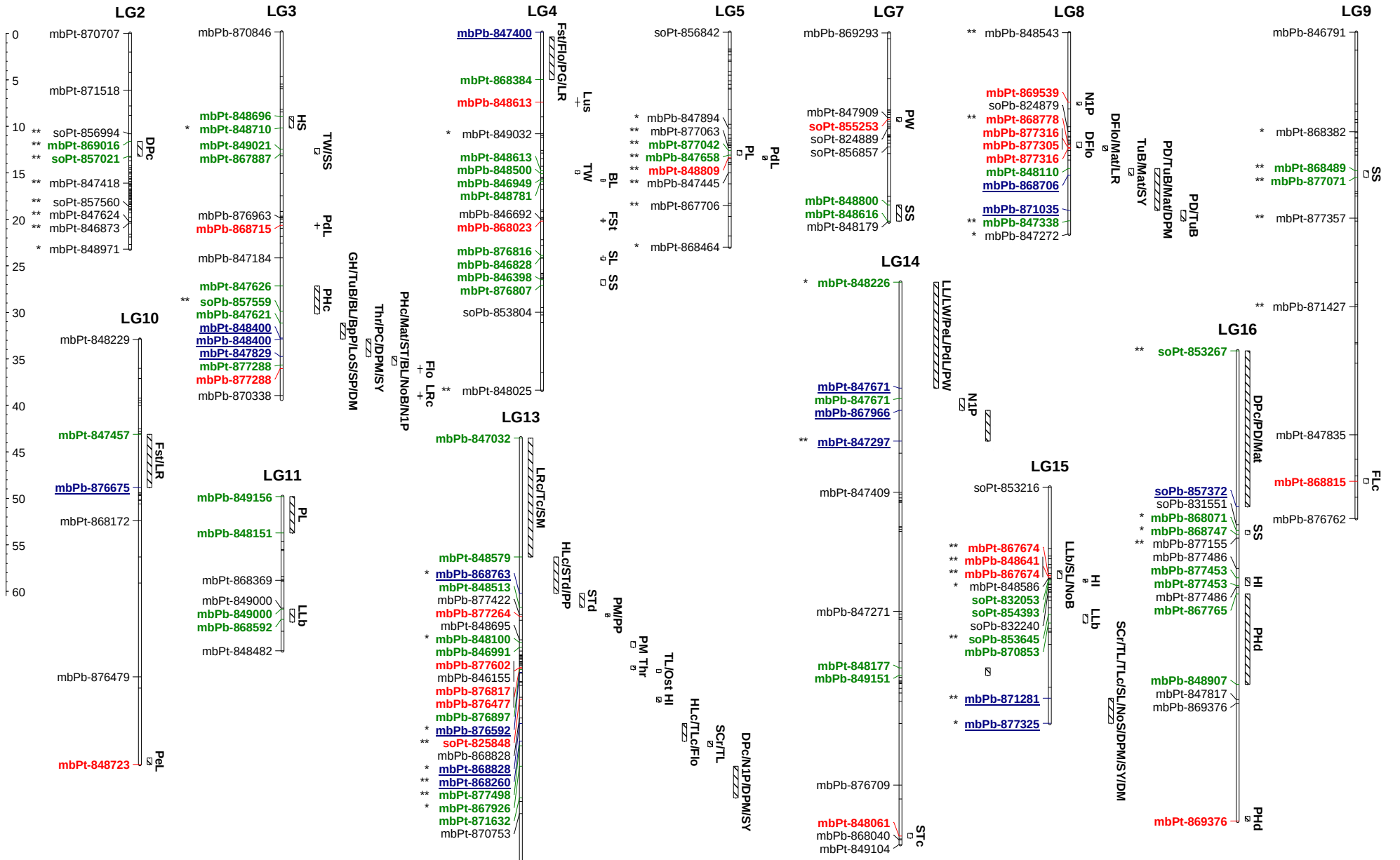


Figure 5.6. Partial DArT linkage map showing QTLs detected in the mungbean F_5 RILs of the cross ACC 87 x Kiloga.

A subset of 13 LGs containing QTLs is shown. QTLs detected by SLM ($P \leq 0.001$) are in bold and red. QTLs detected by ICIM – ADD are in bold and green. Overlapped QTLs are in bold, blue and underlined. Traits are indicated next to QTLs (see Table 5.1. for trait abbreviation).

QTLs associated with qualitative seed traits

The total number of QTLs conditioning visual seed traits varied, with 16 in 1xB, 15 in 1xK, 18 in 87xB and 27 in 87xK. Of those, eight QTLs in each of the 1xB, 87xB and 87xK crosses and six QTLs in 1xK significantly contributed to phenotypic variation (Figure 5.2).

Eleven QTLs (including common QTLs) were associated with testa colour across the four mungbean populations (Table 5.9e). Nine of those QTLs enhanced expression of the wild type seed trait (positive PVE). SML QTL mbPb-868147 was common to the 1xB, 1xK and 87xB populations with highly significant explanations of testa colour (40.8% – 79.6%). However, in 87xK, SML QTL mbPt-868032 was significant with PVE of 70.6%. As expected, these two major QTLs were given by the wild parents. Overall, QTL detection indicated the model of a major QTL linked to testa colour in the 1xK, 87xB and 87xK RIL populations, which was not in agreement with the two gene model based on segregation ratios in the F_2 and BC generations (Nguyen 2011) and the F_5 RILs (Table 4.10a). The 1xB cross was an exception with a two major QTL model. The study by Isemura *et al*. (2012) also identified two loci linked to this trait. Meanwhile, it was noticed that most QTLs or loci conditioning testa colour, especially the major QTLs, were also identified for seed mottling in all four RIL populations, indicating these two traits were the same (Appendix 5.5a, b).

Based on the combination of seed trait attributes (testa colour, seed coat ridging, texture layer depth and texture layer colour described by Lawn *et al.* (1988b)), QTL analysis revealed 13 QTLs linked to overall visual seed traits, with the effect being to enhance the wild phenotypes and contributing 2.0 – 67.7% to the phenotypic variation explanation (Table 5.9f). These basically suggested that additive QTLs contributed to overall seed appearance. Congruent QTLs across populations, such as mbPb-868147, mbPb-868763 or mbPt-868032, could be useful in MAS for specific traits.

Potential reasons for the discrepancy between the numbers of genes suggested by the Mendelian segregation ratio approach and the number of detected QTLs for qualitative traits are exemplified as follows: for pigmentation traits such as hypocotyl, leaf rachis and stem colour, transient degrees of pigmentation from green or slightly purple spotted to completely purple were observed. Similar transient degrees of trait expression also occurred for other qualitative traits including growth habit, twining, flower colour, dry pod colour, pod dehiscence and powdery mildew and thrips reaction. While two or more phenotypic categories for traits such as growth habit and leaflet lobing were aggregated when fitting the Mendelian segregation ratios (Table 4.7), separate categories were maintained for the QTL analyses. This implied that caution is needed in applying and interpreting the classical segregation ratio approach to suggest models of gene action.

The number of major QTLs conditioning a trait can vary depending on what level of QTL effect can be considered to reflect 'major' gene action. It is generally suggested that major QTLs will account for ≥ 10% of the PVE (Collard *et al*. 2005). For example, in the study by Hossain *et al*. (2010) on chickpea seed size, both the classical approach and QTL detection implied two major genes for the trait. These two

QTLs together accounted for 20% of the seed size variation. However, in other studies, QTLs were defined as major when PVE was over 20% (Isemura *et al*. 2012) or 25% (Bratteler *et al*. 2006). QTL effects can be biased upwards when both location and phenotypic effects of QTLs are estimated from a single population or be overestimated when sample sizes are low (Beavis 1998; Goring *et al*. 2001). Thus, the present PVE values have to be interpreted with some caution: for qualitative traits, SML QTLs with PVE ≥ 20% and ICIM QTLs with PVE ≥ 30% were considered to be 'major'; for quantitative traits, the 'major' QTLs were those with PVE ≥ 10% and 20% for the SML and ICIM methods respectively.

The effects of segregation distortion can lead to erroneous interpretations of gene action models solely based on classical analyses. This was the case in a study of seed testa colour inheritance in F_2 and F_7 populations of a cross between cv. Berken and the wild mungbean accession ACC 41 (Lambrides *et al*. 2004). Phenotypically, ACC 41 is generally more similar to ACC 1 than to ACC 87. In that study, although classical genetic approaches indicated an excellent fit to 13:3 for the F_2 and 3:1 for the F_7, lending strong support to an epistasis model of two genes, segregation of three markers linked to the testa colour was abnormal. It then was concluded that the testa colour was conditioned by a single locus. In the present study, some QTLs linked to testa colour showed distorted segregation (Figures 5.3 – 5.6; Appendix 5.10). Therefore, the suggested model of a major QTL for the 1xK, 87xB and 87xK RIL populations seemed to be in agreement with Lambrides *et al*. (2004) (Table 5.9e). Segregation distortion also occurred with markers flanking several QTL regions linked to some other traits (Figures 5.3 – 5.6; Appendix 5.10). However, in order to draw conclusions and investigate the mechanisms of segregation distortion in qualitative traits in this study, it is necessary to include genotypic/ marker information of different populations such as F_2 and backcrosses.

5.3.6. QTLs associated with quantitatively inherited traits

Overall, 100, 130, 79 and 113 QTLs were selected for 30 quantitative traits (phenological and quantitative morphological traits, pod and seed traits and yield-related traits) in the 1xB, 1xK, 87xB and 87xK populations respectively, with contributions of 0.4 – 71.8% to phenotypic variation explanation (Figure 5.2). The corresponding numbers of major QTLs (PVE ≥ 10% for SML QTL and ≥ 20% for ICIM QTL) in individual populations were 10, 25, 12 and 12 (Figure 5.2; Appendix 5.6 – 5.9). Numbers of QTLs were somewhat similar between the 1xB, 1xK and 87xK crosses while those in the 87xB population were least. Generally, suggested models were minor additive QTLs for most quantitative traits. QTL results for representative traits/ traits of interests are shown in Table 5.10 while QTLs for all traits are in Appendices 5.6 – 5.9.

Table 5.10. Locations and effects of QTLs associated with representative quantitative traits detected by the SML (*P*-value ≤ 0.001) and ICIM-ADD methods in the four mungbean F_5 RIL populations derived from crosses between cultivated plants (Berken and Kiloga) and wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on linkage group (in cM); LOD = Logarithm of odds score; PVE = phenotypic variation explanation (%) (+ve or -ive depending on whether the QTL increased or decreased the trait effect

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. Time to flowering	3	85.1	soPt-855371 a		**15.7**	7	40.0	mbPt-846324/ mbPt-868542	10.6	**27.2**	9	23.0	soPb-824730/ soPb-825660	5.1	**21.0**	3	36.2	mbPb-877288 a		0.9
	8	32.8	mbPb-870338 a		2.5											4	1.0	mbPb-847400/ mbPt-868384	3.7	11.6
	8	39.7	mbPb-877288 a		-2.0	11	35.2	soPb-825660 a		-3.0	16	15.0	mbPt-847782/ mbPt-847390	3.0	11.2					
						11	36.0	soPb-824755/ mbPt-867926	11.6	**28.6**						13	30.7	mbPt-868828 a		1.0
											-	-	mbPb-871145 a		2.8					
						19	27.0	mbPb-846990/ soPb-832109	3.7	-8.1	-	-	mbPt-847829 a		1.3	13	31.0	mbPt-868828/ mbPt-868260	8.6	**29.9**
																-	-	soPb-853363		0.4
						-	-	mbPb-870338 a		**18.5**										
						-	-	mbPb-876778 a		-3.3										
b. Seed size	3	62.6	mbPb-846816 a		-5.2	2	27.0	mbPb-848616/ mbPt-848179	6.7	-14.3	-	-	mbPb-847295 a		6.5	3	13.0	mbPt-849021/ mbPt-867887	8.4	**-20.1**
	-	-	mbPb-848652 a		2.3						-	-	mbPt-847817 a		-3.8					
	-	-	mbPt-868152 a		-2.2	4	23.0	mbPt-868741/ mbPt-868438	3.9	-8.5						4	27.0	mbPb-846398/ mbPt-876807	6.1	-19.8
						7	38.0	soPb-856649/ mbPt-868516	3.7	-7.4						7	19.0	mbPt-848800/ mbPt-848616	6.3	-15.4
						8	33.7	mbPt-847771 a		-0.6						9	15.0	mbPt-868489/ mbPb-877071	3.9	-8.3
						8	34.0	mbPt-847771/ mbPt-848008	10.1	**-26.7**										
						9	4.0	mbPb-868634/ mbPb-846522	6.3	-14.1						16	19.0	mbPb-868071/ mbPb-868747	4.1	-8.8
																-	-	mbPb-849005 a		3.3
						13	4.9	mbPb-847923 a		-0.5						-	-	mbPt-876465 a		-1.7
						-	-	soPt-855434 a		0.6						-	-	soPt-832048 a		-1.7

♪: Underlined LG and Pos are position of SML QTLs which are assigned based on the genotype correlation ($r \geq 0.84$) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Markers highlighted in the same colours are overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold number indicated major QTL with PVE % ≥ 10% for SML and ≥ 20% for ICIM methods respectively

a: QTLs detected by SML method at significant *P* – value ≤ 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for a QTL region

Table 5.10. Continued...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
c. Hard-seededness	7	22.0	mbPt-848539/ mbPt-848588	4.5	**-25.3**	16	48.3	mbPt-870410 a		-1.0	5	10.1	mbPb-846147 a		4.2	3	10.0	mbPt-848696/ mbPt-848710 †	5.1	16.3
						-	-	soPb-825426 a		-1.6	8	27.0	mbPb-848482/ mbPt-868592 †	3.9	11.5					
	13	27.4	mbPb-877550 a		1.2	-	-	soPb-824603 a		-1.1						-	-	mbPt-876644 a		-1.1
	-	-	mbPt-849188 a		1.8						17	23.4	mbPt-848216 a		9.4	-	-	mbPt-848087 a		0.7
	-	-	mbPb-849188 a		1.5											-	-	mbPb-847506 a		-0.7
d. Seed yield	1	28.0	mbPt-877268/ mbPt-869029 †	12.3	**63.0**	7	19.0	mbPb-847372/ mbPb-877288	4.6	**24.1**	9	29.0	soPt-832041/ mbPb-868828	5.2	**28.8**	3	34.0	mbPb-848400/ mbPt-847829	6.2	**25.4**
	1	52.3	mbPb-870825 a		-16.1	7	19.6	mbPt-877288 a		7.5	11	2.3	mbPt-846602 a		-1.3	8	15.0	mbPt-848110/ mbPb-868706	3.5	12.7
						11	35.0	soPb-824730/ soPb-825660	6.9	**23.5**	17	46.5	mbPt-847488 a		1.7					
	3	58.0	mbPt-868143/ mbPt-848973 †	5.1	-19.6											13	36.0	mbPt-867926/ mbPt-871632	3.3	13.3
						-	-	mbPb-848954 a		6.7										
	7	96.0	soPt-825622/ soPt-825646 †	6.2	-2.2	-	-	mbPt-871009 a		-2.9						15	25.4	mbPb-877325 a		1.1
	8	20.7	mbPt-846832 a		-2.1											-	-	soPb-853497 a		-1.6
	-	-	soPb-824730 a		2.0											-	-	mbPb-848797 a		-1.3
e. Harvest index	8	32.8	mbPb-870338 a		-3.7	2	20.0	mbPt-876710/ mbPt-847660	3.7	10.9	9	22.0	mbPt-867926/ soPb-824730	3.5	17.8	13	28.1	soPt-825848 a		-1.5
	8	39.7	mbPb-877288 a		-4.4											14	42.0	mbPt-848177/ mbPb-849151	3.3	10.9
	8	39.9	mbPt-877288 a		-3.0	7	43.0	mbPt-848267/ mbPt-876614	6.3	**20.2**	-	-	mbPt-876830 a		-2.3					
											-	-	mbPb-847671 a		-1.2	15	10.0	soPt-832053/ soPt-854393	4.9	15.6
						8	24.1	mbPt-846792 a		2.8										
						8	26.0	mbPt-868381/ mbPt-847654	5.1	15.8						16	24.0	mbPb-877453/ mbPt-877453	4.2	13.1
						-	-	mbPb-876778 a		3.3						-	-	soPb-857025 a		-4.7
						-	-	soPb-855926 a		3.1						-	-	soPb-855100 a		2.5

QTLs associated with phenological traits

The cultivars contributed alleles conditioning earlier flowering and shorter durations of flowering, pod growth and total plant growth, while the wild parents contributed alleles conditioning longer durations. In total, 12, 23, 13 and 14 QTLs linked to phenological traits were identified in the 1xB, 1xK, 87xB and 87xK crosses respectively, with PVE range of 0.4 – 66.1% (Figure 5.2; Appendix 5.6).

A total of 17 QTLs associated with time to flowering were identified across the four populations (Table 5.10a). Except for four minor QTLs in the ACC 1 crosses with negative PVE %, other QTLs enhanced later flowering. In the ACC 1 crosses, where late flowering was most strongly expressed, one and three major QTLs, with PVE of 15.7% – 28.6%, were revealed for 1xB and 1xK, respectively. A SML QTL, mbPb-870883, while only mapping on LG8 in 1xB, was common to both ACC 1 crosses and had relatively large effects (15.7% in 1xB and 18.5% in 1xK). The presence of major and positive additive effect QTLs originating from ACC 1 suggested relatively few major genes may control late flowering in these crosses. Prior to this research, there has been little information reported on QTLs associated with late flowering in mungbean. Three QTLs associated with earliness - a domestication-related trait - were identified by Isemura *et al.* (2012), and four by Kajonphol *et al*. (2012).

QTLs associated with morphological traits

As expected for quantitatively inherited traits, various numbers of additive QTLs linked to morphological traits such as leaflet size, petiole length, stem length, peduncle length or node of first pod, with PVE range of 0.6 – 49.2% were identified: 46 in the 1xB, 63 in the 1xK, 37 in the 87xB and 49 in the 87xK populations (Figure 5.2; Appendix 5.7). More often, QTLs had the effect of enhancing the wild type phenotype, such as decreasing leaflet size and floral standard size (negative additive effects) (Appendix 5.7a, f) or increasing stem length, branch length or higher node of first pod formed (positive additive effects) (Appendix 5.7g, h, n). Isemura *et al*. (2012) reported one QTL linked to stem diameter and 13 QTLs with both decreasing and increasing effects on stem length.

QTLs associated with pod and seed traits

In total, 24, 27, 18 and 29 additive QTLs linked to pod and seed traits were identified in the 1xB, 1xK, 87xB and 87xK populations respectively, with contributions of 0.4 – 62.6% to phenotypic variation explanation (Figure 5.2; Appendix 5.8).

While most QTLs linked to number of pods per peduncle and total number of pod clusters had the effect of increasing the number (Appendix 5.8a, b), those linked to pod size and seed size enhanced the wild type phenotype, i.e. decreasing the pod width and length, and the weight of 100 seeds (Table 5.10b; Appendix 5.8d, e). In particular, 19 QTLs were identified for seed size (one common locus between 1xK and 87xK) across the four RIL populations (Table 5.10b). Humphry *et al.* (2005)

reported 11 QTLs conditioning seed weight in a cross between Berken and the wild accession ACC 41 and explaining 4.9% to 18.8%, or collectively up to 80% of the phenotypic variation. Other studies by Fatokun *et al*. (1992), Kajonphol *et al*. (2012) and Isemura *et al*. (2012) respectively identified four, six and seven QTLs linked to seed size. The detected QTLs in this study clearly suggested seed size to be quantitatively inherited, consistent with previous mungbean studies (Fery 1980; James *et al*. 1999; Humphry *et al*. 2005).

Thirteen QTLs linked to hardseededness were identified across the four populations. This was in agreement with high heritability estimates (99 – 100%), which indicated the trait was genetically controlled (Table 5.10c). As expected, most of these QTLs originated from the wild parents and had the effect of increasing hardseededness, the exception being the major QTL in 1xB, which decreased hardseededness.

QTLs associated with yield related traits

The total number of QTLs associated with yield related traits (dry pod mass, seed yield, standing dry biomass and HI) varied with 18 in the 1xB, 17 in the 1xK, 11 in the 87xB and 21 in the 87xK populations with PVE varying from 1.0 to 71.8% (Figure 5.2; Appendix 5.9). The corresponding numbers of major QTLs were 5, 6, 2 and 2. Most major QTLs contributed to increased seed yield, dry biomass and HI, except for two major QTLs conditioning pod dry mass in 1xB (Table 5.10d; Appendix 5.9). Meanwhile, most QTL regions linked to total pod mass were also linked to seed yield (Appendix 5.9a, b), indicating these were effectively two measures of the same trait.

5.3.7. Distribution, common and co-localization of QTLs in the four mungbean F_5 RIL populations

As might be predicted from the large number of statistically significant phenotypic correlations among the traits (Table 4.20), many linked, co-located or perhaps some pleiotropic QTLs were detected (Figure 5.7; Appendix 5.10). The total numbers of significant QTLs located on the maps were 84 on 11 LGs, 74 on 16 LGs, 47 on 15 LGs and 59 on 13 LGs for 1xB, 1xK, 87xB and 87xK respectively (Figure 5.7). The details of common and co-located QTLs across the traits in each population are shown in Appendix 5.10.

In each individual RIL population, a few LGs contained only one QTL such as LG9 and LG13 of the 1xB or LG13 and LG17 of 1xK. Most of these QTLs conditioned qualitative morphological traits (e.g. dry pod colour, twining and leaf rachis colour) and quantitative pod and seed traits (e.g. seed size and hardseededness). Most LGs harboured 2 – 7 QTLs. There were a few LGs which harboured large numbers of QTLs (> 7) such as LG1, LG3, LG6 and LG11 of 1xB, LG7, LG8 and LG11 of 1xK, LG9 of 87xB and LG3 and LG13 of 87x K (Figure 5.7). QTLs were generally distributed within narrow regions on the LGs, especially those habouring several QTLs (Figures 5.3 – 5.6). This was

possible due to the close inter-marker distances in the high-density mungbean linkage maps that were constructed by DArT markers.

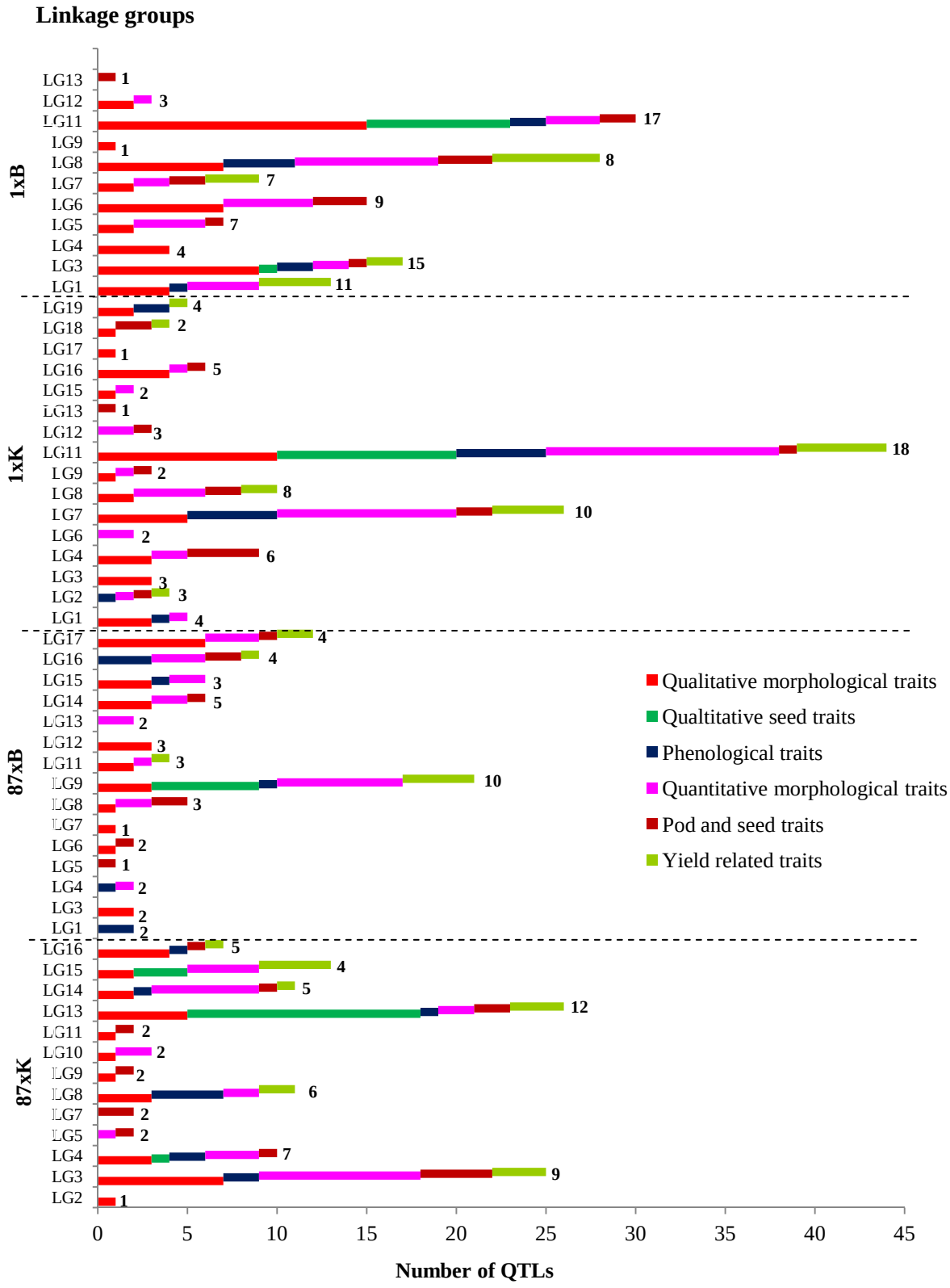


Figure 5.7. Number and distribution of QTLs associated with the six trait categories on each linkage group (LG) in the four mungbean F_5 RIL populations derived from crosses between cultivated plants (Berken and Kiloga) and wild accessions (ACC 1 and ACC 87).
(The number next to each bar indicates the actual number of QTLs on each LG when common QTLs across traits were accounted for).

QTLs associated with qualitative and quantitative morphological traits were scattered on various LGs in all four populations (Figures 5.3 – 5.7). There were cases where co-localization of these QTLs indicated wild-wild links as observed in phenotypic evaluation. Examples were QTLs conditioning twining, prostrate habit, long stem and long branches co-located on LG3 of 87xK.

QTLs conditioning visual seed traits tended to be distributed and co-localize on one (1xK and 87xB), two (1xB) or three (87xK) LGs. In 1xB, six QTLs conditioning wild-type seed traits such as black testa colour, seed mottling, pigmented texture layer and hilum colour co-localized on LG3 and LG11. This supported significant wild-wild associations between testa colour, texture layer and hilum pigment reported by Nguyen (2011) in the F_2 generation of the same cross. In 1xK and 87xB, these QTLs were found only on LG11 and LG9 respectively.

Except for a few QTLs linked to phenological, pod and seed and yield-related traits which were scattered on different LGs in each population, most of these QTLs co-located on the same LGs, i.e. LG8 (1xB), LG7 and LG11 (1xK), LG15 and LG16 (87xB) and LG6 and LG8 (87xK) (Figure 5.7). Their co-localization again supported the significant pairwise phenotypic correlations observed between many of these traits (Table 4.20). For example, in the 1xB RIL population, the correlations between seed yield and time to flowering and total pod clusters were r = 0.44, and r = 0.60 respectively (Table 4.20a). Similarly, in 87xK, correlations between total pod clusters and, respectively, seed size, seed yield and dry biomass were r = 0.31, r = 0.79 and r = 0.61 (Table 4.20d).

Some QTLs for traits of interest did not co-locate with QTLs associated with undesirable traits, implicating their potential use in mungbean improvement. In the Berken crosses, QTLs linked with increased hardseededness (on LG7 and LG13) did not co-localize with any of the QTLs for wild type visual seed traits such as dull lustre or seed coat mottling. This was also the case with the QTLs conditioning the late flowering in 1xB (LG8) and perenniality in 87xB (LG8, LG15 and LG17). However, this did not occur in the Kiloga crosses where QTLs for late flowering and perenniality co-localized with wild type visual seed traits (1xK) and pod dehiscence (87xK) respectively.

Common QTLs between traits in a population were observed on some LGs, i.e. LG7 and LG11 (1xK), LG9 (87xB) or LG3 (87xK) (Figure 5.7; Appendix 5.10). Most common QTLs were for qualitative seed traits and a few were for traits across the different categories. For example, a common QTL in 87xB for hilum colour and seed coat ridging supported the significant linkage between hilum colour and presence of a surface texture layer reported by Nguyen *et al*. (2012), based on the same cross and by James *et al*. (1999) based on other crosses between wild and cultivated mungbean. In the 87xK population, the QTL region on LG8 was common for perenniality, pod dehiscence, growth duration and total pod mass.

CHAPTER 6. PHYSIOLOGICAL DROUGHT STRESS RESPONSE TRAITS IN SOYBEAN

6.1. Introduction

As noted in Chapter 2, drought stress is a major constraint to the yield of soybean. Considerable efforts have been directed towards understanding mechanisms as well as identifying feasible/ applicable physiological traits, and their QTLs, for improving drought tolerance in soybean.

Drought tolerance assessment is complex and various measures have been reviewed elsewhere for plants in general (e.g. Kirkham 2004; Blum 2005; Jones 2007; Xoconostle-Cazares 2011; Munns 2011; Anjum 2011) and for soybean in particular (e.g. Manavalan 2009; Ku *et al*. 2013). Various physiological traits have been proposed as offering improved performance in drought prone environments (Tuberosa 2012). However, decisions about which physiological traits warrant study is not straightforward and depends in part on both the germplasm and the nature of the drought. The research here focussed on physiological traits identified in previous studies by James *et al*. (2008a, b, c) and Lawn and Likowse (2008), and used a similar experimental approach to screen for drought stress response in plants grown in large cylindrical pots in a partly controlled aerial environment.

Of necessity, drought stress response traits are highly environmentally sensitive – either they only express during drought or their expression is influenced by drought. But drought environments are highly complex in space and time. Even in pots, where the environment can be controlled to an extent, the drought environment to which each plant is progressively exposed depends in part on its previous growth response. In addition, a number of distinct limitations characterise pot experiments and should also be carefully considered and managed to obtain meaningful results relevant to field conditions (Passioura 2006; Salekdeh *et al*. 2009; Poorter *et al*. 2012). Because of this dynamic situation, reliable phenotyping is fraught with difficulty which is one reason why reliable molecular markers or QTL detection would be useful.

Availability of sufficient genetic variability for the target traits is one of pivotal factors contributing in informative and efficient phenotyping. This in turn is crucial also for genetic understanding of the traits at molecular levels (Tuberosa 2012). The narrow genetic base of soybean (Gizlice *et al*. 1994, 1996; Cui *et al*. 2000; Zhou *et al*. 2000) is a potential limitation in the identification of molecular markers or QTLs associated with drought tolerance. Conversely, the good levels of polymorphic DArT markers identified for soybean during the soybean and mungbean library creation process (Chapter 3) may enhance the opportunity for QTL detection.

The research described below used three sets of RILs developed from hybrid populations in which genetic variation for drought stress response had been observed in the parents and the F_2 generation (James 2004). These hybrid populations were developed from two oilseed types, CPI 26671 and cv. Valder and an Indonesian landrace variety G2120. The F_2 progeny exhibited apparently normal distribution for plant water status traits including relative water content (RWC) and epidermal conductance (g_e), indicating quantitative inheritance with a high degree of additive gene action (James *et al*. 2008c). For RWC, in two crosses involving G2120, the range in F_2 progeny values was greater than the combined range of the parental plants and estimates of broad-sense heritability were moderate (0.73 – 0.74). For g_e, F_2 plants with low g_e were recovered at the highest frequency from crosses where G2120 was a parent. Estimates of broad sense heritability were moderate to high for this trait in all three F_2 populations (0.60 – 0.86).

Phenotypic evaluation of the three soybean RIL populations and their parents was undertaken under drought stress conditions in the glasshouse, during which leaf material was collected for DNA extraction and analysis. Subsequently, these DNA samples were used for the identification of QTLs associated with drought response using DArT markers.

6.2. Materials and methods

The research in this chapter was necessarily undertaken in three different locations to facilitate access to the requisite experimental facilities needed for each phase of the work. The initial development of the soybean RIL populations was conducted in Townsville, the evaluation of their phenotypic responses to drought stress and the extraction of leaf DNA was undertaken in Brisbane, and the DArT marker research was undertaken in Canberra.

6.2.1. Plant materials and population development

The three germplasm sets comprised one F_7 and two F_6 RIL populations, developed from crosses between CPI 26671, cv. Valder and G2120 (Table 6.1). The parents and F_2 seeds of crosses CPI 26671 x G2120 (CG), CPI 26671 x Valder (CV) and F_4 seeds of cross Valder x G2120 (VG) were provided by Dr Andrew James, CSIRO Plant Industry, Brisbane, from a previous PhD study into genotypic variation in soybean for drought stress traits (James 2004). The parental genotypes were originally chosen on the basis that they had demonstrated different capacity for leaf survival and different combinations of three leaf traits (epidermal conductance, osmotic adjustment (OA) and critical relative water content (RWC_c) under drought stress), and also had different genetic backgrounds based on knowledge of their ancestry (James 2004). In general, leaf and plant survival during the advanced stages of drought stress were enhanced by low g_e, high OA capacity and low RWC_c, so that leaf maintenance during drought was greater in G2120 than in the other two genotypes (James *et al*. 2008b).

Table 6.1. Soybean germplasm used to generate three hybrid populations for study.

Genotype	Maturity group♪	Region of origin	Pedigree information	Traits of interest‡
CPI 26671	IV	USA via Morocco	Selection from Nanking	weak capacity for leaf survival: moderate to high g_e, moderate capacity for OA, low to moderate RWC_c
Valder	IV	Australia	Williams x Calland	weak capacity for leaf survival: high g_e, low to moderate capacity for OA, low to moderate RWC_c
G2120	X	Indonesia via Taiwan	Selection from an Indonesian landrace	stronger capacity for leaf survival: low g_e, moderate to high capacity for OA, low to moderate RWC_c

♪: Maturity group: IV: adapted to warm temperate regions; X: adapted to the tropics

‡: Based on James (2004) (g_e = Epidermal conductance; OA = Osmotic adjustment; RWC_c = Critical relative water content)

The RILs were developed using the single seed-descent breeding method, without selection, in the glasshouse at the CSIRO Davies Laboratory, in Townsville, Queensland. The CV and CG were advanced from F_2 to F_3 while the VG was advanced from F_4 to F_5 in 2008 (RJ Lawn, personal communication). Subsequently, these F_3 and F_5 RILs were respectively advanced to the F_6 and F_7 generations during 2009 – 2010. During generation advancement, the plants were grown with very favourable water supply and sprayed with pesticides as necessary to control insect pests and mites. The advanced generation seeds were sent to CSIRO Plant Industry in Brisbane, where the experiment for phenotyping plant response to drought stress was conducted in 2011.

6.2.2. Phenotyping drought response

The study of phenotypic response of the three RIL populations and their parents to drought stress was undertaken on plants grown in large cylindrical pots in the glasshouse, so that the environmental conditions could be at least partly controlled. The plants were initially well-watered, then droughted, and finally re-watered, so that responses to drought stress and recovery after re-watering could be documented. Even in pots in a glasshouse, the drought environment experienced by individual plants is temporally complex, and likely to be influenced at least in part by the growth and water use of each individual plant. Therefore, to try to obtain a better description of the 'drought environment' to which the plants were exposed, it was decided also to grow a 'reference' plant in each pot, and to compare the responses of each RIL and its parents against those of the respective reference plants.

6.2.2.1. Cultural details and experimental design

A total of 52, 66 and 70 RIL lines for the CG, CV and VG RIL populations respectively, and four plants of each parent, were tested for drought stress response. The plants were grown in soil-filled PVC pots 1.00 m tall and 0.25 m in diameter. Since G2120 was shown to possess traits conditioning better drought tolerance (James 2004) (Table 6.1), it was used as the reference plant in each pot in the

experiment, to provide a measure of the environmental effects experienced in each pot during the tests.

Two runs of the drought phenotyping study were conducted, with a total of 100 pots in each run. The first run was in the glasshouse at the CSIRO Long Pocket Laboratories, Brisbane (LP glasshouse) during the period January to April, 2011. The second run was in the CSIRO Plant Industry glasshouse at the Queensland Biosciences Precinct, Brisbane (QBP glasshouse) during the period June to August, 2011. Mean glasshouse temperatures and relative humidity for both runs were maintained at 25 ^{o}C and 57% respectively. In order to inhibit flowering and maintain plants in the vegetative phase, day length was extended to 16 hr using incandescent lights. In each run, the pots were randomly ordered within the available glasshouse space.

Pot tare weights were recorded before they were filled with 64 kg of soil. Field capacity was determined by saturating the soil with 10 L of water and then leaving it to freely drain for 3 days before the pots were weighed. The same amount of soluble Aquasol fertiliser (NPK – 23: 3.95: 14) was applied to each pot to ensure nutrients were adequate for growth. Two plants of the same population (either RILs or parental plants) were grown in a pot with one G2120 reference plant. For each of the three genotypes in a pot, three seeds were initially sown and one seedling was randomly kept for the drought evaluation. Plants were well watered to the stage of having two fully-expanded trifoliolate leaves (c. 21 days after sowing). This was also the time for last watering, with the same amount of water (3 L for every pot), which brought the soil up to field capacity again.

In the first run, re-watering was commenced from 37 days after the water stress was first imposed, based on observations of the degree of stress exhibited by the reference plants. The aim was to expose all plants to a uniform severe stress, without killing them. Individual pots received a single re-watering when the reference plant was permanently wilted, the remaining live leaflets on the reference plant in the pot showed chlorophyll dis-colouration and the leaflet edges had started to shrink. After re-watering, the degree of recovery of all the plants was recorded. Plants were then left to grow until all soil water had been used by the plants (i.e. the plants died). When all the plants in a pot were completely dead, pot weights were again recorded, and plant available water (PAW) was calculated as the difference between the pots at field capacity and when plants had died. This information was used in the second run, to try to achieve a more uniform level of stress when the plants were re-watered.

In the first run, because re-watering was based on the degree of stress evident in the reference G2120 plant, re-watering occurred too late for a few test plants and there was no recovery. In these instances, the average PAW was < 5%, whereas for the plants that recovered, PAW was > 5%. Therefore, in the second run, the re-watering of each pot was adjusted based on PAW, rather than on observations of the G2120 reference plants. Re-watering was undertaken on a pot-by-pot basis when the PAW in each pot, as estimated based on the change pot weight, had been reduced to 5%. In run 2, re-watering

started 24 days after water stress was first imposed, which was sooner than in run 1. The earlier re-watering in run 2 reflected faster water use, as a result of more vigorous plant growth than in run 1.

6.2.2.2. Trait measurement

In both runs, measurements were made on each RIL plant, each parental plant and each reference plant of the responses to the initial drought stress and the recovery after drought stress. Pot weights were recorded every seven days for PAW calculation.

Plant growth responses

The numbers of stem nodes and live leaflets were recorded every seven days.

Leaf water status: relative water content RWC (%) and epidermal conductance g_e (mm/s)

In run 1, measurements of RWC and g_e were made 14 days after the last watering and thereafter on a weekly basis for three weeks. In run 2, because of faster growth, measurement commenced sooner (day 7) and continued weekly thereafter for three weeks. On each measurement occasion, the second youngest fully expanded leaf on each plant was harvested between 10.00 – 11.00 am, put in a sealed plastic bag and taken to the laboratory for the water status determinations. Since these measurements were intensive and time consuming, leaf samples of only one population were collected and measured on any one day.

The RWC was determined on one of the two side leaflets using the method of Barrs and Weatherley (1962) as modified by Turner (1981). Leaflets were weighed to give the fresh weight, then cut in half at the central midrib and floated on de-mineralised water overnight at 25 ^{o}C. The leaflets were gently dried using tissue paper and weighed to obtain re-imbibed weight and then oven-dried at 60 ^{o}C for 48 hr for dry weight records.

The g_e of the leaflet from the other side of the leaf was determined according to Sinclair and Ludlow (1986). The leaflet's area was determined by an electric planimeter. The leaflet was then attached to a hook and suspended in a room of known temperature. An a aspirated psychrometer was used to determine wet and dry bulb temperatures and then relative humidity of the room. Both leaf surfaces were exposed to the air and the leaf was irradiated at about 20 micro-einsteins which is the light compensation point for photosynthesis in most soybean leaves. This is so that the gain in weight from photosynthesis is balanced by the loss in weight from respiration. In practice, however, the weight gain/ loss is inconsequential to the ultimate result. Leaflet plus hook weights were measured every 20 min over 3 hr to obtain 6 – 7 records (depending on whether weight changes were still being observed or not). At the end of the experiment, the hook weight was subtracted from the leaflet weight.

In order to non-destructively estimate the live leaf area of the plants, least-squares regression relations were established between the areas of individual leaves and the length and width of the terminal leaflets. For each parental plant and RIL population that had been sampled for RWC and g_e

measurements as explained above, the areas of ten leaves were determined using the electric planimeter and the lengths and widths of the terminal leaflet were recorded. The relationship between leaf areas and terminal leaflet length and width was used to estimate live leaf area on each plant, based on measures of terminal leaflet lengths and widths, as follows:

$$LA\ (cm^2) = aLL + bLW$$

where: LA = Leaf area (cm^2); LL = Terminal leaflet length (cm); LW = Terminal leaflet width (cm); a and b = coefficients of terminal leaflet length and width respectively.

Recovery assessment

On the day of re-watering, the numbers of stem nodes and remaining live leaflets were recorded. The lengths and widths of the terminal leaflets of the remaining live leaves were also recorded. Recovery assessment was made 10 days after re-watering, by recording the numbers of stem nodes and leaflets and terminal leaflet sizes. In addition, a subjective 'Recovery rating' was scored as follows: 1. No recovery (plant dead); 2. Recovery but growing point dead; 3. Recovery; 4. Recovery with vigorous growth.

6.2.2.3. Data analysis

Leaf area calibrations for each parental plant and RIL population were conducted by using regression between terminal leaflet sizes (length x width) and leaf areas with the intercept forced to be zero. Based on the relations, leaf areas at-rewatering and after re-watering were calculated.

Measures of recovery, which included the numbers of new stem nodes, new leaflets and new leaf area per plant, were calculated for each of the three plants in each pot by subtracting measurements at re-watering from those made 10 days after re-watering.

PAW (%) was calculated as follows:

$$\text{PAW} = \frac{\text{Pot weight} - (\text{Pot tare weight} + \text{Soil weight})}{\text{Pot weight at field capacity} - (\text{Pot tare weight} + \text{Soil weight})} \text{ x } 100\% \quad (1)$$

RWC (%) was calculated as:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Reimbibed weight} - \text{Dry weight}} \text{ x } 100 \quad (2)$$

The epidermal conductance (g_e, mm/s) was calculated from the change in leaflet weight with time once the stomata had closed, following the formula and procedures described by Sinclair and Ludlow (1986), Paje *et al.* (1988) and James *et al*. (2008a):

$$g_e = \frac{\Delta FW}{t} \frac{1}{A} \frac{1}{e_l - e_a} \quad (3)$$

where: ΔFW is change in fresh weight over time t, A is leaf area, and (e_l - e_a) is the absolute humidity gradient between the leaf and the ambient air inside the room.

ΔFW/t was estimated as the slope from the regression of the linear phase where the stomata had closed (Figure 6.1)

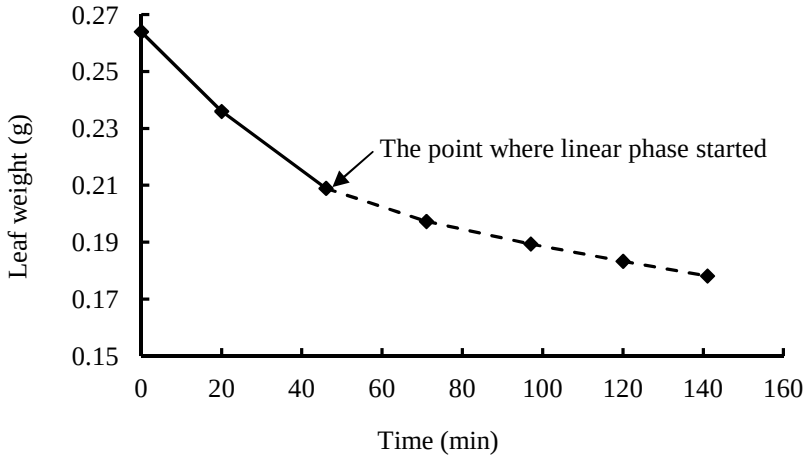


Figure 6.1. Typical plot of leaf weights over time used in determination of epidermal conductance (g_e, mm/s). The linear phase used to estimate ΔFW/t is shown as a dotted line.

Understanding the responses of the G2120 reference plants

Initial analyses were designed to establish a clear understanding of the responses of the G2120 reference plants that were evaluated over the two runs, since these would provide insight into the reliability and repeatability of the drought stress response measurements. For both RWC and g_e, analyses of variance were undertaken to explore the effects of sampling dates in a run. Since a linear inter-relation was assumed by James (2004), the inter-relation between RWC and g_e was also examined. From this relation, the g_e at a RWC of 70% (the stress level where loss of turgor was imminent) was calculated (Sinclair and Ludlow 1986). The inter-relations between traits before and after re-watering were also examined.

Analysis of RILs and parental plants

ANOVA was performed using Genstat V to evaluate the variation among the RILs and parents as follows:

Source	df♪	Mean square	Expected mean square
Runs	r -1	Not relevant	Not relevant
Populations	m -1	Not relevant	Not relevant
Genotypes	m x (n-1)	M_g	$\sigma_e^2 + r\sigma_g^2$
Residuals/Error	f	M_e	σ_e^2

♪: Degrees of freedom generated by ANOVA analysis

Broad sense heritability (H_b^2) and associated standard errors (s.e.) were calculated based on Singh *et al*. (1993) with significant variance partitioned into genetic variance and error variance as follows:

$$H_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

where:

r = Number of runs

m = number of populations

n = number of plant individuals

f = degree of freedom for residuals

$\sigma_e^2 = M_e$ and $\sigma_g^2 = (M_g - M_e)/r$

σ_e^2 = Environmental variance

M_e = Mean square of error

σ_g^2 = Genotypic variance

M_g = Mean square of genotype

$$s.e = \left(1 - H_b^2\right)[1 + (r-1)H_b^2]\sqrt{\frac{2}{rf}}$$

where:

f = Degree of freedom of error

Coefficients of variation (CV%) were calculated based on Gomez and Gomez (1984):

$$CV\% = \frac{\sqrt{\text{Error mean quare}}}{\text{Mean}} \text{ x } 100$$

The CV aims to describe the model fit in terms of the relative sizes of the squared residuals and outcome values. The lower the CV, the smaller the residuals relative to the predicted values. In other words, it expresses the experimental error/ noise as percentage of the mean and thus the higher the CV value, the higher the experimental error/ noise.

The nature of the relation between g_e and RWC that was established for the G2120 reference plants was subsequently used to infer similar relations for the RIL and parent plants. Based on this inter-relation between RWC and g_e, g_e values at RWC of 70 % (g_{e70}) were calculated.

6.2.3. Genetic analysis

6.2.3.1. DNA extraction and genotyping

The terminal leaflets of the leaf samples of the parental plants and the RIL populations that were removed for leaf water status measurements during the two runs were lyophilised and stored for later DNA extraction, which followed the standard DArT protocol (Chapter 3). The only modification was the use of beads for grinding the leaf materials into a fine powder. Genomic complexity reduction with *Pst*I/*Taq*I and *Pst*I/*Bst*NI and the generation, labelling and hybridisation of targets, were conducted as described in Chapters 3 and 5. The soybean RIL populations were genotyped against arrays constructed using both of the previously developed mungbean and soybean DArT libraries.

6.2.3.2. Polymorphic DArT marker selection

DArTsoft version 7.4 was used to identify and score polymorphic clones with "0" for absence, "1" for presence and "x" when DArTsoft was unable to score the sample with sufficient confidence. DArT standard analysis was employed with thresholds of $P > 0$, Callrate > 80 and Reproducibility > 90. In general, polymorphic DArT markers were selected for Average Reproducibility > 90%, Consensus ≥ 95%, Average P > 50%, Discordance ≤ 2% and PIC ≥ 1%. Similarly to mungbean, only markers which consistently scored across replicated parental samples and were polymorphic for the parents in a cross were selected.

6.2.3.3. Construction of genetic linkage maps

Genetic linkage maps of the individual soybean RIL populations were constructed using Joinmap 3.0 (van Oojen and Voorps 2001). Chi-square values for a marker segregation ratio of 1:1 were calculated with "Set χ^2 – Test classification for selected loci" in Joinmap. Markers were placed into linkage groups (LGs) with LOD ≥ 3 and the "Create groups for mapping" command using the Kosambi map function (see details in Chapter 5 – 5.2.4). Marker order was established by the "Calculate map" command using the default settings of mapping parameters. Markers with a mean χ^2 contribution > 5 were excluded.

Polymorphic markers mapped on individual maps were pooled to construct an integrated map using Joinmap, using parameter settings as described for the individual maps. Missing values were assigned

for markers which were not present in the individual populations (Beavis and Grant 1991). Marker orders between individual or component maps and the consensus map were compared and graphically presented by Mapchart 2.2 (Voorips 2002).

6.2.3.4. QTL statistical analyses

Means of trait values over the two runs were used for the QTL analyses. The Statistical Machine Learning method (SML) (Bedo *et al*. 2008) by "Standard marker analysis" (Diversity Arrays Technology P/L, Canberra, Australia) and the Inclusive Composite Interval Mapping (ICIM) method (Wang *et al*. 2011a), described for mungbean in Chapter 5 (5.2.5), were again used for the soybean QTL analyses. Significant QTLs detected by SML (hereafter referred to as SML QTLs) were selected at $P \leq 0.01$.

Pearson correlations (r) among markers linked to QTLs and markers mapped on the integrated map were calculated using marker genotypes. Similarly to mungbean, marker pairs which had significant correlations $r \geq 0.84$ were considered to map on the same LG and target the same QTL.

Positions of SML QTLs detected in each population on the integrated map were included wherever those mapped. In other cases, the position of the marker which was on the map and significantly correlated with the marker linked to a SML QTL ($r \geq 0.84$) was assigned to that SML QTL.

Similar definitions to those used for mungbean (Chapter 5 – 5.2.5) for overlapped, common and co-localized QTLs were used here for soybean.

6.2.4. Sequencing selected DArT markers linked to QTLs for physiological traits associated with drought stress responses

Nineteen DArT markers linked to QTLs for physiological traits associated with drought stress responses were selected and sent to The ACRF Biomolecular Resource Facility (BRF) – Australian National University for Sanger sequencing (Appendix 6.6). Only QTLs detected in the CG and VG populations, which involved G2120 as a drought tolerant parent, were considered for selection.

Pairwise BLAST (Basic Local Alignment Search Tool) (Altschul *et al*. 1997) queries among 19 selected DArT markers were applied to identify markers with redundant sequence.

Sequences of DArT markers were searched against the NCBI database (National Central for Biotechnology Information) (http://blast.ncbi.nlm.nih.gov/) using the nucleotide BLAST (blastn) and *G. max* genome references (1168 sequences) (Schmutz *et al*. 2010). In all cases, several matches were found for each DArT marker sequence. The best alignment/ match was selected based on the lowest E-value, the highest query coverage, the highest total score and the highest identity. The E-value indicates the statistical significance of a given pairwise alignment, and the lower the E-value, the more significant the 'hit'. A sequence alignment that has an E-value of 0.05 means that this similarity

has a 5 in 100 (1 in 20) chance of occurring by chance alone. Query coverage corresponds to the fraction of the query sequence (i.e. DArT marker sequence) that matches a subject sequence (genome reference sequence). The total score is the sum of normalized scores of all aligned sequences. Identity is the extent to which two nucleotide sequences have the same residues at the same positions in an alignment.

Sequences of these DArT markers were also BLASTed against soybean genetically mapped Genebank sequences in the NCBI database (2118 sequences) to confirm whether they were different from other marker types such as RFLPs, SSRs or SNPs.

The BLAST search would be expected to identify the soybean chromosomes aligned with the respective sequences of DArT markers which were linked to QTLs detected in the RIL populations. Based on their aligned positions, the locations of DArT markers were viewed in the genome sequence browser (http://soybase.org/gb2/gbrowse/gmax1.01/). The approximate positions (cM) of these DArT markers/ QTLs on respective LGs of the Soybean Consensus Map 4.0 were converted using their aligned positions and the average recombination rates (cM/Mbp) of the Soybean Consensus Map 4.0 (Hyten *et al*. 2010b; Chapter 2 – Table 2.1; Appendix 6.8).

The results were also compared with previous studies on QTLs associated with drought stress responses in soybean (http://soybase.org/; Mian *et al*. 1996, 1998a; Charlson *et al*. 2009; Du *et al*. 2009; Abdel-Haleem *et al*. 2012; Carpentieri-Pipolo *et al*. 2012; Virginia *et al*. 2012). The positions of these reported QTLs on the Soybean Consensus Map 4.0 were retrieved (Hyten *et al*. 2010b; http://soybase.org/gb2/gbrowse/gmax1.01/). As indicated by Bedo *et al*. 2008, because there are genotyping and linkage mapping errors associated with QTL detection by different QTL analysis methods, QTLs identified using different methods are considered to overlap if they are within 10 cM of each other. As a result, QTLs associated with soybean drought tolerance detected by the SML method in this study were considered to be new if their approximate positions on the Soybean Consensus Map 4.0 were not within 10 cM of previously reported QTLs.

6.3. Results and Discussion

6.3.1. Soil water depletion and reference plant response

The temporal trends in average soil water depletion in the pots after the water stress was imposed in the two runs are illustrated in Figure 6.2a. In the second run, the plants grew more vigorously, as indicated by the more rapid increase and higher peak in the number of stem nodes. The main reason for the faster growth in the second run was that, while the temperature was constant for both runs, more radiation reached the plants in the new QBP glasshouse than in the older LP glasshouse. As a consequence, 95% PAW depletion was reached about 6 d earlier in run 2 than in run 1 (32 compared with 38 d respectively). As expected, the rate of PAW depletion declined as PAW decreased. For example, in run 1, average PAW was depleted by 60% after 12 d (an average rate of 5% per d) but

another 26 d was required to deplete PAW to 95% (1.4% per d). In run 2, PAW was depleted by 70% by day 12 (5.8% per d) and took another 20 d to deplete PAW to 95% (1.3% per d).

While the temporal growth patterns of the G2120 reference plants, as indicated by the numbers of stem nodes, differed markedly between the two runs (Figure 6.2a), the differences were less pronounced when growth was expressed in terms of PAW depletion. In both runs, the growth in live leaflet number was quite similar before PAW depletion reached about 30% (Figure 6.1b). In run 1, active leaflet growth only continued to a PAW depletion of about 60%, whereas in run 2, the growth in live leaflet number continued beyond a PAW depletion of 70%. In both runs, the numbers of live leaflets declined once PAW depletion exceeded 90%. However, the greater number of live leaflets in run 2 contributed to sharper leaflet losses, and as a consequence, at 95% PAW depletion, there were actually fewer live leaflets on the reference plants in run 2 than in run 1 (Figure 6.2b).

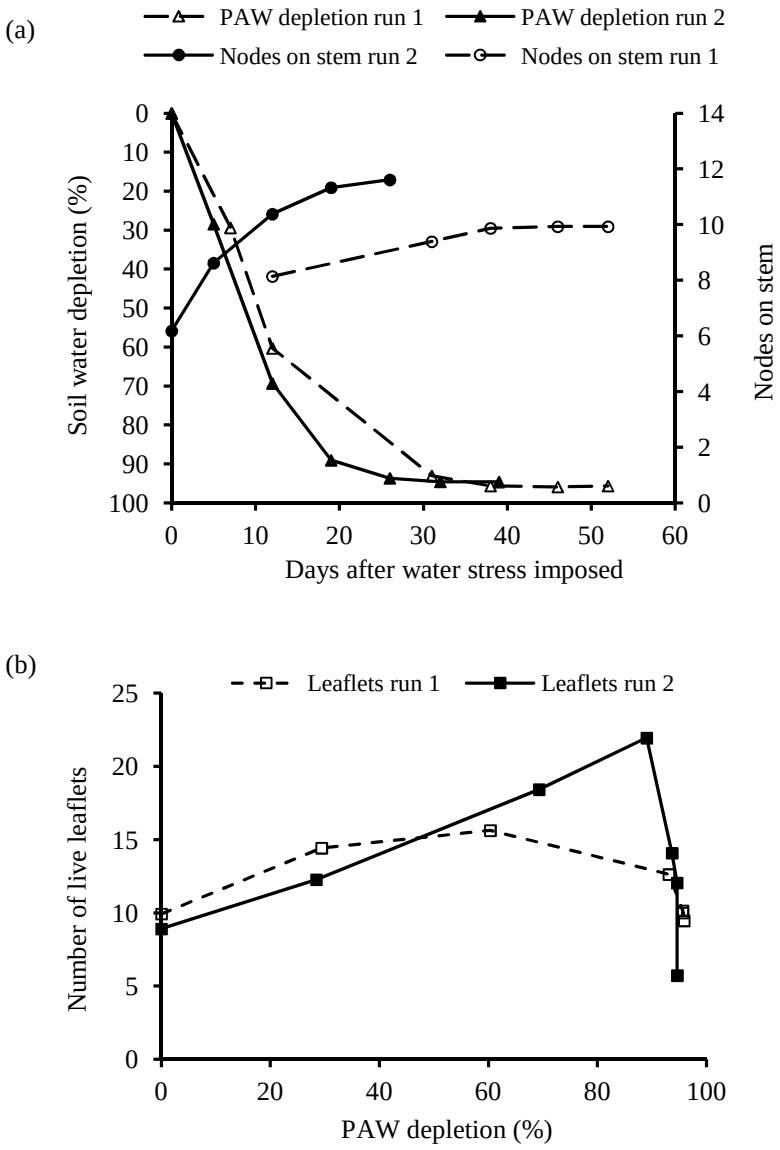


Figure 6.2. Trends in soil water depletion and growth of the G2120 reference plants in the two drought response runs (a) temporal trends in the numbers of stem nodes and in plant avaliable water (PAW) depletion; and (b) trends in numbers of live leaflets as a function of PAW depletion.

As expected, as the PAW depletion increased, both RWC and g_e in the G2120 reference plants declined (Figure 6.3). The broad patterns of change in both RWC and g_e as PAW declined was consistent over the two runs. For example, RWC remained in the 80 – 100% range until PAW depletion reached about 60%. However, as PAW was depleted from 70% to 90%, RWC content declined sharply (Figure 6.3a). The pattern of decline in g_e was more diffuse (Figure 6.3b), but again, there was a broad overlay of data from the two runs. These broad patterns of decreasing RWC and g_e as water stress increased were consistent with observations in soybean by Meyer and Green (1981), Paje *et al*. (1988), Likoswe and Lawn (2008) and James *et al*. (2008a, b, c). Decreases in RWC and g_e in response to drought stress have been noted in wide variety of plants (Praba *et al*. 2009; Anjum *et al*. 2011).

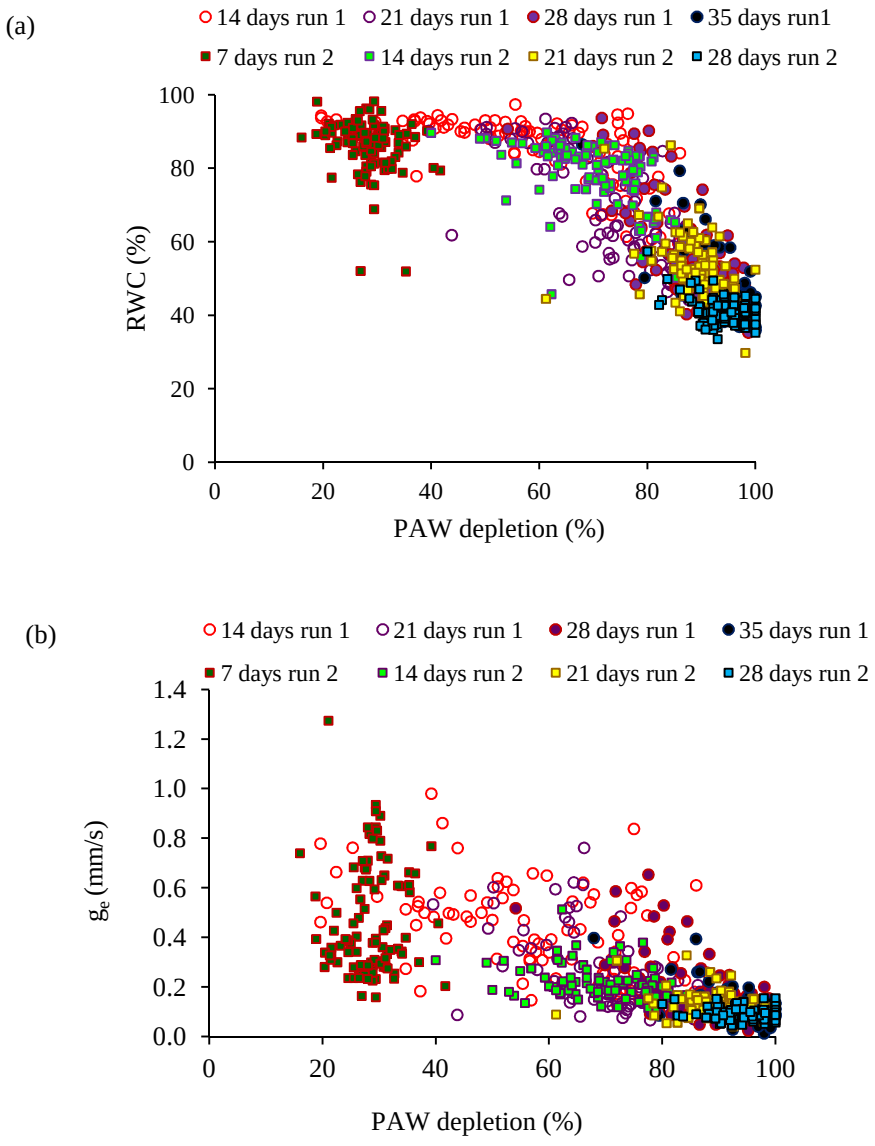


Figure 6.3. Changes in (a) Relative water content (RWC, %) and (b) Epidermal conductance (g_e, mm/s) of the G2120 reference plants as PAW declined

ANOVA revealed statistically significant differences ($P < 0.01$) in mean PAW over the two runs but not in g_e and RWC (Table 6.2). Nonetheless, when the RWC and g_e data were examined in terms of sample times, there were statistically significant and consistent differences between the two runs (Tables 6.2, 6.3). These differences reflected the different temporal trends in PAW depletion in the two runs (Figure 6.2a) which meant that the average PAW at the respective sample times differed somewhat between the two runs. Differences were evident in the second and fourth sample times for RWC but only in the first sample time for g_e. While the PAW depletion at the first sample time differed between the two runs, RWC did not (Table 6.3).

Table 6.2. ANOVA of plant available water (PAW, %), relative water content (RWC, %) and epidermal conductance (g_e, mm/s) in the G2120 reference plants over two runs and sample times

	Sources of variation	**df**	**S.S.**	**M.S.**	**F**	***P***
PAW (%)	Run	1	34649.1	34649.2	406.7	<.001
	Sample	3	604566.5	201522.2	2365.2	<.001
	Run.Sample	3	67023.9	22341.3	262.2	<.001
	Residual	1574	134108.3	85.2		
	Total	1581	833846.1			
RWC (%)	Run	1	34.1	34.1	0.3	0.574 *ns*
	Sample	3	373712.0	124570.7	1153.6	<.001
	Run.Sample	3	28599.1	9533.0	88.3	<.001
	Residual	1454	157014.9	108.0		
	Total	1461	522277.5			
g_e (mm/s)	Run	1	0.01	0.01	0.77	0.379 *ns*
	Sample	3	23.38	7.79	427.28	<.001
	Run.Sample	3	0.82	0.27	15.05	<.001
	Residual	1454	26.52	0.02		
	Total	1461	49.26			

ns = Not significant

df = Degree of freedom, S.S. = Sum of squares, M.S. = Mean square; F = F-value, *P* = Probability

In both runs, the average RWC values at the first sample time (83.9% and 86.3% in runs 1 and 2, respectively) were lower than those reported by James *et al*. (2008b) for well-watered G2120 plants in soil filled beds (96%). In part, the difference may reflect the fact that the current measurements were taken on plants grown in pots rather than soil-filled beds and/ or in drier aerial conditions in the glasshouse. Meanwhile, the average g_e values at the first sample (0.40 – 0.46 mm/s) were higher than those reported by James *et al*. (2008b) for well-watered G2120 plants (0.23 mm/s). However, average RWC values at the last sample time (near lethal stage), were consistent with those reported for stressed G2120 plants by James *et al*. (2008a, b). Likewise, the average g_e at the near-lethal stage in both runs (Table 6.3) were of similar magnitude to those reported by James *et al*. (2008b) for severely stressed G2120 leaves.

Table 6.3. Means of plant available water depletion (PAW depletion, %), relative water content (RWC, %) and epidermal conductance (g_e, mm/s) of the G2120 reference plants over sample times in two runs

Means followed by the same letters are not significantly different ($P > 0.05$) based on one-way ANOVA

	Run 1				**Run 2**			
Sample time	1	2	3	4	1	2	3	4
	Early stress →		near lethal		Early stress →		near lethal	
PAW depletion	59.8^{b}	75.2^{d}	87.7^{e}	94.7^{f}	28.4^{a}	69.3^{c}	89.0^{e}	94.2^{f}
RWC	83.9^{f}	65.7^{d}	56.8^{c}	50.5^{b}	86.3^{f}	78.7^{e}	52.0^{bc}	41.1^{a}
g_e	0.40^{d}	0.21^{c}	0.18^{bc}	0.13^{ab}	0.46^{e}	0.22^{c}	0.13^{ab}	0.09^{a}

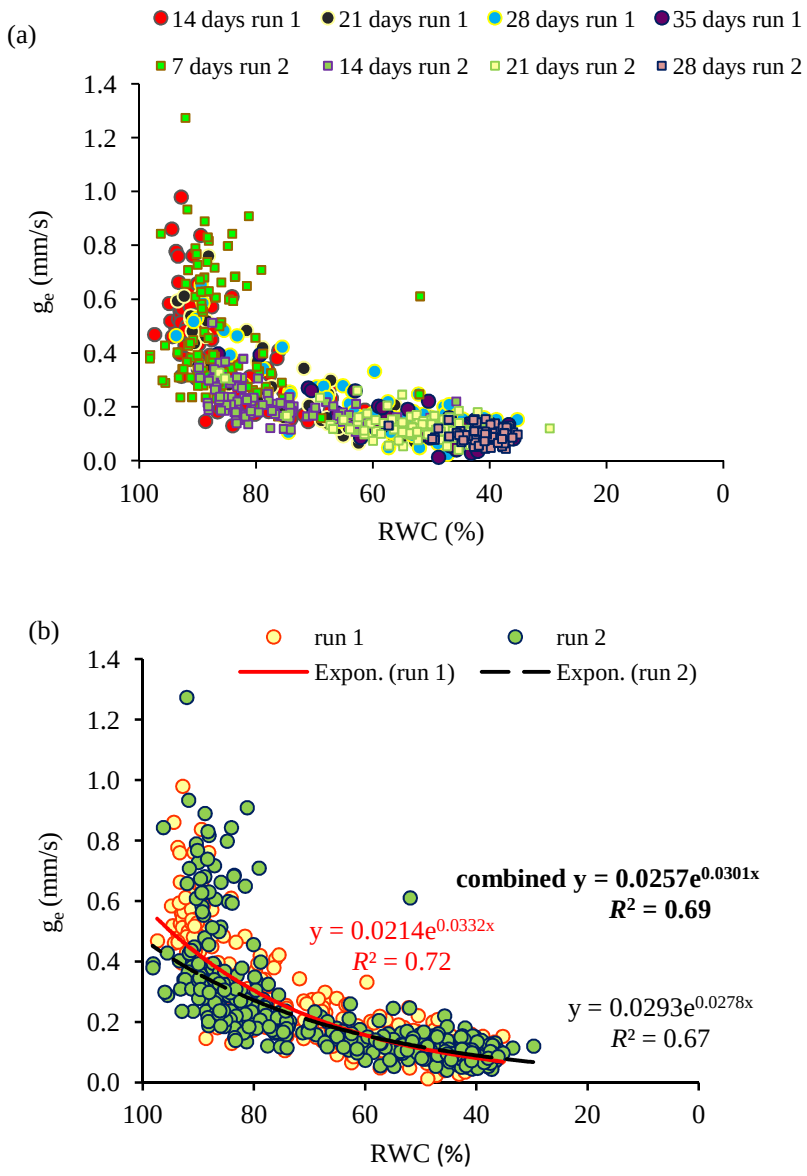


Figure 6.4. Best fit least square relations between relative water content (RWC, %) and epidermal conductance (g_e, mm/s) for 200 G2120 reference plants, (a) Sampled four times in each of two runs to evaluate drought stress response; (b) Exponential relations.

The relation between RWC and g_e over the four sample times in the two runs is illustrated in Figure 6.4. As observed by James *et al.* (2008a), g_e decreased as RWC decreased, with significant linear correlations (r = 0.83 and 0.67 for runs 1 and 2, respectively). However, rather than a linear relation as assumed by James *et al.* (2008a, b, c), an exponential relation provided a better fit to the data (Figure 6.4a, b). The significant R^2-values in both runs show that for the G2120 reference plants, 72% and 67% of the variation in g_e in runs 1 and 2 respectively, was explained by the exponential relation linking it to the decline in RWC. Combined, the two runs provided a relation with a significant R^2-value of 0.69. Based on this relation, g_{e70} for the G2120 reference plants was estimated to be 0.21 mm/s. This value was somewhat higher than the range 0.13 – 0.20 observed in James *et al.* (2008a, b).

In summary, the growth, RWC and g_e responses of the G2120 reference plants to water stress were broadly similar in the two runs, and were also broadly in line with previous studies on soybean. Plant growth continued for some time after the last watering, but slowed as PAW was progressively depleted. Ultimately, when PAW depletion exceeded 90%, leaflets began to senesce rapidly. Both RWC and g_e declined as PAW declined, with a reasonably strong interrelation between these two physiological traits. There were some temporal differences in responses between the two runs, because of differences between the glasshouses where the runs were conducted. However, these temporal differences were dampened when the relations were expressed in terms of changes in PAW. The responses observed in the reference plants indicated that, consistent with previous experience, there was inherent variability or 'noise' associated with the relations between the physiological traits, and between these traits and PAW. In part, this variability simply reflected measurement error. However, it also reflected micro-environmental differences between sample time, runs and pots. It will also have reflected any micro-environmental effects caused by any differences among the RILs/ parental plants grown in the same pots. To the extent that the variability reflected 'real' micro-environmental differences rather than measurement error, the reference plants thus potentially provide an additional measure of the environments to which the RIL lines and their parents were exposed.

6.3.2. Recovery of the G2120 reference plants

As described in Section 6.2.2.1, the criteria for deciding when to re-water differed slightly between the two runs. In run 1, the timing was based on the number of remnant live leaflets on the reference plants whereas in run 2, it was based solely on PAW depletion. Statistically, there was no significant difference ($P > 0.05$) between the mean PAW at re-watering in the two runs (Table 6.4) with means of 2.7% and 3.4% for runs 1 and 2, respectively. In both runs, there was a small number of instances where re-watering was applied too late and the reference plants were not able to recover. The average PAW at re-watering for those cases was 1.5% and 4.8% in the two runs, respectively. Presumably, the fact that some plants were unable to recover at a relatively higher PAW in run 2 was because the plants had grown more vigorously, and had more leaflets and leaf area when the stress became most severe (cf. Figure 6.2b). In run 2, the number of stem nodes was higher, stress was greater and new nodes were fewer.

Table 6.4. ANOVA for water status and traits measured prior to and after re-watering in the G2120 reference plants over two runs

	Traits	M.S.	F	*P*
Water status before re-watering	PAW	26.5	2.9	.089 *ns*
	Last RWC	4119.6	70.3	.000
	Last g_e	.07	21.81	.000
At re-watering	NS1	141.1	130.4	.000
	rLL	239.8	5.6	.019
	rLA	759306.3	26.5	.000
After re-watering	NS2	32.7	11.4	.001
	LL	273.8	1.0	.327 *ns*
	LA	2571118.3	11.9	.001
Recovery	nNS	62.3	30.7	.000
	nLL	4.5	0.0	.854 *ns*
	nLA	442129.1	4.2	.041
	Rc	0.01	0.01	.925 *ns*

NS1/ NS2 = No. of stem nodes before/ after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score

ns = Not significant

M.S. = Mean square; F = F-value, *P* = Probability

Given the variation among the individual G2120 reference plants at the various sample times, and in the two runs, there were inevitably some plant-to-plant differences in plant water status (Last RWC and g_e) and growth immediately prior to re-watering (Table 6.4). These differences were likely to have affected the recovery capacity of individual reference plants following re-watering. Even so, there were no differences between the two runs in average number of leaflets, number of new leaflets and recovery score. In order to examine whether soil water status affected the expression of recovery traits in the G2120 reference plants, the PAW measurement prior to re-watering was adopted as a covariate. The analysis of co-variance over the two runs indicated that the PAW immediately prior to re-watering had no significant effect ($P \geq 0.05$) on the growth parameters (Table 6.5). Thus, there were no average differences between the two runs in PAW just before re-watering (Table 6.4) and no evidence that differences in PAW among pots contributed to pot-to-pot differences in measures of recovery in the G2120 reference plants after re-watering.

Linear correlations of the total number of leaflets and of the new leaflets after re-watering on the number of live leaflets prior to re-watering were statistically significant ($r = 0.82^{**}$ and $r = 0.62^{**}$, respectively) (Table 6.6). Clearly, higher numbers of remnant live leaflets immediately before re-watering resulted in better recovery.

Table 6.5. Analysis of co-variance for traits prior to and after re-watering in the G2120 reference plants over two runs, with the plant available water (PAW, %) prior to re-watering as a covariate

	Traits	M.S.	F	$P_{covariate}$
At re-watering	NS1	2.7	1.5	.218
	rLL	60.4	1.6	.205
	rLA	13322.5	0.5	.495
After re-watering	NS2	7.6	2.5	.115
	LL	44.8	0.2	.668
	LA	52786.8	0.3	.606
Recovery	nNS	1.2	0.6	.434
	nLL	0.5	0.0	.946
	nLA	14690.4	0.2	.693
	Rc	0.08	0.11	.739

NS1/ NS2 = No. of of stem nodes before/ after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No.of leaflets; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score

M.S. = Mean square; F = F-value, *P* = Probability

Table 6.6. Pairwise linear correlation coefficients (*r*) among traits measured before and after re-watering in the G2120 reference plants

	At re-watering			After re-watering			Recovery				Last RWC
	NS1	**rLL**	**rLA**	**NS2**	**LL**	**LA**	**nNS**	**nLL**	**nLA**	**Rc**	
rLL	0.31**										
rLA	0.45**	0.95**									
NS2	0.61**	0.42**	0.48**								
LL	0.21**	0.82**	0.78**	0.54**							
LA	0.32**	0.84**	0.86**	0.56**	0.96**						
nNS	-0.26**	0.33**	0.24**	0.66**	0.51**	0.43**					
nLL	0.12	0.62**	0.59**	0.49**	0.95**	0.89**	0.51**				
nLA	0.21**	0.70**	0.70**	0.52**	0.95**	0.96**	0.47**	0.95**			
Rc	0.05	0.68**	0.64**	0.56**	0.81**	0.80**	0.63**	0.75**	0.78**		
Last RWC	-0.07	-0.14*	-0.18*	0.25**	-0.05	-0.13	0.36**	0.02	-0.08	0.02	
Last g_e	-0.03	-0.16*	-0.16*	0.17*	-0.03	-0.08	0.23**	0.06	-0.04	-0.01	0.65**

*, **: Correlation significant at $P \leq 0.05$ and $P \leq 0.01$ respectively

NS1/ NS2 = No. of stem nodes before/ after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score

The linear regression of total leaf area after re-watering (LA) with remnant live leaf area prior to re-watering (rLA) was stronger (R^2 = 0.74) than that for new leaf area (nLA) after re-watering (R^2 = 0.48) although both were highly statistically significant (Figure 6.5). Clearly, higher remnant live LA resulted in better subsequent recovery with more LA on plants (Table 6.6; Figure 6.5).

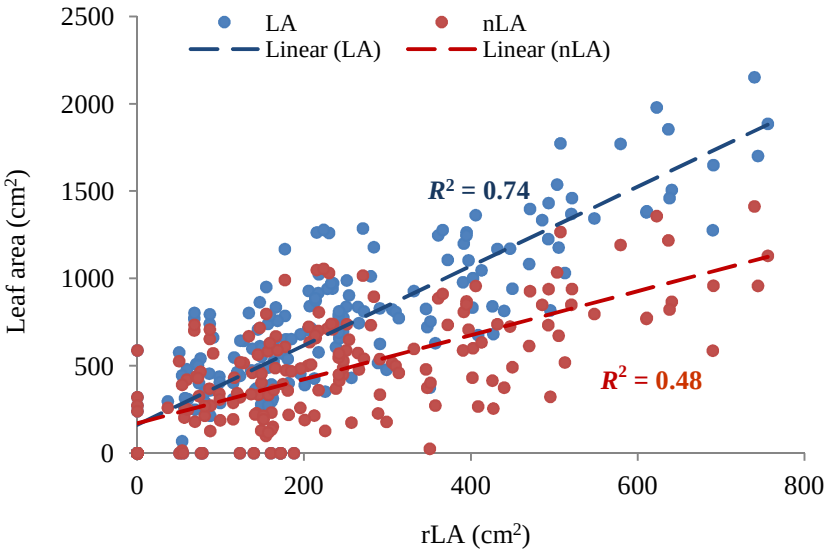


Figure 6.5. Relations indicating there was a strong effect of remnant leaf area before re-watering (rLA, cm^2) on both the leaf area after re-watering (LA, cm^2) and the new leaf area after re-watering (nLA, cm^2).

In general, there were significant positive correlations between the growth in node and leaf traits in the ten days following re-watering and the remnant leaf at re-watering, strongly indicating that those plants with more live leaflets and leaf area at re-watering recovered more strongly. These recovery patterns were broadly consistent with observations on soybean by James *et al.* (2008b) and on G2120 in particular, by Lawn and Likoswe (2008). The correlations between the measures of recovery and the number of stem nodes at re-watering were weaker than those with remnant leaflet numbers and area. Prior to re-watering, there were small but significant ($P < 0.05$) negative correlations between both numbers of live leaflets and remnant leaf area and RWC and g_e (Table 6.6). In part, the fact these relations were negative may indicate that where remnant leaf area was high, it was difficult for plants to maintain high RWC and as RWC declined, so did g_e.

6.3.3. Phenotypic variation in the parental plants and the three soybean RIL populations

6.3.3.1. Relative water content RWC (%) and epidermal conductance g_e (mm/s)

Analysis of variance revealed significant differences ($P < 0.05$) among the three RIL populations and lines within a populations for both RWC and g_e averaged over runs and sample times (Table 6.7a, b). PAW had significant effects on g_e which indicates the effects of stage of water stress on the determination of genotypic variation in g_e (Table 6.7c).

Table 6.7. ANOVA and relative contribution (%) of variation components for relative water content (RWC, %) and epidermal conductance (g_e, mm/s) in the three soybean RIL populations derived from crosses between two oilseed cultivars (CPI 26671 and Valder) and a landrace variety (G2120), averaged over two runs and sampling times♪

Pops = Populations; Pops/Gen = Genotypes within populations

Traits	Sources of variation	d.f	S.S.	M.S.	F	*P*	Relative contribution (%)‡
(a) RWC	Pops	3	5853.5	1951.2	24.1	<.001	1.1
	Pops/Gen	187	50519.0	270.2	3.3	<.001	9.8
	Residual	1266	102643.6	81.1			20.0
	Total	1463	514255.4				
(b) g_e	Pops	3	6.78	2.26	81.0	<.001	6.1
	Pops/Gen	187	11.97	0.06	2.3	<.001	10.8
	Residual	1265	35.30	0.03			31.8
	Total	1462	111.04				
(c) g_e (PAW as a covariate)	Pops	3	6.63	2.21	88.3	<.001	6.0
	Pops/Gen	187	7.09	0.04	1.5	<.001	6.4
	Covariate	1	3.58	3.58	143.1	<.001	3.2
	Residual	1262	31.61	0.03			28.5
	Total	1460	110.86				

♪: Only part of the ANOVA table is presented to illustrate significant differences at the population and plant (genotype) stratums/ levels. See Appendix 6.1 for complete ANOVA table details.

‡: Sums of squares expressed as a percentage of the total SS, including those due to runs, samples times and their interaction (i.e. environmental effects).

df = Degree of freedom, S.S. = Sum of squares, M.S. = Mean square; F = F-value, *P* = Probability

The components of variation due to genetic factors i.e. due to populations and to genotypes within populations were smaller than that due to environment or error (i.e. the residual component) for both RWC and g_e (Table 6.7a, b). In addition, the variations among genotypes within a population were larger than that among the populations (i.e. 9.8% compared with 1.1% for RWC and 10.8% compared with 6.1% for g_e respectively). When PAW was adopted as a covariate for g_e (Table 6.7c), the proportion of variation due to genotypes was reduced from 10.8% to 6.4%, indicating that some of the apparent genotypic effects might be really due to environmental effects on water status.

The mean RWC and g_e of the G2120 reference plants declined significantly at the later sampling times, reflecting PAW depletion (Table 6.3; Figure 6.3) and the effects of stage of water stress on these traits. In an attempt to remove those environmental effects from the determination of genotypic variation in RWC and g_e, the RWC of each tested plant was expressed as a response relative to the RWC of the corresponding reference plant in the same pot (hereafter referred to as relative response RWC, R_RWC) and g_e values were adjusted by adopting PAW as a covariate (hereafter referred as adjusted g_e).

In addition, in light of the exponential relation between g_e and RWC observed in the G2120 reference plants, similar relations were fitted for all the individual RILs and parental plants, using the pooled

data for these two traits over runs and sample times. These relations were then used to estimate g_e at a RWC of 70% (g_{e70}). At that stage, which corresponded to the start of the linear decline in the RWC in the G2120 reference plants (Figure 6.3a), average PAW depletion was around 70% and there already had been considerable acclimation in g_e (Figure 6.4).

The average RWC was higher in Valder than in G2120 and CPI 26671. However, when expressed as response relative to the reference plants, Valder fell between the other two parents (Table 6.8a, b). G2120 tended to have lower g_e values than CPI 26671 and Valder, while Valder was slightly higher than CPI 26671, except g_{e70} (Table 6.8c – e). These findings were broadly consistent with previous studies (Paje *et al.* 1989; James *et al*. 2008a; Lawn and Likoswe 2008). In general, the magnitude of the g_e values was also consistent with previous studies.

In each of the RIL populations, RWC, relative response RWC (R_RWC), g_e and g_{e70} were normally distributed (Figure 6.6) consistent with quantitative inheritance. There was also transgressive segregation, wherein some of the RIL lines felt outside the parental ranges.

RWC means in all three RIL populations were lower than in the parental plants (Table 6.8a; Figure 6.6a), indicating that on average, more RIL lines had lower RWC values than the parents. However, when expressed relative to the RWC of reference plants, the means of R_ RWC fell in the range of the parental means, except for the VG population (Table 6.8b; Figure 6.6b). Similarly, the mean values of g_e, adjusted g_e and g_{e70} for the RILs were generally in the range of the parental means (Table 6.8c – e), except for g_e of CV and g_{e70} of VG (Table 6.8c, e; Figure 6.6c, d). On average, RWC was largest for the VG population, as were the means of g_e, and adjusted g_e. The higher mean values for g_e in the VG population were unexpected given the lower g_e for G2120. However, when adjusted for RWC, the resultant g_{e70} values for VG were closer to expectation based on the parental values.

The ranges observed in the F_6 and F_7 generations for RWC and g_e were broadly comparable to the ranges in the F_2 generation of these same three crosses in the study by James *et al*. (2008c). However, in CG and CV, there were lines with significantly lower g_e than observed in the F_2 generation (g_e of 0.09 and 0.12 mm/s – Table 6.8c).

The CV% was lower for RWC than g_e (13.8% and 47.2% respectively) (Table 6.8), indicating more environmental noise in the g_e determinations. However, adopting PAW as a covariate for g_e seemed not to be a useful adjustment, because the CV% was only marginally improved while the broad sense heritability was lowered (from 0.33 to 0.14) (Table 6.8c).

Table 6.8. Ranges, means, coefficients of variation (CV, %) and broad sense heritability estimates (H^2_b, %) of relative water content (RWC, %), relative response RWC (R_RWC), epidermal conductance (g_e, adjusted g_e and g_{e70}, mm/s) in the parental plants and the three soybean RIL populations derived from crosses between two oilseed cultivars (CPI 26671 and Valder) and a landrace variety (G2120)

Traits		CPI 26671	G2120	Valder	CG	CV	VG	LSD	CV%	H^2_b ± s.e.
a. RWC	Range				48.6 – 75.6	50.2 – 76.4	53.0 – 82.2	1.9	13.8	0.54 ± 0.02
	Mean	71.5	73.0	74.1	63.6	64.2	67.7			
b. R_RWC	Range				0.95 – 1.12	0.92 – 1.17	0.85 – 1.15	0.02	–	–
	Mean	1.05	0.96	1.01	1.04	1.02	1.02			
c. g_e	Range				0.12 – 0.40	0.09 - 0.69	0.24 – 0.59	0.04	47.2	0.33 ± 0.02
	Mean	0.38	0.21	0.45	0.25	0.37	0.41			
d. Adjusted g_e	Range				0.13 – 0.37	0.14 – 0.62	0.24 – 0.56	0.03	44.7	0.14 ± 0.03
	Mean	0.38	0.24	0.42	0.25	0.37	0.42			
e. g_{e70}	Range				0.17 – 0.37	0.20 – 0.56	0.23 – 0.51	–	–	–
	Mean	0.40	0.21	0.32	0.25	0.37	0.33			

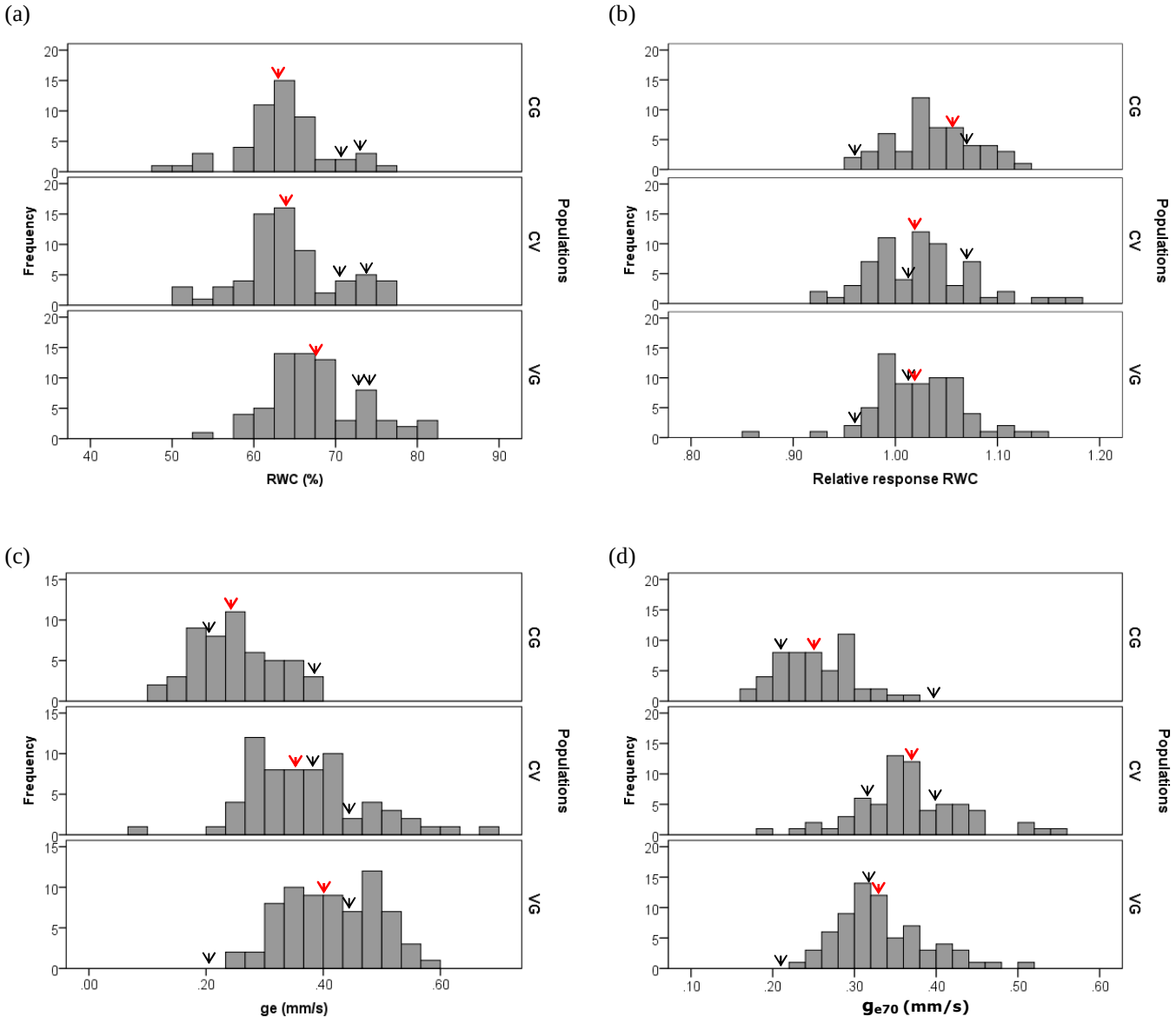


Figure 6.6. Frequency distributions for each of the three soybean RIL populations derived from crosses between two oilseed cultivars (CPI 26671 and Valder) and a landrace variety (G2120) for (a) RWC (%), (b) Relative response RWC, (c) g_e (mm/s) and (d) g_{e70} (mm/s)
(Black arrows indicate parental means; Red arrows indicate population means)

6.3.3.2. Recovery following re-watering

Analysis of variance revealed significant differences ($P < 0.05$) among the RIL populations and among lines within populations for all traits except remnant live leaf area (rLA) prior to re-watering and leaf area (LA) after re-watering (Table 6.9a – j). In all cases, variation components due to genotypes within the population were largest (in the range of 42.2 – 82.9%), followed by components due to environment or error (28.8 – 43.4%) and among populations (1.7 – 8.2%) (Table 6.9). Proportions of variation due to errors in leaf area measurement (rLA, LA and new LA – Table 6.9c, f, i) were nearly as large as those due to genotypes, which probably reflected the fact that leaf area was estimated by regression using leaflet width and length.

In order to remove environmental effects on trait determination, PAW was adopted as a covariate for the recovery score, which was an overall evaluation of plant recovery capacity. There was no significant difference in the recovery score at the population level (Table 6.9k). Although PAW had a statistically significant effect as a covariate, the proportion of additional variation accounted for was small (1.1%). Therefore, the variation components due to populations and genotypes did not change compared to the analysis with no covariate (Table 6.9j).

Table 6.9. ANOVA and relative contribution of variation components for recovery traits in the three soybean RIL populations derived from crosses between two oilseed cultivars (CPI 26671 and Valder) and a landrace variety (G2120) over two runs♪

Pops = Populations; Pops/Gen = Genotypes within populations

Traits	Sources of variation	df	S.S.	M.S.	F	*P*	Relative contribution (%)‡
a. NS1	Pops	3	49.8	16.6	15.1	<.001	6.3
	Pops/Gen	187	333.7	1.8	1.6	<.001	42.2
	Residual	207	227.4	1.1			28.8
	Total	398	790.0				
b. rLL	Pops	3	1056.6	352.2	6.3	<.001	3.8
	Pops/Gen	187	15302.2	81.8	1.5	0.004	54.8
	Residual	207	11634.7	56.2			41.7
	Total	398	27908.8				
c. rLA	Pops	3	1661330	553777	10.4	<.001	6.5
	Pops/Gen	187	12320754	65886	1.2	0.064*ns*	47.9
	Residual	207	10978450	53036			42.7
	Total	398	25732362				
d. NS2	Pops	3	145.6	48.5	19.2	<.001	40.9
	Pops/Gen	187	1026.3	5.5	2.2	<.001	82.9
	Residual	141	355.9	2.5			28.8
	Total	332	1238.1				
e. LL	Pops	3	8258.7	2752.9	9.9	<.001	5.8
	Pops/Gen	187	75420.6	403.3	1.5	0.004	52.7
	Residual	207	57266.6	276.7			40.0
	Total	398	143208.7				
f. LA	Pops	3	9428000	3143000	13.1	<.001	8.2
	Pops/Gen	187	55050000	294400	1.2	0.077*ns*	48.1
	Residual	207	49750000	240300			43.4
	Total	398	114500000				

NS1/ NS2 = No. of of stem nodes before/ after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score

♪: Only part of the ANOVA table is presented to illustrate significant differences at the population and plant genotype levels. See Appendix 6.2 for complete ANOVA table details.

‡: Sums of squares expressed as a percentage of the total SS, including those due to runs, samples times and their interaction (i.e. environmental effects).

ns = Not significant

df = Degree of freedom, S.S. = Sum of squares, M.S. = Mean square; F = F-value, *P* = Probability

Table 6.9. Continued...

Traits	Sources of variation	df	S.S.	M.S.	F	*P*	Relative contribution (%)
g. nNS	Pops	3	18.4	6.1	3.9	0.010	2.3
	Pops/Gen	187	372.1	2.0	1.3	0.049	47.1
	Residual	192	300.4	1.6			38.0
	Total	383	790.1				
h. nLL	Pops	3	3291.7	1097.2	9.8	<.001	5.6
	Pops/Gen	187	28964.3	154.9	1.4	0.011	49.7
	Residual	206	23000.2	111.7			39.5
	Total	397	58290.1				
i. nLA	Pops	3	3428836	1142945	12.8	<.001	8.0
	Pops.Geno	187	21132755	113009	1.3	0.048	49.4
	Residual	206	18357428	89114			42.9
	Total	397	42788038				
j. Rc	Pops	3	10.4	3.5	2.9	0.038	1.9
	Pops.Geno	187	336.1	1.8	1.5	0.003	60.5
	Residual	192	232.6	1.2			41.9
	Total	383	555.9				
k. Rc (PAW as covariate)	Pops	3	9.3	3.1	2.6	0.053*ns*	1.7
	Pops.Geno	187	330.3	1.8	1.5	0.003	59.4
	Covariate	1	6.1	6.1	5.1	0.025	1.1
	Residual	191	226.6	1.2			40.8
	Total	383	555.9				

Overall, Valder had the lowest number of stem nodes, number of remnant live leaflets and remnant live leaf area prior to re-watering. Consequently, Valder showed least capacity to recover (Table 6.10). While those traits were somewhat similar in CPI 26671 and G2120 at re-watering, G2120 still revealed greater recovery capacity with high numbers of nodes and new stem nodes, and the highest number of leaflets and new leaflets, and leaf area and new leaf area (Table 6.10b, c).

In general, discounting the plants that had died and so scored only 1, most of the growth traits in the RILs exhibited continuous or near-normal distributions (Figure 6.7). The mean values for the RILs were generally within the range of the parental means except for the CG population (e.g. Figure 6.7a, d, g). As expected, the CV population had the narrowest ranges and lowest means for all recovery traits. The two populations involving the landrace variety G2120 (CG and VG) broadly exhibited similar ranges and means (Table 6.10).

CV% varied among recovery traits from low (10.1% in NS1) to high (96.4% in rLA) (Table 6.10). High CV% suggested that there were large errors associated with the measurements of the traits, perhaps due to environmental effects. However, as with the adjustment in $g_{e,}$ adopting PAW as a covariate for recovery score seemed not to be useful because the CV% was only marginally improved and broad sense heritability was similarly low (0.2) (Table 6.10c). The broad sense heritability estimates were significantly different from zero but low for all traits (0.11 – 0.37).

Table 6.10. Ranges, means, coefficients of variation (CV, %) and broad sense heritability estimates (H^2_b, %) of traits prior to and after re-watering in the parental plants and the three soybean RIL populations derived from crosses between two oilseed cultivars (CPI 26671 and Valder) and a landrace variety (G2120)

	Traits		CPI 26671	G2120	Valder	CG	CV	VG	LSD	CV%	H^2_b± s.e
(a) At re-watering	NS1	Range				9.0 – 13.5	7.0 – 12.0	7.0 – 13.0	0.46	10.1	0.24 ± 0.07
		Mean	12.0	11.0	9.5	10.7	10.0	10.6			
	rLL	Range				0 – 34.5	0 – 24.0	0 – 27.0	3.3	79.8	0.19 ± 0.07
		Mean	9.0	9.0	1.9	11.7	7.3	9.8			
	rLA	Range				0 – 1181	0 – 464.0	0 – 797	100.3	96.4	0.11 ± 0.07
		Mean	363.0	314.0	37.0	303.4	150.8	263.2			
(b) After re-watering	NS2	Range				5.2 – 16.5	6.2 – 14.2	7.2 – 16.2	0.69	13.2	0.37 ± 0.07
		Mean	14.0	13.0	10.1	12.7	11.3	12.4			
	LL	Range				0 – 75.0	0 – 46.5	0 – 58.5	7.2	71.1	0.19 ± 0.07
		Mean	25.5	34.5	6.0	30.0	17.7	24.2			
	LA	Range				0 – 1853.0	0 – 1149.0	0 – 1701.0	213.5	78.7	0.10 ± 0.07
		Mean	919.0	1210.0	123.0	798.5	408.5	684.3			
(c) Recovery	nNS	Range				0 – 3.5	0 – 3.0	0 – 3.5	0.55	82.4	0.12 ± 0.07
		Mean	2.0	2.0	0.8	1.8	1.3	1.6			
	nLL	Range				0 – 40.5	0 – 28.5	0 – 36.0	4.6	74.1	0.16 ± 0.07
		Mean	16.5	25.5	4.9	18.4	10.6	14.6			
	nLA	Range				0 – 1028.0	0 – 695.0	0 – 1062.0	130.0	76.1	0.12 ± 0.07
		Mean	556.0	896.0	95.0	497.0	260.6	429.0			
	Rc	Range				0.8 – 4.0	0.8 – 4.0	0.8 – 4.0	0.48	39.4	0.19 ± 0.07
		Mean	3.5	3.5	1.8	3.0	2.6	2.8			
	Rc with PAW as covariate	Range				0.8 – 4.0	0.7 – 4.0	0.7 – 4.0	0.48	38.9	0.20 ± 0.07
		Mean	3.6	3.4	1.8	2.6	3.0	2.8			

NS1/ NS2 = No. of stem nodes before/after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score;

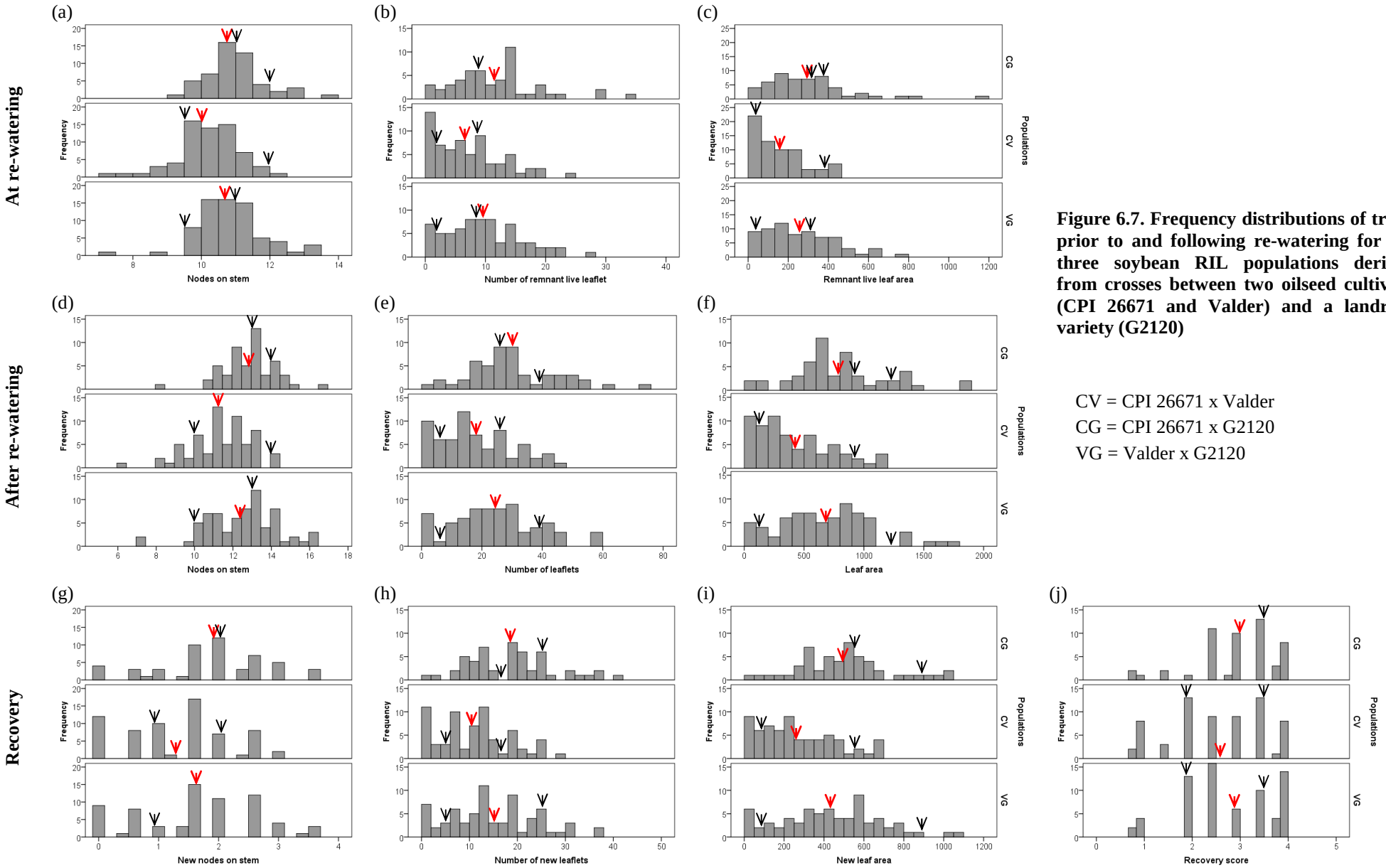


Figure 6.7. Frequency distributions of traits prior to and following re-watering for the three soybean RIL populations derived from crosses between two oilseed cultivars (CPI 26671 and Valder) and a landrace variety (G2120)

CV = CPI 26671 x Valder
CG = CPI 26671 x G2120
VG = Valder x G2120

To summarise, the results of the phenotypic evaluation of the G2120 reference and the parental plants were broadly consistent with previous studies, especially the results reported by James (2004) for RWC and g_e and by Lawn and Likoswe (2008) for recovery after water stress was relieved. As reported by Paje *et al*. (1988), James *et al.* (2008a, b, c) and Abdullah *et al.* (2011), RWC and g_e decreased as water stress increased. The ranking order of G2120, CPI 26671 and Valder for higher RWC and lower g_e was broadly consistent with James *et al*. (2008a). Similar ranges of RWC and g_e were seen in the present studies using F_6 and F_7 RILs as were observed in the parents and the F_2 generation by James *et al*. (2008c). One exception was the g_e values (Table 6.3) which were higher than those observed under well- watered conditions by James *et al*. (2008a). Another was that an exponential rather than a linear relation provided a better fit to the interrelation between RWC and g_e in the G2120 reference plants (Figure 6.4).

Significant differences were observed among the parental plants and the three RIL populations and genotypes within the population in traits prior to re-watering as well as their recovery after re-watering. G2120 retained more live leaflets and leaf area and showed greater recovery (Table 6.10). Generally, recovery in the parental and RIL plants supported the hypothesis of James *et al.* (2008b) and Lawn and Likoswe (2008) that plants with higher leaf survival, as indicated by the retention of live leaf area during the latter stages of water deficit stress, have greater capacity to recover after stress is relieved. Oya *et al*. (2004) also reported that more drought tolerant soybean cultivars maintained larger leaf area during stress periods in the field.

The significant negative correlations between both RWC and g_e with number of remnant live leaflets and live leaf area observed before re-watering in the G2120 reference plants were consistent with James *et al*. (2008b) and Lawn and Likoswe (2008) that leaf survival was enhanced by low g_e (Table 6.6). A number of other studies also showed that species adapted to arid environments tend to have low g_e (Schreiber and Riederer 1996; Heilbsing *et al*. 2000; Riederer and Schreiber 2001), and crop species or varieties with low g_e were often those that survived the longest under severe soil water deficits (Hull *et al*. 1978; Sinclair and Ludlow 1986; Jovanovi *et al*. 1996).

While CV% varied from low to high, depending on the trait, the values for the most important traits (e.g. g_e and recovery responses) were high, indicating lower precision of estimate for these traits. Broad sense heritability estimates were low. The low precisions of estimate and low heritability values presumably reflected large environmental and perhaps G x E effects on the determinations of phenotypic variation in these traits. On the other hand, low heritability could reflect the real small proportion of genetic factors contributing to the phenotypic variation of measured traits (Tables 6.7, 6.9). In addition, PAW effects contributed only small proportions of variation (i.e. 3.2% for g_e and 1.1% for recovery score – Tables 6.7 and 6.9 respectively). Adjustments of g_e values and recovery scores by adopting PAW as a covariate were not useful in reducing the 'noise' around the estimates of genotypic values for these traits. Therefore, the adjusted values were not used in the QTL analyses.

6.3.4. Identification of QTLs associated with drought stress responses in soybean

6.3.4.1. Polymorphic DArT markers and component linkage maps for the three soybean RIL populations

The CG (CPI26671 x G2120) and VG (Valder x G2120) populations which involved the landrace G2120 as a parent had more polymorphic markers than the CV (CPI26671 x Valder) population (Table 6.11), reflecting the fact as a landrace variety, G2120 was genetically more distinct than the two oilseed lines. The level of redundancy was lowest in CG, followed by CV and VG. Despite CG having the highest number of selected polymorphic markers (1272), only 32.2% of its selected markers mapped onto linkage groups (LGs). In total, 845 DArT markers mapped onto the three individual linkage maps, of which 758 (89.7%) were unique. Pairwise population comparisons revealed there were 10 (CV and CG), 26 (CV and VG) and 51 (CG and GV) markers in common between the respective pairs.

Table 6.11. Number of selected polymorphic DArT markers with redundancy and mapping statistics of individual genetic linkage and integrated maps for the three soybean RIL populations derived from crosses between two oilseed cultivars (CPI 26671 and Valder) and a landrace variety (G2120)

Attributes		CG	CV	VG	Integrated map
Selected polymorphic DArT markers					
Totals	No.	1272	482	550	845
Redundancy♪	No.	207	99	170	-
	%	16.3	20.5	30.8	-
Linkage maps					
Mapped markers	No.	409	196	327	759
	%	32.2	40.7	67.6	89.8
Markers with segregation distortion‡	No.	71	54	136	-
	%	17.4	27.6	41.6	-
LGs	No.	22	16	15	27
Number of markers on each LG	Range	4 - 72	4 - 49	5 - 105	4 - 195
	Average	18.6	12.3	14.9	28.1
LG size (cM)	Range	8.1 – 39.3	8.2 – 80.6	8.8 – 48.1	7.8 – 83.6
	Average	19.7	32.3	18.6	28.2
Inter-marker distance (cM)	Range	0.01 – 16.0	0.1 – 26.6	0.01 – 28.9	0.05 – 22.3
	Average	1.3	3.4	2.0	1.5
Total size (cM)		434.2	516.7	409.4	762.2

♪: Redundancy obtained from the "Similarity of loci" calculation in Joinmap 3.0; Markers with similarity in scoring pattern ≥ 98% were considered to be redundant.

‡: Marker segregation ratio different from 1:1 at $P < 0.05$.

Segregation distortion occurred in all three RIL populations, the level ranging from 17.4% to 41.6% and occurred on 7, 12 and 13 LGs in the VG, CV and CG respectively (data not shown). The levels of segregation distortion in the CV and CG were somewhat higher than observed for other marker types

used for soybean genetic linkage maps e.g. 9.6% for SSR and AFLP (Liu *et al*. 2007) and 25% for RFLP (Zhang *et al*. 1997). These observed levels of DArT marker segregation distortion could affect mappability and reduce the number of mapped markers, as the case in CV. In the CV and VG populations, the number of identified LGs was fewer than the chromosome number of soybean (20), whereas with the CG population, there were 22 LGs. However, there were cases where either LGs had very short lengths (< 5 cM) or markers clustered only at the two ends of the LG in all three populations. These LGs then were not selected.

The total sizes of these DArT genetic linkage maps were similar to the size of the very first soybean linkage map (420 cM with 57 markers) (Palmer and Kilen 1987). However, these map sizes were much shorter than subsequent other published maps for soybean, variously based on SSR, AFLP, RFLP and RAPD markers e.g. 1200 cM (Keim 1990), 2276 cM (Song *et al*. 2004), 3312 cM (Du *et al*. 2009) and 2169 cM (Abdel-Haleem 2012). Reasons for the smaller sizes of soybean DArT linkage maps could include the small size of the RIL mapping populations and the genetic similarity of the parental plants.

The individual linkage maps contained broadly similar average numbers of markers per LG within the range of 12.3 – 18.6. Markers were positioned at a sub-cM average inter-marker distance of 1.3 – 3.4 cM, which was less than 10cM and therefore adequate for QTL scanning (Doerge 2002). The large ranges in the numbers of markers per LG, LG size and in inter-marker distance also indicated uneven distribution of DArT markers along the chromosomes, as was observed in mungbean (Chapter 5).

6.3.4.2. Soybean integrated map from pooled datasets

To construct integrated or consensus maps, several different approaches, using various software packages, have been described in the literature (e.g. Stam 1993; Wu *et al*. 2002; Yap *et al*. 2003; de Givry *et al*. 2005; Ronin *et al*. 2012). In this study, preliminary integrated map construction was tested by joining those LGs of the three component maps that shared at least two common markers, using the "Combine Groups for Map Integration" in Joinmap. However, the small number of common markers (10 – 51) over four (CV and CG) to six LGs (CG and VG; CV and VG) resulted in the creation of only seven joined groups. Even when seven joined groups were created, each joined group remained divided into several subset LGs (data not shown). Therefore, an alternative approach was applied whereby component datasets were pooled and missing values were assigned to markers not present in a population (Beavis and Grant 1991). The existence of common parents among the three RIL populations made this pooling possible.

The integrated map contained 759 DArT markers and 27 LGs with an average LG size of 28.2 cM and an average inter-marker distance of 1.5 cM (Table 6.11; Figure 6.8). Although the integrated map expanded to 762.2 cM, the size was still shorter than most published linkage maps for soybean and still did not resolve the 20 LGs that would equate to the diploid chromosome number in soybean.

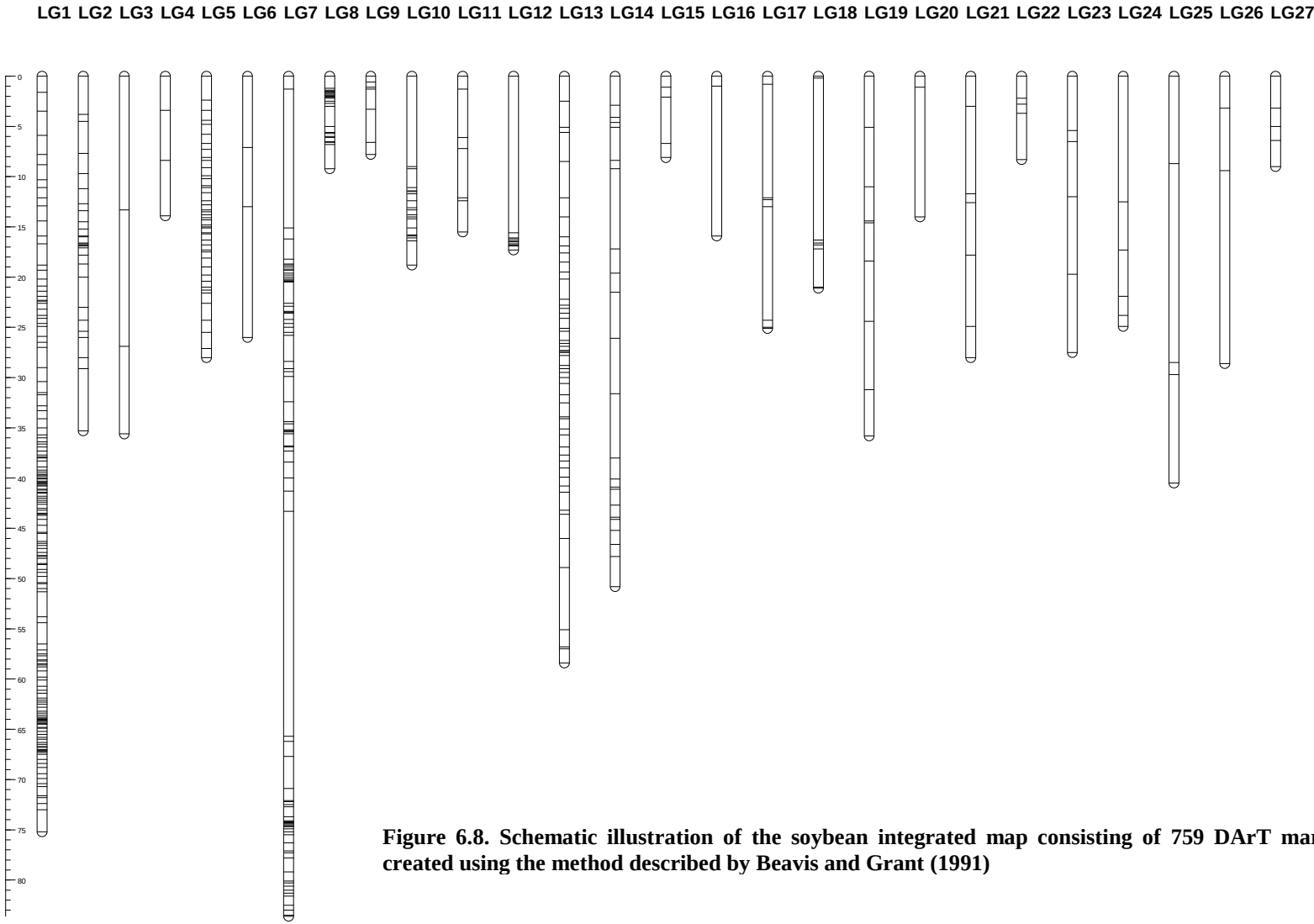


Figure 6.8. Schematic illustration of the soybean integrated map consisting of 759 DArT markers, created using the method described by Beavis and Grant (1991)

Eight LGs (namely, LG1, LG5, LG7, LG9, LG10, LG11, LG13 and LG14) comprised of segments or joins of different groups within individual component maps or from different component maps (Appendix 6.3, 6.4). For example, LG1 of the integrated map consisted of LG6CV of the CV map and 7 LGs (LG1CG – LG7CG) from the CG map (Figure 6.9; Appendix 6.3). Although marker orders on these eight LGs were generally maintained, there were cases of marker re-ordering. For example, most marker orders on LG2CG were in the reverse order on LG1 (Figure 6.9). The remaining groups in the integrated map were simply un-joined groups from the component maps with the locus order well maintained (Appendix 6.3).

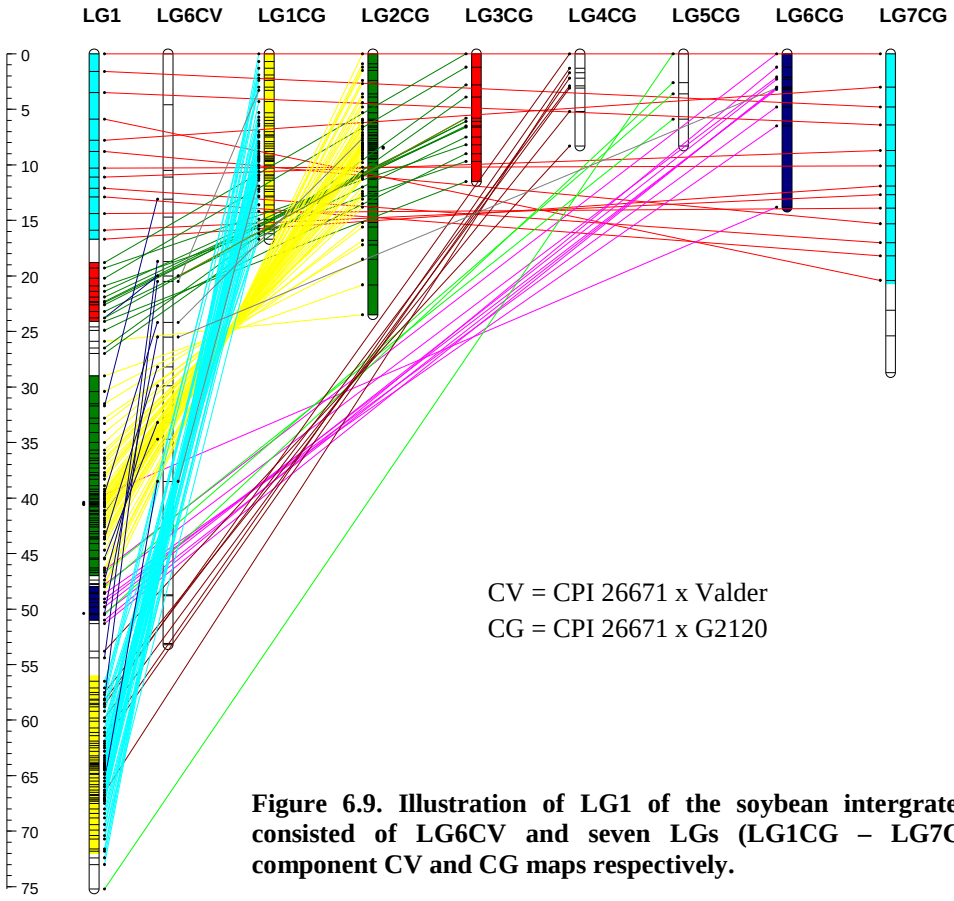


Figure 6.9. Illustration of LG1 of the soybean intergrated map which consisted of LG6CV and seven LGs (LG1CG – LG7CG) from the component CV and CG maps respectively.

6.3.4.3. QTL detection for physiological drought stress response traits in the soybean RIL populations

Similar to the putative QTL detection result in mungbean (Chapter 5), the SML method again was more robust than the ICIM method. While ICIM did not identify any putative QTLs at either a PIN of 0.001 or a relaxed PIN of 0.01, SML detected 6 – 14 putative QTLs for each trait (14 traits) in the CG, CV and VG populations respectively, all with relatively minor effects (PVE < 10%) (Appendix 6.5).

The parental plants CPI 26671 and Valder were known not to possess traits of interests which conditioned drought tolerance (Table 6.1; James 2004). In addition, there was no coincidence between LGs harbouring QTLs in the CV and the other two soybean populations, CG and VG (Appendix 6.6). For example, while QTLs in the CV population were detected on five LGs (LG13, LG14, LG17, LG21 and LG25), those in the VG population were located on more LGs (LG5, LG7, LG8 ,LG11, LG16, LG18, LG23, LG25, LG26 and LG29). The results might suggest that different regions of the genome (maybe via different pathway) affect the measured traits.Nonetheless, DArT markers associated with QTLs detected in the CV were not discussed and selected for sequencing.

The total number of QTLs detected in the CG population was higher than that in the VG population (106 compared with 34) (Appendix 6.6). This was possibly due to the higher number of polymorphic DArT markers selected for CG than for VG (Table 6.11). However, similar numbers of QTLs (18) mapped on the integrated linkage map in both populations (Table 6.12).

Similarly to mungbean (Chapter 5) and other QTL analysis studies (e.g. Du *et al*. 2009; Hossain *et al*. 2010; Negeri *et al*. 2011; Ding *et al*. 2011; Li *et al*. 2012b), different QTLs were detected for the same traits in the CG and VG populations, even though G2120 was involved as a common parent. The fact that different sets of polymorphic DArT markers were selected for each RIL population and used for the SML analysis necessarily dictated that for some traits, different QTLs would be detected for the one trait (Table 6.12; Appendix 6.5). In addition, the main reason for the limited number of congruent QTLs was the very limited number of common loci, only 51, between the CG and VG. The inability to identify sequencing redundancy of DArT markers may also have resulted in an underestimate of the presence of congruent markers between the CG and VG populations.

In both the CG and VG populations, 10 LGs harboured QTLs associated with various traits such as RWC, g_e and recovery ability (Table 6.12). Despite the limited number of congruent QTLs, there was coincidence between CG and VG in that similar LGs contributed to drought tolerance. Five LGs, LG5, LG7, LG8, LG18, LG23 and LG25, were identified in both populations. These common LGs harboured most of the QTLs associated with RWC, R_RWC and growth traits before and following re-watering (e.g. number of stem nodes before and after re-watering, number of remnant live leaflets and remnant live leaflet areas, number of new leaflets). For example, QTLs linked to the number of stem nodes before re-watering were located on LG5 in both CG and VG. Similarly, QTLs conditioning R_RWC were located on LG23. LG7 contributed the highest number of QTLs (5) which mainly conditioned recovery ability. QTLs associated with g_e and g_{e70} were located on different LGs such as LG4 and LG10 in CG and LG7 and LG20 in VG (Table 6.12). There was also a number of QTLs that did not map on the integrated map (Appendix 6.6).

Table 6.12. Distribution on the integrated map of putative QTLs for physiological traits associated with drought stress responses in the two soybean RIL populations involving G2120 as a parent

LG = Linkage group; Pos = QTL position on LG (in cM); No. QTLs = Number of QTLs on the LG

CPI 26671 x G2120 (CG)					Valder x G2120 (VG)				
LG	Pos	Traits	Marker	No. QTLs	LG	Pos	Traits	Marker	No. QTLs
1	71.6 71.8	RWC	mbPb-871580 mbPb-869437	2	**5**	27.1	RWC NS1 NS2	soPb-853611 or soPb-856104	1
3	26.9	RWC	soPb-825841	1					
4	8.4	g_e	mbPb-877452	1		0.0	Rc	soPt-856127	5
5	21.0	NS1 nLL	soPb-853729	1			RWC NS1		
		NS1	soPb-856127	5		34.4 or	rLL	soPb-855130 or soPb-855129	
	0.0	NS2 Rc	or soPt-856127		**7**	34.6 or	rLA NS2	or soPb-853608	
	1.3	NS1	soPb-856183			35.6	LL		
7	37.3	NS2	soPb-825453				LA		
	40.0 or 41.3	NS2	soPb-825761 or soPb-831844				nLA		
						65.7	NS1	soPb-856137	
	74.1 or 74.4	nNS nLL Rc	soPb-854948 or soPb-857058			66.2	RWC	soPb-855445	
						70.9	RWC g_e	soPb-855545	
8	1.8	rLL	soPb-855377	1	**8**	1.2	NS1	soPb-856335	1
10	12.4 16.1 16.4	g_{e70}	soPb-856842 soPb-831699 soPb-853348	3	**11**	6.1 or 7.2	LA nLA	soPt-854224 or soPt-824436	1
					16	0.0	nNS	soPt-855872 or soPt-856479	1
18	16.8	LA nLL nLA	soPt-825083	1	**18**	21.1	NS1 rLL	soPb-825062	1
23	6.5	R_ RWC nLA	soPb-853730	1	**20**	0.0	g_{e70}	soPb-853371	1
						5.4	rLA Rc	soPt-853730	3
25	0.0	R_ RWC	soPb-854858	2	**23**	12.0	R_ RWC	soPb-831556	
	29.7	R_ RWC	soPt-854547 or soPt-856082			19.7	R_ RWC	soPt-854142	
						28.5	rLL	soPb-825425	3
					25	29.7	rLL rLA LL nLL Rc	soPt-854547 or soPt-856082	
						40.5	rLA	soPb-854943	
					26	28.6	nNS nLL	soPb-853334	1
Number of QTLs on the integrated map				**18**					**18**
Total number of detected QTLs				**106**					**34**

RWC = Relative water content (%); R_RWC = Relative response relative water content; g_e = Epidermal conductance (mm/s); g_{e70} = Epidermal conductance (mm/s) at relative water content of 70%;

NS1/ NS2 = No. of stem nodes before/ after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets after re-watering; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score

In the study by James *et al*. (2008c) using F_2 progeny, and in this study using F_6 and F_7 progeny (Figure 6.6), the progeny distributions for both g_e and RWC were consistent with quantitative inheritance, with a high degree of additive gene action. The evidence of many QTLs with minor effects detected by the SML method was consistent with this observation. Similarly, the presence of various minor QTLs associated with g_{e70} supported the observed continuous distribution (Figure 6.6d) and the genotypic variation observed by James *et al*. (2008a).

For other traits related to growth before and following re-watering, QTL detection also indicated additive genetic factors, which again were in agreement with the observed continuous distributions (Figure 6.7). In addition, QTLs detected for the number of remnant live leaflets and remnant live leaf area supported the observations of James *et al*. (2008b) and Lawn and Likowse (2008) that there were genetic factors contributing to leaf area maintenance during stress.

No QTLs were detected by the ICIM – ADD method and this may have been due partly to the relatively narrow genetic diversity among the parental plants for the traits of interest. In addition, the size of the RIL populations (only 50 – 70 RIL lines) may have simply been too small since the ICIM method is much more sensitive to population size than SML (Bedo *et al*. 2008). Consistent with this interpretation, genotypic variation in the trait determinations generally accounted for only a modest amount of the total observed variation in both the stress response traits and the recovery traits. For example, for RWC and g_e, the variation among lines within RILs accounted for only about 10% of the observed variation (expressed as a percentage of total sums of squares – Table 6.7). There were large environmental (E) effects due to runs and sample times, and also presumably G x E effects which would have been confounded in the residual error terms. With the recovery traits (Table 6.9), the genotypic component of variation, while again statistically significant, was modest relative to the large unexplained variation (residual sums of squares). For all traits, estimates of heritability were generally modest to low (Tables 6.8, 6.10). More QTLs may have been uncovered using a larger number of genotypes/ lines/ populations. That said, attempts to screen for genotypic variation for these physiological traits may still have failed because their measurement is sophisticated, laborious and time consuming (e.g. see Ashraf 2010; Sinclair 2011).

6.3.4.4. Sequences of selected DArT markers linked to QTLs associated with drought stress response

Pairwise BLAST comparisons among the sequences of 19 selected DArT markers indicated only two out of 19 were sequencing redundant (99% of identities); these were soPb-825639 and soPb-832207 (hereafter referred to only as soPb-825639) with the same size of 290 bp (Table 6.13) and the same alignment position on chromosome 2 (Chr. 2) (Appendices 6.7, 6.8). In fact, these two good quality markers also had identical scoring and were selected for sequencing to confirm that identical scoring DArT markers would have the same sequences.

Thus, there were 18 DArT markers representing unique DNA sequences. The BLAST search found that all 18 selected DArT markers shared highly significant similarity to one or more reported sequences of the soybean genome reference stored in the NCBI database (E- values were either zero or extremely small, while identities of the best-fitting matches ranged from 91.8% to 100%) (Table 6.13). The BLAST searches indicated that the 18 DArT markers, which included both non-mapped and mapped markers, were aligned to 12 soybean chromosomes. There were cases where markers mapped on the same LGs in the present study (e.g. LG5 and LG7), but were aligned best to different chromosomes based on the selection criteria. For example, markers soPb-853729 and soPb-856104 mapped on LG5 in the present study, but their best matched sequences were on Chr.7 and Chr.15 respectively (Table 6.13). However, a significant match to soPb-853729 was also found on Chr.15 (E-value of 3e-25 and Identities of 88%) (data not shown). Similarly, soPb-855130, soPt-856127 and soPb-855545 mapped on LG7 in the present study but their best alignments were on Chr.1, Chr.15 and Chr.18 respectively. However, two other significant alignments with lower identities, one for soPb-855130 (E-value of 1e-22 and Identities of 86%) and the other for soPb-855545 (E-value of 2e-45 and Identities of 92%) were also on Chr.15 (data not shown). The reason could be large gaps between markers. For example, marker soPb-855130, soPt-856127 and soPb-855545 positioned at 34.4 cM, 0 cM, and 70.9 cM on LG7. Moreover, there are often duplicate genes in plant genomes, and each DArT marker sequence was found to have several matches. However, only the best match was selected.

The BLAST search for DArT markers against genetically mapped sequences found significant matches (E-value $\leq$ -20) in some cases, such as soPt-854142, soPt-856127 or soPt-856479. However, the alignments had very low query coverage (< 13%) (data not shown). This indicated that the 18 selected DArT markers were different from any other marker types registered in the NCBI. In addition, based on alignment position (Appendix 6.8) and comparison with the Soybean Consensus Map 4.0 (Hyten *et al*. 2010b) in the genome sequence browser, the DArT markers were located in regions with various densities of other marker types, such as SSRs or SNPs. Nine out of the 18 selected DArT markers, including soPb-825017 and soPb-853729, were located in between or close to other markers. Thus, although soybean genetic maps are said to be saturated, DArT markers could still be used to fill gaps in both the physical and genetic linkage maps.

Table 6.13. Sequence alignment and chromosome location from BLAST searches of 19 selected DArT markers linked to QTLs for physiological traits associated with drought stress responses in the two soybean RIL populations involving G2120 as a parent

LG = Linkage group; Chr./LG = Soybean chromosome/assigned corresponding linkage group (http://www.soybase.org/sbt/) with which each DArT marker was aligned; App.Pos = Approximate position of DArT markers/QTLs on the Soybean Consensus Map 4.0

Marker	Size (bp)	LG	BLAST search			App. Pos (cM)‡	Traits with which DArT QTLs were associated	Other drought stress response traits for which QTLs were identified on the same chromosome≠	
			Chr./ LG	E-value♪	Identities (%)			Pos (cM)¶	Traits
soPb-855130	446	7	1/D1a	0	95.0	29.5 *n*	rLL, rLA, LA, nLA	50.0 - 53.3	Drought susceptibility index (DSI) (iii)
soPt-856479	437	16		0	99.3	3.4 *n*	nNS		
soPb-825639	290	-	2/D1b	7e-147	100	15.0 *n*	NS1, rLL, rLA, LL, LA, nLL, nLA, Rc	61.4	Slow canopy wilting (iv)
soPb-832207	290	-		9e-146	99.7	15.0 *n*		116.5 - 126.5	RWC (vi)
soPb-825017	449	-	3/N	0	99.6	82.5 *n*	g_{e70}	60.2 - 64.9	Limited hydraulic conductance (v)
soPb-855967	525	-		0	100	21.0 *n*	R_RWC, NS2	66.3 - 73.2	RWC (vi)
soPt-854142	703	23	5/A1	0	99.1	66.0	R_RWC	41.6 - 50.3 or 51.6 - 58.5 18.9 31.9 - 41.6	DSI (iii); Slow canopy wilting (iv); Limited hydraulic conductance (v)
soPt-854224	755	11	6/C2	0	99.1	102.7	LA, nLA	103.3 - 103.9	DSI (iii)
soPt-856082	705	25		0	99.9	17.6 *n*	R_RWC, rLL, rLA, LL, nLL		
soPb-853729	415	5	7/M	0	98.6	113.2	NS1, nLL	103.6 - 125.6	DSI (iii)
soPb-825062	215	18	8/A2	2e-102	98.6	32.9 *n*	NS1, rLL	21.9	Slow canopy wilting (ii)
soPb-856104	551	5	15/E	0	99.6	96.7 *n*	RWC, NS2	-	-
soPt-856127	335	7		1e-169	99.7	20.4 *n*	NS1, NS2, Rc	-	-
soPb-853348	400	10	16/J	2e-161	91.8	33.5 *n*	g_{e70}	10.6 - 12.2 72.5	DSI (iii) Water use efficiency (i)
mbPb-877452	504	4	17/D2	0	94.2	68.3	g_e	22.4 - 46.8 or 56.7 - 62.9	DSI (iii)
soPb-857280	511	-		0	98.2	0.0 *n*	g_e	36.1 or 25.5	Slow canopy wilting (ii, iv)
soPb-855545	555	7	18/G	0	99.8	80.6	RWC, g_e	-	Water use efficiency (i)
soPb-853488	406	-		0	100	109.1 *n*	g_e, g_{e70}, NS2	52.0 - 75.0	RWC (vi)
soPt-854382	344	-	20/I	3e-176	100	101.3 *n*	Rc	55.5 - 66.8	DSI (iii)

RWC = Relative water content (%); R_RWC: Relative response relative water content; g_e = Epidermal conductance (mm/s); g_{e70} = Epidermal conductance (mm/s) at relative water content of 70%; NS1 / NS2 = No. of stem nodes before / after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets after re-watering; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score;

♪: E-value indicates the probability that the match is by chance alone; e is the exponent; ‡ The new/ non-overlapped QTLs were subscripted with *n*; ¶: Position of other QTLs in previous studies on the Soybean Consensus Map 4.0;

≠: Sources: soybean linkage/QTL maps in Soybase (http://soybase.org/); i. Mian *et al.* 1996, 1998a; ii. Charlson *et al*. 2009; iii. Du *et al*. 2009; iv. Abdel-Haleem *et al*. 2012; v. Carpentieri-Pipolo *et al*. 2012; vi. Virginia *et al*. 2012.

When compared with previous studies, five DArT QTLs fell into the 10 cM interval and overlapped with reported QTLs. For example, soPt-854142 linked to R_RWC overlapped with the QTL for drought susceptibility index (Du *et al*. 2009) on Chr.5 (Table 6.13). Similarly, soPt-854224, soPb-853729, mbPb-877452 and soPb-855545 overlapped with QTLs for drought susceptibility index (Du *et al*. 2009) and water use efficiency (Mian *et al*. 1996, 1998a) on Chr.6, Chr.7, Chr.17 and Chr.18 respectively. Thus, of the 18 selected DArT markers linked to QTLs for physiological traits associated with drought stress responses and reported in this study, 13 are not overlapped with any other reported QTLs (Table 6.13). This maye be due to the fact that parents/ populations and traits used in this study are different from previous studies.

However, the BLAST search (Table 6.13) suggested that most of the DArT QTLs for growth traits before and after re-watering (e.g. the number of remnant live leaflets and new leaf area) were located on Chr.1, Chr.2 and Chr.6 while the DArT QTLs governing RWC, g_e and g_{e70} were on Chr.3, Chr.16, Chr.17 and Chr.18. These findings were consistent with some other studies on QTLs associated with drought stress responses in soybean, to the extent that traits have been linked to the same chromosomes as in the present study (e.g. Mian *et al.* 1996, 1998a; Charlson *et al*. 2009; Du *et al*. 2009; Abdel-Haleem *et al*. 2012; Carpentieri-Pipolo *et al*. 2012; Virginia *et al*. 2012). Indeed, most of the chromosomes with which DArT QTLs in the CG and VG populations were associated (Table 6.13) also harboured previously identified QTLs associated with drought response traits. The exception was Chr.15, which in the present study harboured two DArT markers associated with recovery traits, but which has not been found to harbour drought stress response traits in previous studies.

There are also some apparent consistencies between the present study and previous studies at a more detailed level. For example, in the present study, markers for the number of remnant live leaflets and remnant live leaf area were linked with Chr.2 and Chr.8 (Table 6.13). These same chromosomes had been previously linked with slow canopy wilting (Charlson *et al*. 2009; Abdel-Halemm *et al*. 2012). One of the attributes associated with the putative drought tolerance of G2120 was slower wilting (James *et al*. 2008b) which it was agued contributed to improved leaf area maintenance during drought (see also Lawn and Likoswe 20008). Similarly, two QTLs associated with low g_e – which is believed to contribute to enhanced leaf water status during drought (James *et al.* 2008b) – were linked to Chr.17, where previous research indicated a QTL associated with slow canopy wilting (Charlson *et al*. 2009; Abdel-Halemm *et al*. 2012). Both this study and Virginia *et al*. (2012) identified QTLs for RWC on Chr.3 and Chr.7.

CHAPTER 7. GENERAL CONCLUSIONS AND IMPLICATIONS

7.1. Introduction

The broad aim of the work reported in this thesis was to evaluate the utility of Diversity Array Technology (DArT) markers in two crop legume species; mungbean and soybean. DArT technology had not previously been applied to these crops. The subsidiary aim was to evaluate whether DArT markers could be a potentially useful adjunct to traditional methods of genetic improvement in these crops. In particular, the question to be explored was whether DArT markers could be useful for tagging traits in these crops – tagging qualitative and quantitative trait loci (QTLs). Some traits are particularly difficult to select for, either because their expression is environmentally sensitive and / or their measurement is time-consuming and/ or technically difficult. Many physiological traits combine all three attributes, making the use of molecular markers especially attractive. The premise underlying the study was that since DArT markers had been successfully applied in other crop species such as rice (Jaccoud *et al*. 2001), the model plant *Arabidopsis* (Wittenberg *et al*. 2005), Triticale (Tyrka *et al*. 2011) and pigeonpea (Yang *et al*. 2006), DArT should also be applicable for mungbean and soybean.

As noted in previous chapters (Chapters 1, 2, 4, 5 and 6), two genetic populations were chosen for study:

a) In mungbean, four hybrid populations between two cultivated and two wild accessions. The wild germplasm had been identified as sources of potentially interesting traits, including perenniality and a late-flowering trait (Table 4.1). Both of these physiological traits would be easier to manage if molecular markers were available to tag them.

b) In soybean, three hybrid populations between an Indonesian landrace variety and two oilseed varieties. The landrace variety had been identified as a source of low epidermal conductance which enhanced leaf area maintenance during drought stress (Table 6.1). Drought stress response by definition is environmentally sensitive, and traits like relative water content (RWC) and epidermal conductance (g_e) are complex and time-consuming to measure.

Given the structure of these genetic populations, some specific hypotheses were formulated at the start of the study. Due to their greater inherent genetic diversity, more polymorphic DArT markers, or polymorphisms, would be detected in the progeny of cultivated x wild genotypes than in the progeny of cultivated x cultivated genotypes. Thus,

a) more polymorphisms should be detected in the cultivated x wild mungbean progenies than in the cultivated soybean progenies; and
b) within soybean, more polymorphisms should be present in the landrace variety x oilseed cultivar progeny than in the oilseed cultivar x oilseed cultivar progeny

In addition to QTL analysis, the use of DArT markers was explored in a preliminary analysis of genetic diversity among selected *Vigna* species and in the construction of genetic linkage maps in mungbean and soybean.

7.2. Development of DArT for mungbean and soybean and its application to study genetic diversity

DArT was successfully developed for both soybean and mungbean (Chapter 3). Similar to most other plant species that DArT have been developed for, such as barley (Wenzl *et al*. 2004), wheat (Akbari *et al*. 2006), and pigeonpea (Yang *et al*. 2006), two genomic complexity reduction methods, utilizing *Pst*I/*Taq*I and *Pst*I/*BstN*I restriction digests, were selected for DNA clonal library development for soybean and mungbean. Generally, both methods produced good levels of polymorphisms although the *Pst*I/*BstN*I method produced slightly more polymorphisms than *Pst*I/*Taq*I in soybean (Table 3.2). In total, 7680 DArT clones from genomic representations of pooled DNA samples were isolated for each species array (Table 3.3). The proportion of polymorphic DArT markers identified in the soybean (10.4% – 11.8%) and mungbean (22.9%) arrays were both similar to reports on the development of the DArT platform for other species (Xia *et al*. 2005; Yang *et al*. 2006; Risterucci *et al*. 2009; Sansaloni *et al.* 2010; Thudi *et al*. 2011). However, marker redundancy including genetic and sequencing redundancy, can lead to overestimates of numbers of polymorphic markers, as shown in Tinker *et al.* (2009) and Petroli *et al*. (2012).

The level of genetic diversity within the metagenome pool used for generation of the discovery array affects the efficiency of the DArT method (Jaccoud *et al.* 2001; Kilian *et al*. 2005). The full size array of soybean was generated using five individual soybean genotypes and three *Glycine* species. The full size mungbean array was developed using pooled samples of two cultivated and two wild mungbeans and another 19 accessions of *Vigna*. As a result, the polymorphism frequency in the mungbean array was much higher, around twice that in the soybean array (Table 3.3). Even in the initial and smaller arrays where the parental plants of the soybean and mungbean hybrid populations were mainly used, more polymorphisms were revealed in the mungbean array than in the soybean array (Table 3.2.), indicating greater diversity in the mungbean samples.

Similar to other marker types employed for legumes, transferability of DArT markers was also observed between soybean, mungbean and several *Vigna* spp., ranging from 3.1% to 20.2% (Table 3.5). Although these levels of DArT marker transferability were less than the levels reported for other markers in legumes (e.g. Pandian *et al*. 2000; Choi *et al*. 2004; Choudhary *et al.* 2009; Zhao *et al*.

2010), they suggest the potential to use DArT markers for comparative genomic studies. In particular, it indicates the potential to use soybean and mungbean DArT markers for other legume species.

The consistency in genetic relationships among 11 diverse *Vigna* spp. samples (Figure 3.1) revealed by DArT markers with published information on the taxa (Verdcourt 1970; Lawn and Watkinson 2002; Damayanti *et al*. 2010a) suggested the useful and successful application of DArT markers in the genetic diversity study. Because the mungbean array included a much higher number of clones generated from mungbean than from other *Vigna* spp. (5376 clones compared with 768 clones for the other two *Vigna* spp.), the apparent degree of relatedness between the cultivated and wild mungbean was probably more distinguishable than that between accessions of *V. vexillata* and of *V. lanceolata*. This may explain the minor inconsistency whereby the cultivated and wild mungbean were more distant at molecular level but were strongly genetically compatible in terms of cross-breeding whereas accessions of *V. vexillata* and of *V. lanceolata* were less distant based on genetic markers but were less genetically compatible in terms of crossability (Figure 3.1). The use of large numbers of DArT markers should increase the ability to distinguish among species/ samples/ accessions and even closely related cultivars, as found by Akbari *et al*. (2006).

The overall results indicated that for soybean and mungbean, the DArT technique is an effective tool for marker generation in terms of speed and the numbers of markers identified. The transferability of markers between soybean and mungbean indicated that DArT may be useful for comparative genomic studies, while the ability of the mungbean array to discriminate between related *Vigna* taxa suggested that DArT also may be useful for studies of genetic diversity.

7.3. Application of DArT in linkage map construction in mungbean and soybean

As a result of greater polymorphism in the developed array/libraries through the use of wild accessions to create the hybrid populations, the number of polymorphic DArT markers was higher in the mungbean than in the soybean RIL populations in this study. Generally, the number of polymorphic DArT markers identified and mapped in the four mungbean RIL populations (Tables 5.2, 5.3) were around twice those in the three soybean RIL populations (Table 6.11). This was also consistent with the polymorphism levels evaluated during array development and reflected the larger genetic distances between the two cultivated and two wild mungbean accessions, compared with that between the two soybean cultivars and the Indonesian landrace accession. Moreover, the redundancy level in soybean was higher than that in the mungbean populations, perhaps because of duplicated gene features in the soybean genome (Schmutz *et al*. 2010).

DArT marker segregation distortion was observed in both the mungbean and soybean RIL populations. While the levels of segregation distortion in mungbean were in the range observed for other markers in mungbean mapping projects (e.g. Lambrides *et al.* 2000; Humphry *et al*. 2005; Zhao

et al. 2010), those in soybean were somewhat higher than in other studies (e.g. Zhang *et al*. 1997; Liu *et al*. 2007). However, they were still within the reported ranges for other marker types on other species, such as for DArT markers in Triticale mapping (Alheit *et al*. 2011) and for RFLP and SSR markers in *Brassica* (Wang *et al*. 2011b). In addition, more severe segregation distortion occurred in populations involving wild and landrace parents: i.e. segregation distortion levels were more severe in the four mungbean populations than in the three soybean RIL populations, and in the RILs that involved the landrace G2120 as a parent than in the RIL from the two oilseed cultivars. This phenomenon has been reported in other studies in which mapping populations were derived from wide genetic crosses (Paterson *et al*. 1991; Lambrides *et al.* 2000; Wang *et al*. 2012). The high levels of polymorphisms together with severe segregation distortion in the four mungbean RIL populations, even though the RILs were intra-specific crosses, could suggest that the Australian forms of *sublobata* are distinct from cultivated mungbean (Lawn and Cottrell 1988; Rebetzke 1994; Lambrides *et al*. 2000). Moreover, because segregation distortion was lower in the crosses involving ACC 87 than ACC 1, it also suggests that there is diversity among the wild Australian forms. This is consistent with the observations that ACC 1 and ACC 87 are distant genetically (Figure 3.1) and morphologically different (Table 4.1; Nguyen 2011). Phenotypic variation was also reported for other accessions in *V. radiata* spp. *subolata* collected in Australia (Lawn and Cottrell 1988; James *et al*. 1999; Rebetzke and Lawn 2006a, 2006b, 2006c), suggesting diversity within the collection.

While the DArT mungbean linkage maps lengths (Table 5.2) were comparable with published linkage maps (Menancio-Hautea *et al.* 1993; Boutin *et al.* 1995; Lambrides *et al.* 2000; Humphry *et al.* 2002; Zhao *et al.* 2010; Kajonphol *et al.* 2012; Isemura *et al*. 2012), those for soybean (Table 6.11) were shorter. Indeed, the soybean linkage maps were only a quarter to a half the size of previous maps published by Keim (1990), Song *et al*. (2004) and Abdel-Haleem *et al*. (2012) and the Soybean Consensus Map 4.0 (Hyten *et al*. 2010b). The narrow genetic base of the soybean genetic materials utilized for creating the three RIL populations resulting in areas of the genome which are identical by decent, together with the small population sizes, were probably the main reasons for the small number of polymorphic markers and the small linkage map sizes (Kumar 1999; Collard *et al*. 2005). However, the differences in design, size, marker density, as well as technical errors of genotyping using manually performed, low-plex assays which cause significant map expansions could also result in genetic map variation among multiple population maps (Wang *et al*. 2011b). In addition, genomic structural variations (i.e. translocations, inversions and deletions) can contribute to variation observed in lengths, number of LGs and marker orders among multiple population maps (Khan *et al*. 2012), i.e. among the four mungbean F_5 RIL populations, among the three soybean RILs populations or with other populations in previous studies.

The uneven patterns of marker distribution and clustering in different chromosome regions observed with DArT markers in this study have been observed with other marker types. For example, similar occurrences have been observed using RFLP and SSRs in mungbean (Fatokun *et al.*1992; Kajonphol

et al. 2012) and soybean (Cregan *et al*. 1999), and in other species such as wheat and Triticale (Crossa *et al*. 2007; Alheit *et al.* 2011). It has been shown in other crops that map resolution can be improved by the incorporation of DArT markers with other marker types e.g. barley (Wenzl *et al*. 2006) and Triticale (Tyrka *et al*. 2011). Thus, it would be expected that these DArT linkage maps for mungbean and soybean might well be improved, in terms of both their resolution and the identification of LGs corresponding to the respective chromosome numbers, by incorporating other markers that have been used in mungbean and soybean mapping projects e.g. RFLPs (Keim *et al*. 1990; Lambrides *et al.* 2000; Song *et al*. 2004; Humphry *et al*. 2005), and STS and SSRs (Funatski *et al*. 2005; Zhao *et al*. 2010; Kajonphol *et al.* 2012; Isemura *et al*. 2012; Abdel-Haleem *et al*. 2012). This approach would also allow the congruency of marker orders and mapping positions to be compared, but was beyond the scope of this study.

Although Joinmap was used for constructing the individual maps for both mungbean and soybean and for the integrated map in soybean, it is extremely computer intensive and time consuming, especially when large numbers of markers are involved. For instance, the most recent version of Joinmap took three months of computation to construct a consensus map from three individual maps of barley containing a total of 1,800 markers which were divided into seven linkage groups of roughly equal sizes (Wu *et al*. 2008b). Similar approaches applied in the construction of the soybean integrated map in this study were also tested on the four related mungbean RIL populations. The first approach joined LGs in individual/component maps which shared at least two common markers. However these joined LGs remained divided into several subset LGs as observed in soybean. The alternative approach which pooled component DArT datasets worked well in soybean but not in mungbean. A large number of markers (~ 1000 out of 1883 DArT markers at a high LOD cut of 10) were clumped on the very first LG in mungbean (data not shown). The severe segregation distortion in the mungbean RILs could possibly cause extensive re-arrangement of marker orders in the integrated map. Alheit *et al*. (2011) illustrated other possible consequences of segregation distortion which not only affect genetic map distances and ordering of loci, but can even result in complete chromosomes being absent from genetic maps. To this time, there have not been any reports on the construction of a mungbean integrated/ consensus map from more than two hybrid populations. Given that in the present study, 1883 DArT markers were mapped across the four mungbean RIL populations, there should be ample information available to underpin the construction of an integrated mungbean map in the future. However, construction of the mungbean integrated map will need to be done cautiously and to take into account the effects of segregation distortion and genomic structural variation mentioned above.

7.4. Application of DArT to identify putative QTLs associated with physiological traits in mungbean and soybean

Important factors which have been found to affect the success of QTL detection include the population size (i.e. the number of genotypes sampled), the distribution of markers, distances between

consecutive markers along the chromosomes, and the heritability of the trait (Asıˊns 2002; Erickson *et al*. 2004; Tuberosa 2012; Würschum 2012). In the present study, the DArT linkage maps for mungbean and soybean were based on populations with a minimum of 50 individuals, large numbers of polymorphic DArT markers and inter-marker distances averaging less than 10 cM. In broad terms, therefore, the maps met the main requirements for QTL detection (Darvasi *et al*. 1993; Young 1994; Mohan *et al*. 1997; Doerge 2002).

There can be false discovery rates in QTL analysis, as described in *Li et al*. (2012a). Therefore, high significance thresholds, which enhance the likelihood that a real QTL was detected, were applied for both the SML ($P \leq 0.01$) and ICIM methods (PIN of 0.001). In order to enhance the reliability and to not overestimate the effects of putative QTLs for quantitative traits, only QTLs for which PVE ≤ broad sense heritability estimates (H_b^2) were selected. In addition, the precison of QTL detection was confirmed by using the two different statistical methods. As shown in mungbean populations, most ICIM QTLs were also detected by SML.

In mungbean, the RIL populations were generated from parents which were genetically distant and segregated for multiple traits (Chapters 4, 5). As expected, this proved advantageous and allowed QTLs controlling different traits to be located on a single map and therefore putative QTL detection in mungbean was relatively straightforward for both qualitative and quantitative traits. Most of the quantitative traits also had moderate to high broad sense heritability which indicated moderate to large additive genetic effects on the traits. Therefore, not surprisingly, large numbers of QTLs were detected for the vast majority of the evaluated traits in the four populations (Table 5.5). In particular, the levels of QTL congruency across populations were reasonable strong (Tables 5.6, 5.7; Appendix 5.3).

In contrast, the three soybean RIL populations were genetically fairly close and only segregated for a few traits of interest (Chapters 3, 6). As a result, at the same *P*-value ≤ 0.01, the number of putative QTLs identified by the SML method in soybean was much smaller than that in mungbean. There were no QTLs detected by the ICIM method even at relaxed PIN of 0.01 in any of the three soybean populations. Phenotyping under drought conditions for plants in general, and soybean is particular, is challenging in terms of simplifying the evaluation systems and collecting accurate data with minimal experimental ‘noise’. In other words, there is large G x E interaction at the quantitative trait locus level which causes phenotypic plasticity and so inconsistency of QTL effects across environments (Asıˊns 2002; Tuberosa 2012). In comparison to mungbean, there was a very limited number of common DArT markers in pairwise soybean RIL population comparisons, and so a very limited number of congruent QTLs. However, the phenomenon where different QTLs are detected for traits even in genetically related populations is common (e.g. Du *et al*. 2009; Hossain *et al*. 2010; Negeri *et al*. 2011; Ding *et al*. 2011; Li *et al*. 2012b). To the extent that similar LGs contributed to drought tolerance, there was some coincidence in QTL detection in the populations which involved G2120 as a parent (Table 6.12).

In terms of the evaluation of DArT as a marker technology, the approach was effective in both species in generating a large number of polymorphic markers and providing dense linkage maps with close interval marker distances, both of which are key factors increasing the power of QTL detection. There are of course other factors that affect the efficiency of QTL detection. Single approaches are rarely enough to enable a full understanding of the genetic control of traits.

The QTL detection for particular traits and the implications for mungbean and soybean genetic improvement are discussed in more detail in the next two sections.

7.5. Detected QTLs and implications for mungbean genetic improvement

7.5.1. Key traits for which putative QTLs were detected

7.5.1.1. Morphological traits

Many QTLs (hereafter referred to as significant QTLs, with *P*-value ≤ 0.001 for the SML method and PIN of both 0.001 and 0.01 for the ICIM method) associated with both qualitative and quantitative morphological traits were identified in each of the mungbean RIL populations. In general, additive QTL models were suggested for most traits with phenotypic variation explanation (PVE) effects ranging from minor (0.4%) to highly significant (74.4%) (Figure 5.2; Appendices 5.4, 5.7). More often, QTLs had the effect of enhancing the wild type phenotype such as increasing degree of twining and leaflet lobing or decreasing leaflet size and floral standard size.

7.5.1.2. Powdery mildew and thrip resistance

Putative resistance to both powdery mildew and thrips in mungbean appears to be complex, because several additive QTLs from both parental sides were identified in each RIL population (Table 5.9b, c). Generally, most QTLs had the effect of enhancing plant resistance and were donated by the wild parents. Many of these QTLs were not co-located with other QTLs governing wild phenotypes such as prostrate growth habit, twining and seed phenotypes. This raises the possibility of pyramiding different resistance genes to provide wider spectrum resistance in order to develop mungbean varieties resistant to powdery mildew disease and thrip insects.

High genetic variability in reaction to powdery mildew in mungbean landraces was reported by Yohe and Poehlman (1972) and Tickoo *et al.* (1988). Several studies based on phenotypic data have suggested different gene models conferring powdery mildew resistance in mungbean, from single gene action (Khajudparn *et al*. 2007; Nguyen *et al.* 2012) to two genes with both additive and dominant gene actions (Reddy *et al*. 1994; Gawande 2003; Kasettranan 2009; Nguyen *et al*. 2012). At a molecular level, one to three major loci conditioning resistance were identified in earlier studies on mapping powdery mildew resistance (Young *et al.* 1993; Chaitieng *et al*. 2002; Humphry *et al*. 2003).

In contrast to powdery mildew, little is known about resistance to thrips in plants in general and in mungbean in particular. Several studies have reported genetic variability in thrips resistance in cabbage (Stoner *et al*. 1989), cotton (Stanton *et al*. 1992), common bean (Cardona *et al*. 2002) and pepper (Maris *et al*. 2003). Multi-genic resistance mechanisms were suggested with additive, dominance and epistatic gene effects (Omo-Ikerodah *et al*. 2009). Two QTLs associated with thrips resistance have been reported in common bean (Frei *et al*. 2005) and 2, 3, and 6 have been reported in cowpea (Omo-Ikerodah *et al*. 2008; Muchero *et al*. 2010). Although there were some studies screening for thrips resistance of mungbean (Chhabra and Kosoner 1994; Khattak *et al*. 2004b), no other molecular markers appear to be available for thrips resistance in mungbean.

7.5.1.3. Seed appearance traits

Testa colour, hilum colour, lustre and seed coat ridging are visual traits and can be easily screened for selection. However, they are all maternal tissue traits, so that the detection of associated QTLs can still be useful for marker – assisted selection (MAS). Maternally inherited traits require generation advancement before they become evident (Roach and Wulff 1987), making MAS useful since it allows for selection before the seeds are formed, perhaps even before sowing (Mogensen 1996). Most of the seed trait QTLs detected in the present study governed wild seed phenotypes (Table 5.9; Figure 5.2; Appendix 5.5). Further, some of the major QTLs were common among the four populations, indicating greater reliability and consistency for these QTLs. In particular, six QTLs were identified as potentially useful for early screening out of lines that carry undesirable wild seed appearance phenotypes.

7.5.1.4. Pod and seed traits

Pod dehiscence (shattering) prior to harvest is generally an undesirable trait since it is one of the major reasons for loss of seed and low yield in mungbean. Thus breeding for dehiscence – resistant varieties is an important objective in mungbean genetic improvement programs (Fernandez and Shanmugasundaram 1988). In this study, three QTLs linked to pod dehiscence, with the large effect of accelerating pod dehiscence and PVE of 15.6 – 20.7% were detected (Appendix 5.4m). These did not co-localize with undesirable traits, except for twining habit. Since only DArT markers were used, comparison was not possible with other studies where pod shattering QTLs were identified for mungbean (Isemura *et al*. 2012) and for other legumes such as azuki bean (Isemura *et al*. 2007; Kaga *et al.* 2008), rice bean (Isemura *et al.* 2010) and soybean (Luo *et al.* 2012). It is inconclusive whether these three QTLs were either new or specific to mungbean. DArT markers associated with these QTLs could be sequenced and mapped as was done for soybean (Chapter 6). However, to do the mungbean traits in that way was beyond the scope of this thesis.

Hardseededness has been identified as a possible trait in mungbean breeding programs where the aim is to develop weather-resistance (Williams 1989; lmrie *et al*. 1991). Both wild parents showed extreme hardseededness, while the cultivated lines were almost completely soft seeded. The detection

of only 1 – 4 major QTLs for hardseededness (Table 5.10c) was consistent with previous observations that few genes control this trait (Lawn *et al*. 1988b; Williams 1989; Lambrides 1996). A single common QTL that explained 11% to 23% of the hardseededness in field and glasshouse conditions was reported by Humphry *et al.* (2005). In Isemura *et al.* (2012), four QTLs conditioning the loss of seed dormancy or an increase of water absorption were reported. In the present study, three of the four QTLs inherited from the wild parent did not co-locate with undesirable traits, especially with wild type of seed appearance (Appendices 5.5, 5.8f) and as such, provide a potential application for improving weather damage in mungbean.

Domestication of mungbean has resulted in a two- to three-fold increase in pod and seed size. In the present study, seed size ranked in the order Berken > Kiloga > ACC 87 > ACC 1 (Table 4.15d – f). Various QTLs controlling pod size and seed size were detected (Appendix 5.8d, e). Some QTLs related to pod size appeared to be domestication-related, and had major effects on increasing pod length and width. Only four QTLs had a minor effect on increasing seed size. In the Kiloga crosses, some QTLs with the effect of decreasing pod and seed size were co-located on the same LGs, indicating that breeding for larger pod size may also increase seed size.

A number of QTLs linked to pod and seed traits has also been reported in other mungbean studies e.g. Fatokun *et al*. (1992), Humphry *et al*. (2005), Kajonphol *et al*. (2012) and Isemura *et al*. (2012). Unfortunately, direct comparison with those studies was not possible because different types of markers were used. Humphry *et al*. (2005) showed QTLs for hardseededness and seed size/ seed weight were co-localized and that alleles associated with hardseededness and small seed weight were usually inherited together. Consistent with that, in the present study, significant negative phenotypic correlations between hardseededness and seed size (Appendix 4.1) were observed in all four populations. In the 87xK, a QTL with the effect of decreasing seed size was co-localized with a QTL with the effect of enhancing hardseededness on LG3 (Table 5.10c, d). This linkage is likely to make attempts to breed large and hard-seeded mungbean varieties difficult to achieve.

7.5.1.5. Perenniality

While phenotyping in the F_5 generation in both 87xB and 87xK crosses suggested two different gene models for inheritance of perenniality (Table 4.9), the QTL analysis indicated additive QTLs contributing to trait expression (Table 5.9d). These QTLs did not co-localize with QTLs linked to deleterious or undesirable traits. As such, it should be possible for breeders to transfer the trait across into cultivated mungbean backgrounds. Together with the study by Nguyen (2011), the present study is among the first to explore the inheritance of perenniality in mungbean.

7.5.1.6. Phenological traits

In the present study, numerous QTLs, respectively controlling time to flowering, duration of flowering and pod growth and days to maturity were detected across the four mungbean populations

(Appendix 5.6). The co-localization of some of the QTLs was consistent with the significant phenotypic correlations between most of these traits (Table 4.20; Appendix 4.1).

Flowering time is a keystone in plant adaptation (Roux *et al.* 2006) and is an agronomically important trait in many legume species including pea, bean, lentil and chickpea (Wallace *et al.* 1993; Sarker *et al*. 1999; Kumar and van Rheenen 2000). The inheritance of time to flowering is reported to be quantitative and complex (Khattak *et al*. 2004a; Nguyen 2011) and can be influenced by environmental factors especially day length and temperature (Khattak *et al.* 2001; Rehman *et al*. 2010). Various hypotheses on gene action for flowering in mungbean have been suggested, including both additive and non-additive gene action controlling earliness (Rehman *et al*. 2010), or both additive and dominant gene action (Tah 2009). However, there is little information on the genetics of late flowering in mungbean. In the ACC 1 crosses, four major QTLs associated with late flowering were revealed, one of which was common to both crosses. The fact that the ACC 87 crosses also produced a small number of very late flowering genotypes (Table 4.12a) indicated that there are also lateness genes in that accession, but they are likely masked by other genes for earliness. This was supported by the detection of QTLs with the effect of delaying flowering in both ACC 87 populations (Table 5.10a).

Mungbean has not been as intensively studied at the genomic level as other legume crops. As such, the detection of these first QTLs is a start for better understanding of the control of flowering in mungbean. Transferring late flowering from the wild to cultivated mungbean might be useful in mungbean breeding, for example, in broadening latitudinal adaptation, extending the crop duration for higher yield potential or delaying pod maturity until unfavourable weather conditions have ceased (Yeates 1994).

7.5.1.7. Yield related traits

In addition to traits that were significantly correlated with seed yield, such as number of seeds per pod, and total pod clusters (Table 4.20), crop improvement aiming for high yield could be based on selection for yield attributes such as total pod mass, standing dry biomass or HI. In this study, numerous QTLs linking to pod dry mass, seed yield, standing dry biomass and HI were detected (Appendix 5.9). Significant phenotypic correlations were observed between seed yield and standing dry biomass but not between these traits and HI. This was consistent with the co-localization of most of the seed yield and standing dry biomass QTLs on the same linkage groups (Figure 5.7).

7.5.2. Applications of these putative QTLs in mungbean genetic improvement

Measurement of many plant traits, especially quantitatively inherited agronomic traits that are sensitive to environmental effects can be confounded with the effects of the environment, of G x E interaction or even of the evaluator team (Asıˊns 2002). As a result, there can be inconsistencies in the detection of QTLs for particular traits, and in the expression and effects of the QTLs across

environments. For example, different QTLs were identified for agronomic traits in barley across 17 environments (Tinker *et al*. 1996), for seed yield per plant in soybean in different water regimes (Du *et al*. 2009), for leaf blight resistance in maize in two environments (Negeri *et al*. 2011) and for seed quality traits in two related wheat populations across five environments (Li *et al*. 2012b). It has been shown that the effect of the evaluator team may sometimes be as large as that due to G x E interaction *per se*. For example, in a QTL analysis of six traits in tomato in eight different situations, Fulton *et al.* (2000) found the effect of the evaluator team resulted in a mean percentage of disagreement in QTL detection of 52.8% (range of 12.5% for fruit shape to 80% for internal fruit colour), with few common QTLs detected by any two teams at two locations. The lack of consistency in the presence of QTLs and their effects across environments has reduced the interest in QTLs for MAS purposes.

In general, the phenotypic observations in the F_5 generation in the present study on most qualitative traits (e.g. leaflet lobing, growth habit and twining) were broadly consistent with those made in the F_2 generation in the earlier study by Nguyen (2011). The consistency between the two different phenotypic evaluations provides a level of confidence in the repeatability of many of the QTLs detected for mungbean in the present study. For a few of the traits in the present study, however, no measurement was made in the Nguyen (2011) study. For example, traits measured only in the present study included pigmentation of the hypocotyl, stem, petiole and leaf rachis, hair colour and density, and putative resistance to thrip infestation. The QTLs for these traits may therefore be less reliable than for those where similar observations were made by different evaluators.

QTLs identified in this study that may be potentially useful in mungbean genetic improvement include those linked to powdery mildew and thrips resistance, seed appearance traits, perenniality, pod dehiscence, hardseedeedness, pod and seed size, late flowering and dry biomass. The application or transfer of these genes from the wild across into cultivated mungbean backgrounds will be more straightforward for those QTLs that are not located in regions of the genome where severe distortion in favour of cultivated alleles was detected, have large PVE and are not co-localized with undesirable traits. For the putative resistances to powdery mildew and thrips, the resilience of the traits needs to be re-checked in different environments and over years. Also as mentioned above, breeding for large and hard-seeded mungbean could still be challenging as many of the detected QTLs for enhancing hardseededness were co-localized with QTLs decreasing seed size.

Before attempts are made to use these QTLs, it would be desirable that further studies be undertaken in order to replicate/ verify the observations made in this study. As noted in Chapter 2, there are many factors influencing the detection of QTLs segregating in a population. The main factors include the genetic properties of QTLs that control traits, environmental effects, population size and experimental error. Thus, ideally, QTL studies should be independently confirmed or verified (Collard *et al*. 2005; Semagn *et al*. 2006b) before attempts are made to exploit them in a plant breeding program. QTL verification is defined as the repeated detection of the same marker alleles at a similar position on the genetic map of a chromosome, or of a QTL controlling a trait under more than one set of experimental

conditions. While numerous QTLs have been identified from many studies targetting a wide range of traits in diverse crop species, only a few QTLs have been confirmed in multiple studies. Examples include QTLs associated with root-knot nematode and bud blight resistance in soybean (Li *et al*. 2001; Fasoula *et al*. 2003), crown rot resistance in wheat (Collard *et al*. 2006), root rot resistance in snap bean (Navarro *et al*. 2008) and reduced aflatoxin in maize (Mayfield *et al*. 2011).

Thus testing, validation and possible further development of identified markers are usually required before they can be used in MAS (Collard *et al*. 2005). These tests require high resolution QTL mapping, determination of the target phenotype in independent populations and different genetic backgrounds and/ or marker conversion to other marker types. It is also suggested that markers that are tightly linked to no more than three QTLs can be used and that all QTLs selected for MAS should be stable across environments (Tanksley 1993; Ribaut and Betran 1999; Hittalmani *et al*. 2002). These constraints often limit the effective adoption of markers for MAS. Hence, while there is cause for "cautious optimism" that the many mungbean QTLs detected in this study may be potentially useful for mungbean improvement, their use for MAS remains uncertain until the requisite verification, testing and validation has been undertaken.

Despite this, QTL analysis is very useful in the genetic dissection of both qualitative and quantitative traits. The old paradigm is to screen and measure individual plants for a defined character recognizable in the phenotype. However, this conventional approach only works well when one or few genes control traits of interest, i.e. qualitative traits. As previously noted in Chapter 5, there were some discrepancies between the numbers of genes suggested by the Mendelian segregation ratio approach and the numbers of QTLs for qualitative traits such as twining, pod dehiscence and perenniality. Most traits important to agriculture such as yield, seed quality, and flowering time are conditioned not by a single gene but by many genes. QTL analysis not only dissects the number of genes governing the trait but also reveals the effects of the loci (Tanksley and McCouch 1997). Often, the effects of loci are not equal and a substantial portion of the genetic variation in a population can be explained by a few QTLs of moderately large effect. For example, while many QTLs conditioning perenniality were identified in each ACC 87 cross, only one or two had large effects on the trait (Table 5.9d; Appendix 5.2.). Similarly, among the most significant four and six QTLs linked to seed yield in the Kiloga crosses, three additive QTLs explained most of the variation (Table 5.10d).

It is sometimes assumed that a high yielding genotype possesses most of the genes for high yield and the low yielding type has little or nothing to offer and likewise for disease resistance. In fact, although a high yielding or resistant hybrid line often contains a great number of positive contributing alleles, some loci from the inferior parent are always found to contribute to superior alleles (deVicente and Tanksley 1993; Xiao *et al*. 1996; Isemura *et al*. 2010; Isemura *et al*. 2012). This was also the case for some traits in this study where QTLs from both cultivated and wild mungbean parents were identified as contributing to trait expression. For example, QTLs associated with powdery mildew and thrips reaction and perenniality were donated by both parental sides. For the late flowering trait, although

ACC 87 and cultivated mungbean are known not to possess late flowering genes based on phenotypic evaluation, a few progeny lines in the ACC 87 crosses were late flowering and QTL analysis detected locations with effects of delaying flowering time (Table 5.10a). This implies that using phenotypic evaluation to determine the breeding value of parental plants/accessions is likely to be misleading, especially with quantitative traits (Tanksley and McCouch 1997). The phenotype of wild germplasm could be a poor predictor of its genetic potential and contribution to domesticated-related traits such as high yield and high nutritional seed quality, until molecular mapping and QTL analysis are applied to identify genes.

Due to some of the limitations that have emerged with the QTL approach, breeders have increasingly moved towards Genomic Selection (GS) which uses genome-wide molecular markers to predict breeding values and make selections of individuals or breeding lines prior to phenotyping (Meuwissen *et al*.2001; Heffner *et al*. 2009; Lorenz *et al*. 2011). DArT technology, as a cheap, whole genome profile method, was the first that was used in GS for plant species as different as wheat (Charmet and Storlie 2012; Poland *et al*. 2012) and *Eucalyptus* (Resende *et al*. 2012).

7.6. Detected QTLs and implications for improving drought tolerance in soybean

As mentioned in many reports, abiotic stress tolerance in general, and drought tolerance in particular, are complex quantitative traits controlled by many minor genes (polygenes) that have additive effects in their expression (Manavalan *et al*. 2009; Fleury *et al*. 2010; Ashraf 2010; Mir *et al*. 2012; Tuberosa 2012). To address the complexity of plant responses to drought, it is vital to understand both the physiological and genetic basis of this response.

This study on soybean responses to drought stress focused on two water status traits (relative water content RWC and epidermal conductance g_e) and recovery traits. The inclusion of the G2120 genotype as a reference plant in each pot illustrated how environmental/experimental 'noise' can affect determinations of genotypic variation in traits (Tables 6.4, 6.5; Figure 6.2). RWC can vary with plant age and habitat (Barrs 1968); likewise for g_e although it is more reliable and stable than some drought stress response traits (Paje *et al*. 1988; James 2004; Hufstetler *et al*. 2007). The relative contributions of environment or error to sources of variation in water status traits (RWC and g_e) and recovery traits were higher or nearly as large as those due to respective genetic factors (Tables 6.7, 6.9). As a result, coefficients of variation were generally high and broad sense heritability estimates were generally low for most traits (Tables 6.8, 6.10). Low heritabilities were also reported for traits governing crop performance under drought conditions in other studies (e.g. Painawadee *et al*. 2009; Charlson *et al*. 2009; Vikram *et al*. 2011; Merewitz *et al*. 2012). Low heritability is one of the obstacles which reduce the capacity to dissect the genetic basis and effectiveness of phenotypic selection (Tuberosa 2012). In addition, the narrow genetic basis or close genetic distances among parental plants (Table 3.4) could limit the genetic variation observed in the soybean RIL populations.

This in turn could also constrain the QTL detection for physiological traits associated with response to and recovery from water stress in these three populations.

Nonetheless, the use of three RIL populations with shared common parents, a large number of polymorphic DArT markers selected for RIL populations (482 – 1272) and an appropriate QTL analysis method (SML) largely overcame those obstacles. A number of minor QTLs detected for each trait indicates the consistency with several genetic studies in which drought tolerance has been found to be a complex quantitative trait and controlled by a large number of minor genes/ QTLs (Fleury *et al*. 2010; Ravi *et al*. 2011; Fukao and Xiong 2013). Although different QTLs were identified for the same trait in the three soybean mapping populations, five LGs harbouring QTLs were found to be common to both the populations that involved the landrace G2120 as a parent (Table 6.12).

No reports have been found of studies on QTLs associated with g_e or with leaf area maintenance under drought stress in soybean. Some studies have identified up to ten QTLs for various traits related to "drought tolerance" in soybean, such as yield (Spetch *et al*. 2001), water use efficiency (Mian *et al*. 1996, 1998a), wilting (Charlson *et al*. 2009; Du *et al*. 2009; Abdel-Haleem *et al*. 2012), leaf pubescence density, wilting coefficient and excised leaf drying (Du *et al*. 2009) and RWC (Virginia *et al*. 2012). Those reported QTLs explained 4 – 27% of the phenotypic variation (Du *et al*. 2009; Abdel-Haleem *et al*. 2012 and see the review by Mir *et al*. 2012). The most recent study by Virginia *et al*. (2012) identified four QTLs linked to RWC with a very small contribution to phenotypic variation explanation (0.01 – 0.18%). In other crops such as rice, wheat, barley, sorghum, maize and tomato, a large number of studies have been conducted to identify QTLs linked to important drought responses traits including yield, flowering time, root traits, chlorophyll, RWC, osmotic adjustment and stay green (see reviews by Cattivelli *et al*. 2008; Fleury *et al*. 2010; Mir *et al.* 2012). Sequence analysis of selected DArT markers linked to QTLs conditioning relative water content, epidermal conductance and recovery traits in this study indicated matches on 12 out of 20 chromosomes of soybean (Table 6.13). Notably, these chromosomes, except Chr. 15, were also reported to habour QTLs associated with other drought stress response traits in previous soybean studies.

Future studies aiming to identify QTLs associated with drought tolerance, such as RWC, g_e and recovery traits in soybean and perhaps in other crops need to try to sample a wide range of genetic diversity i.e. use more wild accessions and landraces like G2120 which will provide valuable opportunities to enhance the variability for drought-adaptive features and, eventually, yield and avoid uninformative crosses like oilseed x oilseed cultivars (Collard *et al*. 2005; Tuberosa 2012). The environmental conditions need to be carefully controlled so that drought stress conditions can be reliably re-created over different runs/replicates; likewise so that G x E can be reduced. It is also important to take into account and measure physical variables such as pot weight, amount of soils and precise amount of water to each pot (Passioura 2006, 2012). RIL population sizes need to be as large as the demands of measurement allow.

The practical deployment in breeding of QTLs linked to drought tolerance responses, in soybean in particular and in other crops, is challenging. Only a few reports are available on introgressing drought tolerance in plants such as rice (Shen *et al*. 2001; Steele *et al*. 2006, 2007, 2013), maize (Ribaut and Ragot 2007), pearl millet (Serraj *et al*. 2005) and soybean (Chen *et al*. 2007b). Despite a large amount of information on QTLs linked to various drought resistance traits, not many of them have been routinely used in MAS. The relatively low success in manipulating QTLs is due to a number of factors: i.e. the precision of QTL detection; pyramiding several QTLs requires unmanageable population sizes; inconsistency of QTL detection due to large G × E interaction (Swamy and Kumar 2013) and even in some cases, QTLs from a specific genetic background do not show significant effects or cease completely in different backgrounds (Ashraf 2010; Mir *et al*. 2012). Also, in order to use physiological traits and QTLs in breeding programs, in addition to the extent of genetic variation, heritability and G x E, their relative genetic correlations with and effects on yield in target environments are also important and need to be evaluated (Sinclair 2011; Deikman *et al*. 2012; Mir *et al*. 2012).

In this study, the evidence of QTLs with minor effects indicated additive genes governed expression of some physiological traits associated with drought stress response in soybean. As discussed by Blum (2011), drought resistance is labelled a "complex trait" mainly when it is viewed from the genomics platforms with the discovery of hundreds or thousands of genes and/ or too many small effect QTLs. However, it can appear much simpler from a physiological and agronomic crop perspective, with success achieved in field research by genetic and crop management solutions (Welcker *et al*. 2006; Munns and Richards 2007; Blum 2009). A number of options are also available to utilize effectively information collected through phenotypic evaluation of germplasm resources (Richards *et al*. 2007; Tuberosa *et al*. 2011). In fact, a well-informed choice of parental lines based on thorough phenotypic characterization of the traits imparting drought resistance allows for the creation of new populations where segregated lines combining drought adaptive and other desirable features can be identified and selected effectively (Reynolds *et al*. 2005). Thus, the complexity of breeding for drought tolerance in soybean can be broken down into more specific and simpler objectives such as selection for low g_e, low to moderate critical RWC, strong capacity for leaf survival (James *et al*. 2008c; Lawn and Likoswe 2008). QTLs associated with these traits in this study are potentially useful in MAS. The improvement of drought tolerance in soybean therefore still needs a combination of conventional breeding and marker-assisted breeding (Ku *et al*. 2013).

Although the observed QTLs had relatively small or minor effects on total phenotypic variation, their contribution to changes/ improvement in drought tolerance could still possibly be significant or even substantial. For instance, four QTLs explaining between 5 and 30% of the variation of root morphology traits under drought stress were introgressed into an Indian upland rice variety (Steele *et al*. 2006, 2007). As a result, the grain yield of near isogenic lines (NILs) significantly increased by 0.4 – 1 tonne/ha, depending on number of introgressed QTLs (1 – 4) (Steele *et al*. 2013). In addition, the

increase in grain yield was also highest in lines introgressed with the highest number of QTLs (4). Similarly, QTLs associated with drought tolerance-related traits were successfully introgressed into cotton (7 QTLs) (Levi *et al*. 2009) and common bean (9 QTLs) (Schneider *et al*. 1997). In cotton, while introgression of QTLs for higher yield had poor success, a considerable number of NILs introgressed with QTLs for physiological traits exhibited the expected phenotype. This highlights the need to manipulate simultaneously and pyramid several QTLs/genes to achieve a significant impact (Cattivelli *et al*. 2008). In soybean, since major QTLs for domestication-related traits were present on only six of the 20 chromosomes and were not clustered (Liu *et al*. 2007), introgressions of useful genes from the wild or landraces to cultivated soybean can be relatively easily carried out without obstacles. Moreover, the advantage of DArT technology in which large number of markers and lines can be simultaneously genotyped allows identifying effectively and quickly lines introgressed with numerous and various QTLs. These together will make utilization of QTLs in this study for MAS a promising approach.

7.7. Conclusions

The present study is the first to develop and apply DArT markers in mungbean and soybean and the first to use DArT to identify QTLs associated with physiological traits in wild mungbean and drought tolerance in soybean. Overall, DArT development was successful for both species with more polymorphism in the mungbean than in the soybean libraries. The genetic relations among 11 *Vigna* spp samples based on DArT markers were broadly consistent with the available taxonomic information. DArT application in linkage map construction seemed to be more effective and successful in mungbean than in soybean, with map sizes comparable to published maps. Linkage maps constructed from DArT markers had close inter-marker distances which were very suitable for QTL scanning. DArT technology, with its advantages in terms of speed of marker discovery and analysis and high-throughput, therefore has great potential for use in constructing high-resolution genetic maps in mungbean and soybean in combination with other marker systems.

Various QTLs were identified for several traits in mungbean with major effects, especially for qualitative traits. In contrast, minor main-effect QTLs were detected for physiological traits associated with drought tolerance in soybean. The greater success with mungbean relative to soybean was due to the greater genetic variation for the traits of interest in the study populations. In addition, the traits conferring drought tolerance are by nature complex and confounded by large G x E interactions. The small soybean population sizes in the study also limited the power of the QTL scanning. Clearly, the successful detection of QTLs associated with drought stress response traits requires (i). populations with useful variation in the traits of interest; (ii). techniques for accurately and reliably measuring genotypic differences in the traits of interest; and (iii). larger test populations than used in this study.

Together with Nguyen's study (2011), this study has provided improved understanding of the expression and inheritance of a range of wild physiological traits in mungbean at genetic and

molecular levels. It also confirmed two Australian wild mungbean accessions to be part of the primary gene pool of cultivated mungbean and a potential source of useful genetic variation for mungbean improvement. Although most wild traits would be undesirable, some wild traits are potentially useful such as resistance to powdery mildew and thrips, late flowering, hardseededness and perenniality. A number of candidate QTLs/ markers have been identified for many mungbean traits that have the potential to save time and resources, and so contribute to more effective and efficient breeding programs.

This is also the first study to detect QTLs for epidermal conductance, leaf area maintenance and drought recovery traits in soybean. While the individual effects of the QTLs were small, the discoveries confirmed there was a genetic basis to the observed variation. The findings therefore offer hope that by drawing on a broader range of genotypic diversity, as well as perhaps by pyramiding several QTLs, advances in drought tolerance improvement in soybean may be possible.

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APPENDICES

LIST OF APPENDICES

Appendix 4.1. Pairwise phenotypic correlations among a subset of quantitative traits observed in the four mungbean F_5 RIL populations derived from crosses between two cultivars and two wild accessions (a) ACC 1 x Berken; (b) ACC 1 x Kiloga; (c) ACC 87 x Berken; (d) ACC 87 x Kiloga. Entries are the linear correlation co-efficients (*r*) between the respective trait pairs.

$^{*, **}$ Indicate significant correlations at $P < 0.05$, $P < 0.01$ respectively; LL = Leaflet length; LW = Leaflet width; PeL = Petiole length; STd ; Stem diameter; IL = Internode length; FloS = Floral standard width; SL = Stem length; BL = Branch length; PdL = Peduncle length; BpP = No. of branches per plant; LoS = No. of leaves on stem; NoS = No. of nodes on stem; N1P = Node of 1st pod; PP = No. of pods per peduncle; PC = Total pod clusters; SP = No. of seeds per pod; PL = Pod length; PW = Pod width; SS = Seed size (100 seed weight), HS = Hardseededness; Flo = Time to flowering; DFlo = Duration of flowering; PG = Pod growth duration; Mat = Growth duration; DPM = Dry pod mass; SY = Seed yield; DM = Standing dry biomass; HI = Harvest index

(a) ACC 1 x Berken

	LL	LW	PeL	STd	IL	FloS	SL	BL	PdL	BpP	LoS	NoS	NoB	N1P	PP	PC	SP	PL	PW	SS	HS	Flo	DFlo	PG	Mat	DPM	SY	DM
LW	.65**																											
PeL	.38**	.47**																										
STd	.29**	.26*	.51**																									
IL	.07	.16	-.08	-.35**																								
FloS	.13	.40**	.03	-.14	.44**																							
SL	-.04	-.18	-.05	-.01	.09	-.10																						
BL	-.03	-.26	.04	.18	-.24*	-.35**	.81**																					
PdL	.06	.32**	.11	-.12	.38**	.40**	-.02	-.20																				
BpP	.09	-.19	.13	.48**	-.66**	-.58**	.05	.43**	-.58**																			
LoS	-.04	-.23*	.20	.48**	-.57**	-.54**	-.04	.30**	-.37**	.76**																		
NoS	.21	.02	.34**	.58**	-.49**	-.45**	.08	.30**	-.37**	.67**	.72**																	
NoB	.02	-.27*	.15	.50**	-.62**	-.64**	.03	.42**	-.50**	.92**	.86**	.73**																
N1P	.07	-.12	.32**	.62**	-.65**	-.49**	.00	.38**	-.52**	.81**	.81**	.74**	.87**															
PP	.31**	.45**	.22*	.21	.15	.23*	-.19	-.28*	.21	-.17	-.30**	.04	-.22*	-.12														
PC	-.06	-.37**	.05	.34**	-.54**	-.66**	.13	.45**	-.49**	.83**	.69**	.58**	.87**	.68**	-.23*													
SP	-.06	-.13	.14	.15	-.21	-.21	.11	.25*	.03	.19	.25*	.07	.21	.24*	-.38**	.14												
PL	.25*	.38**	.39**	.27*	.02	.31**	-.27*	-.21	.07	.00	.04	.04	-.04	.09	-.02	-.16	.30**											
PW	.36**	.57**	.38**	.30**	.23*	.47**	-.25*	-.36**	.20	-.21	-.20	.03	-.25*	-.05	.25*	-.38**	-.14	.65**										
SS	.35**	.51**	.36**	.20	.21	.43**	-.36**	-.49**	.12	-.25*	-.20	.03	-.27*	-.11	.22*	-.35**	-.23*	.67**	.81**									
HS	-.13	-.27*	-.35**	-.34**	-.07	-.27*	.31**	.31**	-.36**	.14	.01	-.04	.11	.03	-.28*	.21	-.03	-.26*	-.29**	-.36**								
Flo	-.03	-.23*	.23*	.54**	-.59**	-.52**	.00	.40**	-.44**	.73**	.81**	.67**	.84**	.94**	-.19	.62**	.27*	.03	-.11	-.19	.04							
DFlo	-.15	-.42**	-.12	.11	-.41**	-.61**	.20	.47**	-.29**	.53**	.54**	.43**	.62**	.47**	-.29**	.62**	.14	-.27**	-.44**	-.48**	.23*	.55**						
PG	.02	-.15	.18	.51**	-.39**	-.38**	-.14	.19	-.40**	.59**	.69**	.52**	.69**	.75**	-.14	.53**	.10	.19	.10	.11	.01	.79**	.39**					
Mat	.23*	-.03	.17	.39**	-.36**	-.41**	.15	.35**	-.25*	.61**	.50**	.48**	.56**	.54**	-.10	.52**	.26*	.09	-.10	-.18	.01	.48**	.37**	.37**				
DPM	.28**	.11	.40**	.57**	-.52**	-.42**	.10	.32**	-.21	.62**	.50**	.64**	.59**	.59**	.04	.62**	.30**	.16	-.06	-.09	-.02	.48**	.31**	.30**	.63**			
SY	.31**	.14	.40**	.57**	-.51**	-.39**	.07	.28*	-.20	.59**	.47**	.64**	.57**	.56**	.07	.60**	.28**	.18	-.02	-.03	-.03	.44**	.27*	.28*	.61**	.99**		
DM	-.01	-.29**	.19	.49**	-.48**	-.58**	.05	.44**	-.35**	.77**	.81*	.64**	.90**	.81**	-.18	.73**	.22*	-.02	-.21	-.24*	.02	.86**	.60**	.76**	.46**	.45**	.42**	
HI	.10	.33**	-.08	-.28*	.34**	.47**	-.18	-.51**	.26*	-.56**	-.62**	-.40**	-.68**	-.64**	.28**	-.47**	-.19	.17	.33**	.43**	-.10	-.75**	-.55**	-.53**	-.35**	-.16	-.10	-.81**

(b) ACC 1 x Kiloga

	LL	LW	PeL	STd	IL	FloS	SL	BL	PdL	BpP	LoS	NoS	NoB	N1P	PP	PC	SP	PL	PW	SS	HS	Flo	DFlo	PG	Mat	DPM	SY	DM
LW	.72**																											
PeL	.47**	.53**																										
STd	.30**	.19*	.49**																									
IL	.08	.15	-.11	-.38**																								
FloS	.14	.27**	-.07	-.35**	.39**																							
SL	.18	.03	.08	.17	.15	-.06																						
BL	.01	-.09	.15	.32**	-.16	-.26**	.81**																					
PdL	.23*	.33**	.12	-.31**	.49**	.49**	.06	-.13																				
BpP	.02	-.06	.28**	.55**	-.68**	-.58**	.12	.37**	-.60**																			
LoS	-.06	-.13	.28**	.59**	-.64**	-.63**	.06	.35**	-.54**	.82**																		
NoS	.12	.04	.29**	.58**	-.50**	-.40**	.30**	.43**	-.43**	.70**	.75**																	
NoB	-.08	-.15	.22*	.56**	-.65**	-.62**	.12	.42**	-.59**	.91**	.89**	.77**																
N1P	-.04	-.10	.27**	.63**	-.64**	-.62**	.13	.46**	-.67**	.85**	.87**	.76**	.90**															
PP	.16	.07	.03	-.15	.03	.00	-.01	-.13	.06	.08	-.08	.13	-.05	-.06														
PC	-.16	-.23*	.18	.51**	-.56**	-.61**	.13	.39**	-.58**	.83**	.86**	.74**	.93**	.84**	-.03													
SP	.14	.15	.21*	.14	.07	-.08	-.26**	-.13	-.12	.04	.08	-.03	.05	.09	-.25*	.07												
PL	.33**	.49**	.38**	.16	.02	.37**	-.31**	-.31**	.11	-.05	-.04	-.01	-.14	-.07	-.11	-.16	.36**											
PW	.43**	.50**	.29**	.11	.09	.39**	-.27**	-.34**	.29**	-.21*	-.21*	-.16	-.28**	-.26**	.05	-.31**	.07	.68**										
SS	.46**	.50**	.36**	.19*	.07	.29**	-.32**	-.37**	.24*	-.14	-.11	-.14	-.21*	-.16	.03	-.28**	.07	.67**	.85**									
HS	-.31**	-.30**	-.25*	-.26**	.19*	-.07	.06	.10	-.01	-.21*	-.18*	-.18	-.14	-.12	-.17	-.07	.01	-.18	-.35**	-.43**								
Flo	-.10	-.19*	.18	.61**	-.58**	-.66**	.14	.45**	-.64**	.80**	.88**	.68**	.88**	.94**	-.12	.81**	.10	-.15	-.32**	-.23*	-.06							
DFlo	-.03	-.14	.13	.35**	-.31**	-.44**	.28**	.41**	-.32**	.49**	.59**	.57**	.61**	.59**	-.25*	.66**	.03	-.21*	-.31**	-.36**	.04	.61**						
PG	-.05	-.18	.13	.55**	-.46**	-.55**	.06	.28**	-.48**	.70**	.80**	.62**	.78**	.82**	-.07	.76**	.01	-.08	-.16	-.11	-.14	.88**	.59**					
Mat	.17	-.01	.13	.35**	-.32**	-.44**	.14	.34**	-.20*	.48**	.49**	.48**	.56**	.50**	.02	.55**	.15	.06	-.11	-.09	.05	.52**	.42**	.46**				
DPM	.04	-.02	.29**	.47**	-.55**	-.50**	.11	.35**	-.35**	.75**	.79**	.65**	.83**	.68**	.06	.81**	.08	-.06	-.14	-.04	-.23*	.66**	.55**	.58**	.57**			
SY	.08	.01	.32**	.47**	-.54**	-.47**	.07	.30**	-.35**	.74**	.77**	.65**	.81**	.66**	.05	.78**	.13	.00	-.07	.03	-.25*	.63**	.52**	.55**	.56**	.99**		
DM	-.09	-.17	.20*	.54**	-.48**	-.61**	.204*	.50**	-.51**	.70**	.79**	.68**	.83**	.85**	-.12	.88**	.06	-.13	-.28**	-.22*	-.05	.84**	.71**	.82**	.53**	.69**	.66**	
HI	.27**	.30**	.00	-.28**	.23*	.45**	-.46**	-.60**	.26**	-.37**	-.43**	-.40**	-.45**	-.53**	.16	-.50**	.14	.37**	.50**	.53**	-.18	-.59**	-.55**	-.55**	-.33**	-.22*	-.14	-.65**

(c) ACC 87 x Berken

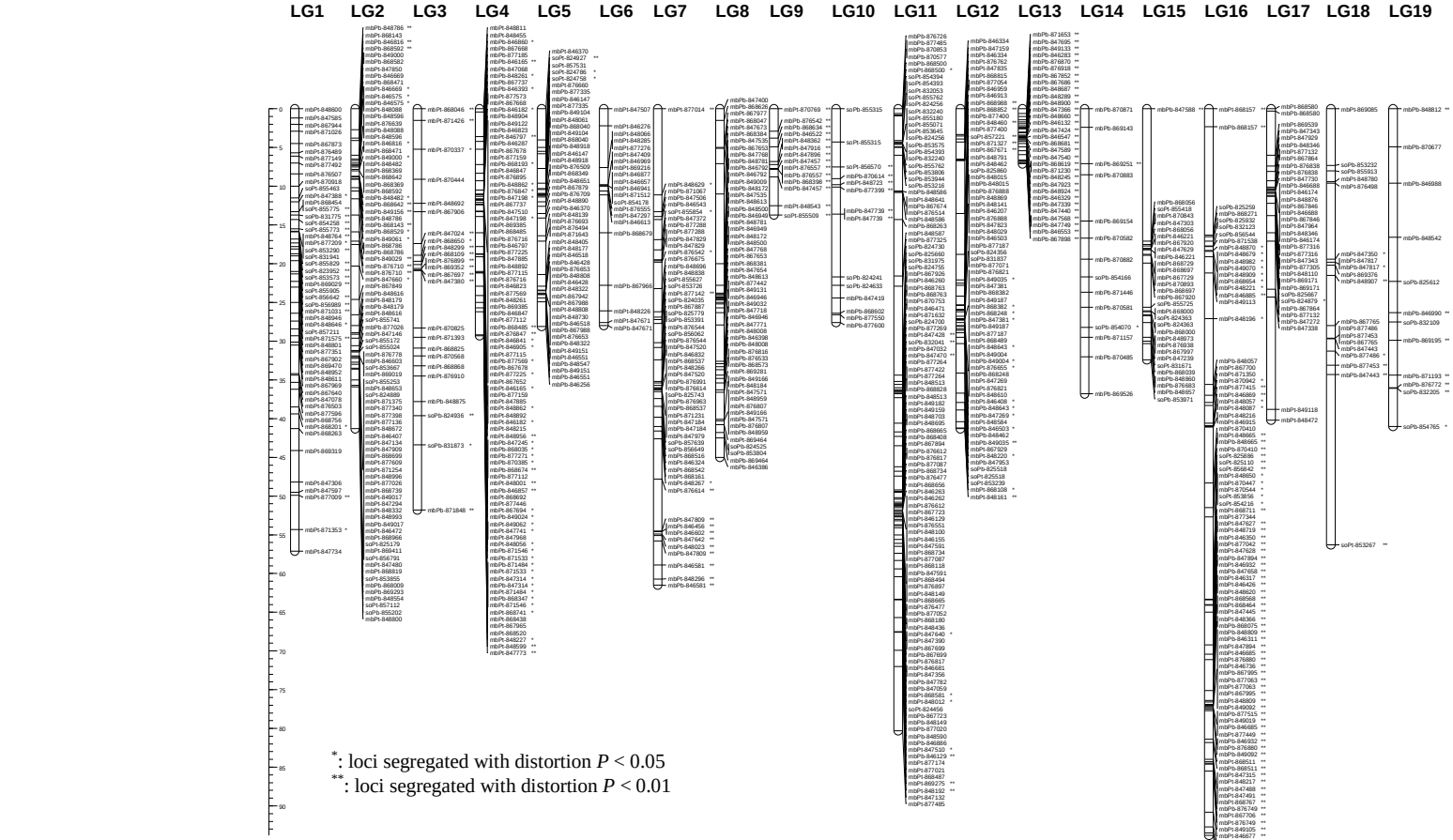
	LL	LW	PeL	STd	IL	FloS	SL	BL	PdL	BpP	LoS	NoS	NoB	N1P	PP	PC	SP	PL	PW	SS	HS	Flo	DFlo	PG	Mat	DPM	SY	DM
LW	.75**																											
PeL	.65**	.59**																										
STd	.52**	.43**	.68**																									
IL	.30**	.32**	.21*	-.08																								
FloS	-.19*	.04	-.11	-.30**	.15																							
SL	.40**	.27**	.52**	.42**	.21*	-.02																						
BL	.39**	.15	.56**	.58**	-.07	-.22*	.82**																					
PdL	.36**	.32**	.39**	.23*	.16	.08	.29**	.24*																				
BpP	.44**	.32**	.51**	.60**	-.18*	-.36**	.51**	.73**	.15																			
LoS	.44**	.27**	.49**	.67**	-.28**	-.32**	.40**	.63**	.19*	.79**																		
NoS	.46**	.29**	.60**	.65**	-.21*	-.37**	.56**	.71**	.27**	.77**	.74**																	
NoB	.43**	.25*	.54**	.72**	-.26**	-.44**	.47**	.74**	.16	.90**	.85**	.82**																
N1P	.41**	.22*	.46**	.67**	-.35**	-.32**	.40**	.64**	.05	.77**	.76**	.76**	.83**															
PP	.36**	.42**	.34**	.32**	-.16	-.15	.13	.20*	.26*	.46**	.43**	.38**	.39**	.31**															
PC	.46**	.30**	.48**	.68**	-.22*	-.45**	.42**	.68**	.14	.86**	.84**	.79**	.95**	.78**	.42**														
SP	.39**	.42**	.35**	.25*	.04	-.05	.31**	.32**	.15	.44**	.36**	.42**	.37**	.40**	.34**	.31**													
PL	.45**	.55**	.53**	.35**	.16	.07	.24*	.15	.14	.35**	.33**	.34**	.29**	.38**	.23*	.24*	.59**												
PW	.25*	.26**	.34**	.27**	.37**	.06	.09	-.07	.06	-.02	.01	.06	.00	.01	-.27**	-.03	-.07	.39**											
SS	.19*	.21*	.29**	.30**	.22*	.01	-.07	-.17	-.01	.06	.06	.03	.08	.04	-.15	.06	-.25*	.40**	.75**										
HS	-.09	-.13	-.06	-.21*	.00	.26**	.07	.12	-.08	.07	.00	.03	.00	-.07	-.01	.01	.15	-.19*	-.24*	-.37**									
Flo	.25*	.07	.24*	.55**	-.39**	-.24*	.30**	.55**	-.05	.61**	.61**	.63**	.71**	.86**	.19*	.66**	.35**	.27**	-.01	.00	-.10								
DFlo	.29**	.16	.35**	.43**	-.20*	-.40**	.27**	.45**	.05	.53**	.50**	.59**	.61**	.49**	.27**	.61**	.23*	.16	-.09	-.03	.13	.30**							
PG	.14	.03	.03	.39**	-.31**	-.30**	.05	.20*	-.19*	.29**	.37**	.41**	.45**	.50**	.01	.46**	.07	.17	.21*	.25*	-.15	.64**	.18						
Mat	.53**	.50**	.51**	.52**	-.03	-.27**	.49**	.55**	.18	.72**	.58**	.63**	.67**	.47**	.47**	.71**	.41**	.40**	.04	.13	-.01	.30**	.49**	.17					
DPM	.54**	.35**	.60**	.77**	-.14	-.49**	.41**	.65**	.19*	.80**	.84**	.79**	.93**	.78**	.40**	.93**	.34**	.34**	.11	.19*	-.15	.68**	.55**	.47**	.68**				
SY	.53**	.35**	.59**	.77**	-.14	-.48**	.39**	.63**	.18	.79**	.84**	.78**	.93**	.78**	.40**	.93**	.34**	.35**	.13	.21*	-.15	.68**	.53**	.49**	.67**	.99**			
DM	.34**	.18	.44**	.74**	-.23*	-.44**	.30**	.58**	.09	.69**	.74**	.71**	.88**	.78**	.26**	.87**	.22*	.17	.07	.13	-.13	.76**	.50**	.59**	.44**	.89**	.89**		
HI	-.23*	-.19*	-.17	-.28**	.14	.02	-.30**	-.42**	-.03	-.13	-.15	-.14	-.19*	-.43**	.04	-.17	-.05	-.05	.21*	.21*	.18	-.50**	-.09	-.19*	-.05	-.17	-.14	-.26**	

(d) ACC 87 x Kiloga

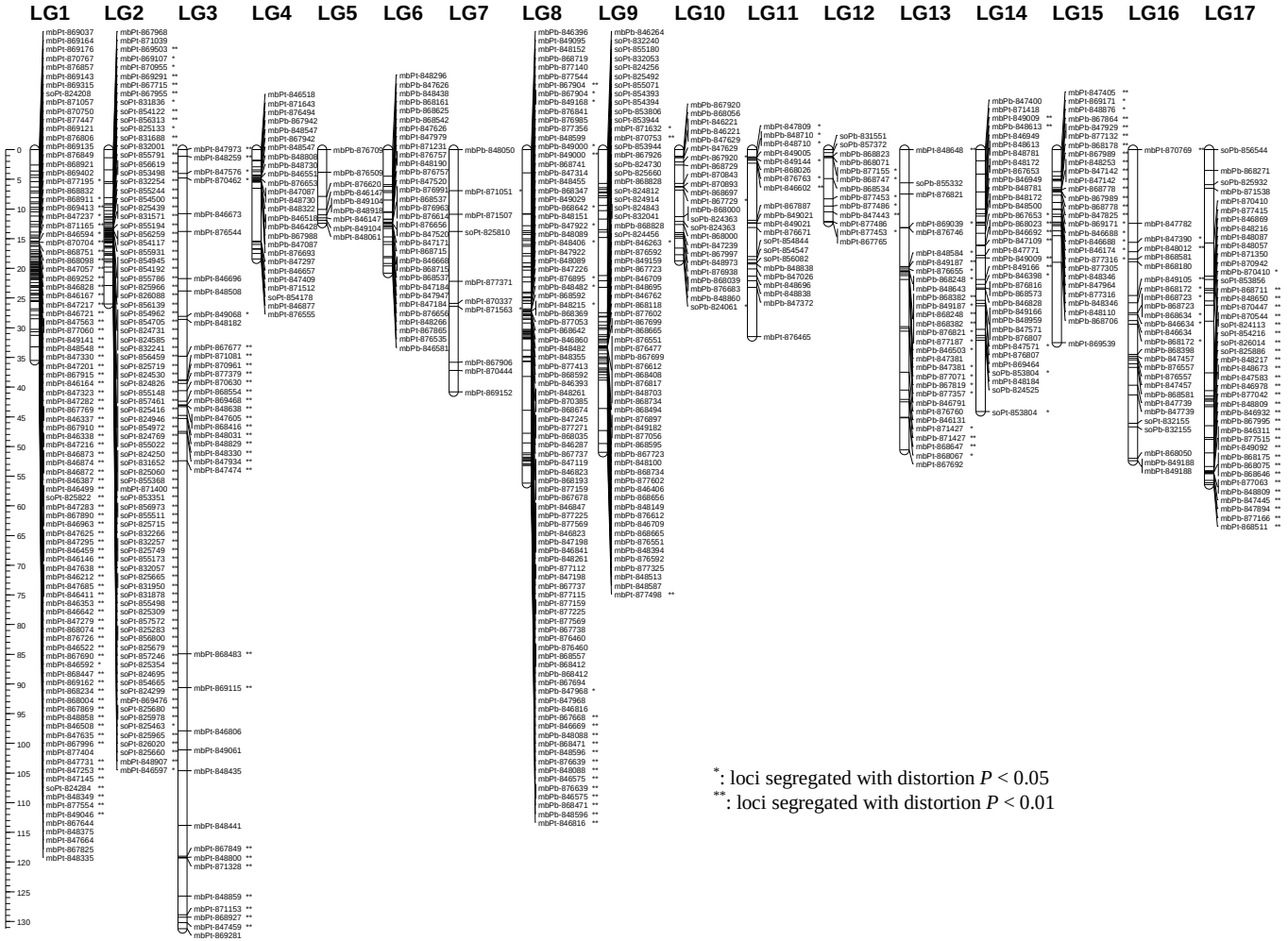
	LL	LW	PeL	STd	IL	FloS	SL	BL	PdL	BpP	LoS	NoS	NoB	N1P	PP	PC	SP	PL	PW	SS	HS	Flo	DFlo	PG	Mat	DPM	SY	DM
LW	.80**																											
PeL	.64**	.67**																										
STd	.55**	.47**	.51**																									
IL	.15	.14	.05	.01																								
FloS	.32**	.43**	.31**	.18	.13																							
SL	.43**	.35**	.36**	.32**	.18	.07																						
BL	.51**	.31**	.29**	.25*	.10	.12	.79**																					
PdL	.45**	.45**	.43**	.19*	.12	.25*	.31**	.19																				
BpP	.50**	.42**	.40**	.42**	-.05	.00	.55**	.56**	.21*																			
LoS	.38**	.35**	.47**	.47**	-.20*	.05	.31**	.26*	.17	.57**																		
NoS	.51**	.53**	.55**	.53**	-.03	.24*	.59**	.44**	.21*	.70**	.52**																	
NoB	.46**	.34**	.41**	.38**	-.07	-.08	.58**	.66**	.23*	.90**	.55**	.68**																
N1P	.46**	.49**	.56**	.54**	-.12	.36**	.57**	.49**	.18	.66**	.51**	.71**	.59**															
PP	.46**	.51**	.54**	.37**	-.05	.13	.33**	.21*	.39**	.42**	.41**	.54**	.34**	.43**														
PC	.41**	.32**	.35**	.30**	-.06	-.05	.49**	.53**	.11	.84**	.55**	.65**	.90**	.43**	.37**													
SP	.37**	.26**	.32**	.17	.08	.17	.36**	.48**	.36**	.49**	.35**	.29**	.46**	.40**	.34**	.37**												
PL	.40**	.39**	.49**	.43**	.10	.34**	.33**	.26*	.27**	.40**	.31**	.40**	.29**	.57**	.32**	.16	.61**											
PW	.23*	.26**	.30**	.40**	-.04	.27**	.04	-.06	.10	-.03	.14	.17	-.08	.30**	.12	-.16	-.07	.43**										
SS	-.04	.12	.14	.21*	-.02	.22*	-.18	-.31**	-.18	-.29**	-.01	.04	-.32**	.12	-.08	-.31**	-.32**	.32**	.70**									
HS	.25*	.16	.07	-.02	.18	.21*	.38**	.53**	.12	.33**	.05	.28**	.35**	.22*	.22*	.33**	.41**	.09	-.29**	-.47**								
Flo	.28**	.26**	.31**	.38**	-.20*	.27**	.44**	.43**	.08	.49**	.33**	.43**	.45**	.78**	.22*	.26**	.35**	.43**	.16	-.09	.12							
DFlo	.41**	.20*	.13	.23*	.09	.05	.40**	.50**	.24*	.44**	.17	.34**	.48**	.10	.26**	.50**	.26*	-.01	-.14	-.36**	.52**	.01						
PG	-.23*	-.22*	-.17	.05	-.12	-.15	-.08	.01	-.30**	-.14	.00	.03	-.11	-.10	-.17	-.09	-.14	.07	.10	.25*	-.19*	-.02	-.01					
Mat	.58**	.42**	.31**	.36**	.11	.09	.44**	.46**	.37**	.64**	.48**	.46**	.63**	.34**	.44**	.67**	.50**	.30**	-.02	-.30**	.31**	.25*	.50**	-.20*				
DPM	.57**	.44**	.47**	.44**	-.11	.04	.58**	.63**	.31**	.77**	.61**	.65**	.81**	.56**	.50**	.78**	.51**	.40**	.07	-.19*	.31**	.39**	.44**	-.13	.76**			
SY	.54**	.43**	.48**	.43**	-.11	.05	.56**	.59**	.29**	.77**	.63**	.65**	.81**	.56**	.51**	.79**	.50**	.41**	.09	-.16	.29**	.37**	.41**	-.14	.75**	.99**		
DM	.54**	.38**	.460*	.54**	-.08	.15	.62**	.68**	.35**	.68**	.55**	.66**	.76**	.65**	.39**	.61**	.49**	.44**	.14	-.14	.26**	.62**	.46**	.10	.61**	.82**	.79**	
HI	-.04	.10	.03	.07	.07	.02	-.09	-.23*	-.08	.08	.13	.18	.06	.01	.21*	.18	.05	.18	.06	.28**	.03	-.29**	-.05	.00	.02	.08	.14	-.17

Appendix 5.1. Three individual genetic linkage maps of mungbean derived from F_5 RILs of cultivars and wild mungbean crosses (a) ACC 1 x Kiloga; (b) ACC 87 x Berken; (c) ACC 87 x Kiloga. A centiMorgan scale is on the left.

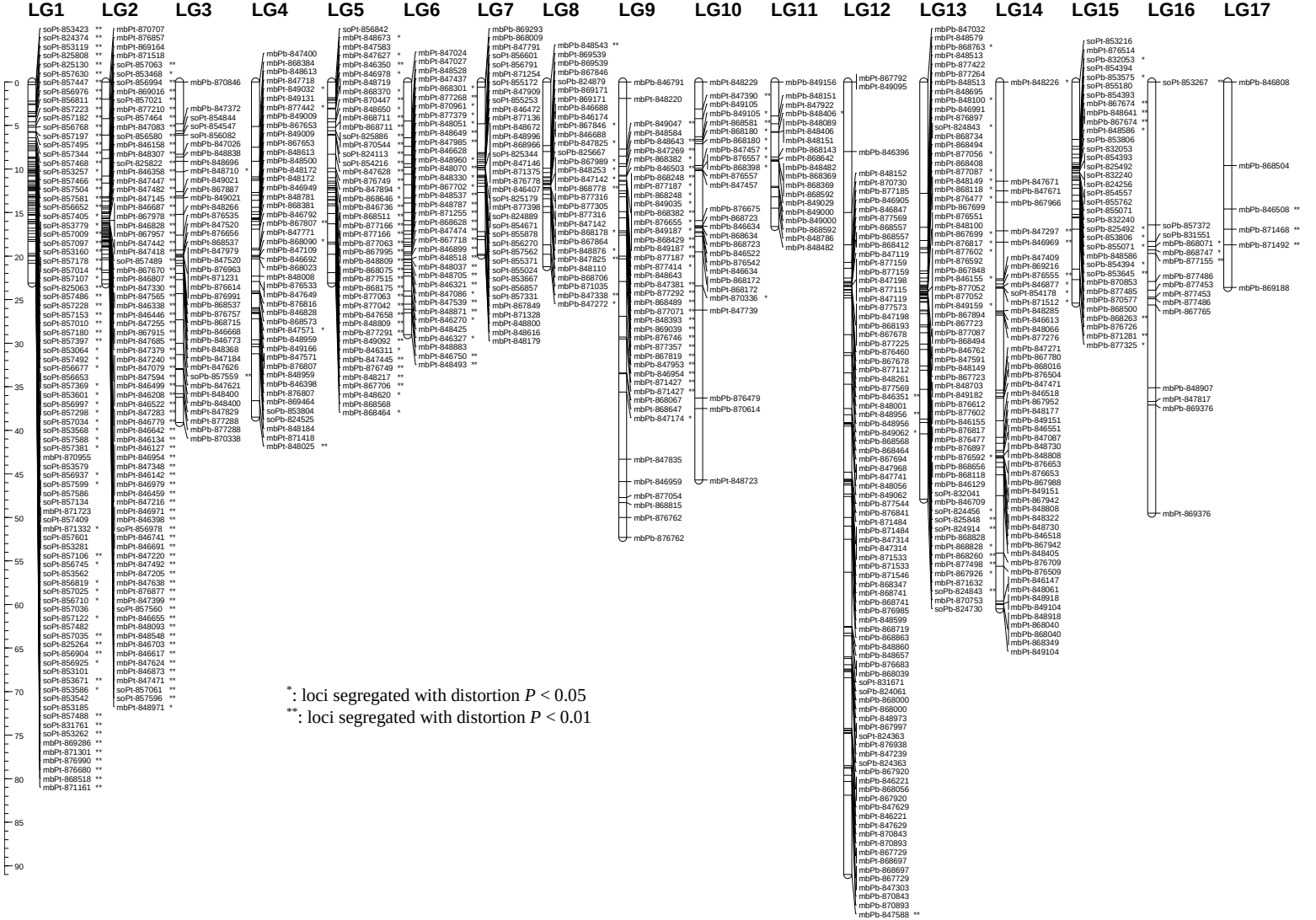
(a) ACC 1 x Kiloga



(b) ACC 87 x Berken



(c) ACC 87 x Kiloga



Appendix 5.2. List of all QTLs detected by the SML and ICIM - ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on LG (in cM); PVE = Phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect); SML QTLs are listed before ICIM QTLs; Sequence in the table of markers/ QTLs detected by the SML method is in order of increasing *P*-value

♪: Overlapped QTLs for a trait in a population are highlighted in the same colour; Congruent markers/ QTLs for a trait across populations are underlined;

‡: Reported SML QTLs were selected at *P*-value ≤ 0.01;

/: separates the left and right flanking markers for an ICIM QTL region; †: ICIM QTLs detected with relaxed PIN of 0.01

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker♪	PVE %	*P*-value‡	LG	Pos (cM)	Marker	PVE %	*P*-value	LG	Pos (cM)	Marker	PVE %	*P*-value	LG	Pos (cM)	Marker	PVE %	*P*-value
Hypocotyl pigment	11	14.5	mbPt-846225	-1.7	0.0000	-	-	mbPb-868147	-5.8	0.0000										
	-	-	mbPb-868147	-1.1	0.0005	11	42.3	mbPb-847032	1.7	0.0005										
	-	-	mbPt-868147	-1.0	0.0010	-	-	soPb-825015	1.7	0.0010										
	-	-	mbPb-846466	0.9	0.0015	-	-	soPb-855332	1.5	0.0015										
	7	121.2	soPt-853099	-0.7	0.0020	-	-	mbPb-847601	-1.4	0.0020										
	-	-	soPb-825103	-0.6	0.0025	3	9.1	mbPt-870444	-1.3	0.0025										
	-	-	soPt-824297	-0.6	0.0030	-	-	mbPb-870679	-1.1	0.0030										
	-	-	mbPb-846448	0.6	0.0035	11	80.3	mbPt-877485	-1.1	0.0035										
	-	-	mbPb-846917	-0.6	0.0040	19	36.0	mbPb-876772	-1.1	0.0040										
	-	-	mbPt-877146	0.5	0.0045	3	12.2	mbPt-848692	-1.1	0.0045										
	5	44.4	mbPb-867966	-0.4	0.0050	-	-	mbPb-877560	1.0	0.0050										
	6	22.9	mbPt-848025	-0.3	0.0055	-	-	mbPt-847098	0.9	0.0055										
	6	69.7	soPt-853267	0.3	0.0060	-	-	mbPt-876758	0.8	0.0060										
	-	-	mbPt-869156	0.2	0.0065	-	-	mbPb-847717	0.5	0.0065										
	-	-	mbPt-870923	0.1	0.0070	-	-	mbPt-848813	-0.3	0.0070										
	-	-	mbPt-869128	0.1	0.0075	-	-	soPt-824694	-0.2	0.0075										
	1	34.3	mbPt-871031	0.1	0.0079	19	41.0	soPb-854765	-0.2	0.0080										
	-	-	mbPt-869368	0.1	0.0084	-	-	soPb-854533	-0.2	0.0085										
	-	-	mbPt-868457	0.1	0.0089	-	-	soPt-853630	-0.1	0.0090										
	-	-	mbPt-876815	0.1	0.0094	-	-	soPb-853734	-0.1	0.0095										
	-	-	soPt-855304	-0.1	0.0099	-	-	soPb-855637	0.1	0.0100										
						3	10.0	mbPt-870444/ mbPt-848692	-15.8											
						11	66.0	mbPt-877021/ mbPt-868487	-18.6											
						19	40.0	soPb-832205/ soPb-854765	-15.5											
Stem colour	1	87.1	mbPb-849011	4.2	0.0000	7	32.4	mbPb-849021	-1.3	0.0000	-	-	mbPt-847817	1.0	0.0000	14	59.6	mbPt-848061	3.0	0.0000
	1	24.6	soPt-856642	-3.3	0.0005	7	32.4	mbPt-867887	-1.1	0.0005	17	21.3	mbPt-877415	0.9	0.0009	-	-	mbPb-870682	-2.5	0.0005
	5	7.5	mbPt-848651	-2.8	0.0010	-	-	mbPt-868086	-1.0	0.0010	8	27.2	mbPt-848215	-0.8	0.0019	-	-	mbPt-868732	1.7	0.0011
	11	40.4	soPb-824843	2.4	0.0015	-	-	soPt-853484	1.0	0.0015	8	24.8	mbPb-848089	0.7	0.0028	-	-	mbPb-870610	-1.5	0.0016
	13	29.2	mbPb-868398	2.0	0.0020	4	22.9	mbPt-868741	-0.9	0.0020	-	-	soPt-831606	0.6	0.0038	-	-	mbPb-849168	1.4	0.0021
	-	-	soPt-856705	-2.0	0.0025	-	-	mbPt-849021	-0.8	0.0025	11	21.3	mbPt-848696	0.5	0.0047	14	60.0	mbPb-848918	1.3	0.0026
	4	20.6	soPt-853856	-1.5	0.0030	-	-	soPb-856203	0.7	0.0030	11	19.1	soPt-856082	-0.5	0.0056	-	-	soPt-854615	-1.2	0.0032
	-	-	soPt-855889	-1.2	0.0035	-	-	soPt-854866	-0.5	0.0035	-	-	mbPb-848128	-0.5	0.0066	-	-	mbPb-869410	-1.1	0.0037
	3	71.3	mbPt-847660	-1.0	0.0040	-	-	mbPt-868136	-0.5	0.0040	17	21.9	mbPt-846869	0.5	0.0075	14	22.8	mbPt-869216	1.0	0.0042
	-	-	mbPt-877146	0.7	0.0045	8	0.0	mbPb-847400	0.4	0.0045	4	16.8	soPt-854178	0.4	0.0085	-	-	mbPb-869222	-0.8	0.0048

			1xB					1xK					87xB					87xK		
	8	39.9	mbPt-877288	0.6	0.0050	14	32.0	mbPb-870485	-0.3	0.0050	-	-	mbPt-877276	0.4	0.0094	-	-	mbPb-869382	0.7	0.0053
	12	18.7	soPt-857221	0.5	0.0055	-	-	soPb-832055	-0.3	0.0055	-	-	mbPt-848756	0.4	0.0104	-	-	mbPt-867904	0.6	0.0058
	-	-	mbPt-848611	-0.5	0.0060	-	-	mbPt-876671	-0.3	0.0060						14	60.0	mbPt-868040	0.5	0.0064
	4	20.7	soPt-825886	-0.5	0.0065	2	33.5	mbPb-868009	-0.2	0.0065						14	26.6	mbPt-848066	0.5	0.0069
Stem colour	11	19.2	mbPb-846886	-0.4	0.0070	15	26.7	mbPt-847239	-0.2	0.0070						14	47.5	mbPt-848405	0.4	0.0074
(cont…)	3	55.9	mbPt-849061	-0.4	0.0075	4	21.0	mbPb-871546	-0.2	0.0075						10	16.7	mbPt-868634	0.4	0.0079
	-	-	mbPb-847457	0.3	0.0079	2	33.6	mbPb-869293	-0.2	0.0080						14	60.0	mbPb-868040	0.3	0.0085
	3	57.9	mbPt-868143	-0.3	0.0084			mbPb-876550	-0.2	0.0085						-	-	mbPt-849168	0.3	0.0090
	-	-	soPt-854216	-0.3	0.0089	4	29.2	mbPt-848599	-0.2	0.0090						10	16.5	mbPt-868723	0.3	0.0095
	-	-	soPt-854506	-0.3	0.0094	2	35.9	soPb-855202	-0.2	0.0095						14	23.5	mbPt-846877	0.3	0.0101
	8	39.9	mbPt-847829	0.3	0.0099	4	29.3	mbPt-847773	-0.2	0.0100										
	1	87.0	mbPt-847742/ mbPb-849011	-12.3		7	33.0	mbPt-867887/ soPb-825779 †	21.0											
	3	71.0	mbPb-846221/ mbPt-847660	12.8		19	5.0	mbPb-870677/ mbPb-846988 †	16.6											
	11	40.0	mbPt-847459/ soPb-824843	14.9																
	-	-	mbPt-869240	2.4	0.0000	11	34.4	soPb-824730	-1.3	0.0000	17	21.3	mbPt-877415	1.4	0.0000	13	0.0	mbPb-847032	1.0	0.0000
Leaf rachis colour	8	39.9	mbPt-877288	1.6	0.0005	-	-	soPt-855536	1.2	0.0005	17	21.9	mbPt-846869	1.0	0.0009	3	39.1	mbPb-870338	0.8	0.0005
	8	39.7	mbPb-877288	1.6	0.0010	11	36.5	mbPt-870753	-1.1	0.0010	-	-	mbPt-847829	0.9	0.0019	-	-	mbPb-846720	0.6	0.0011
	-	-	soPb-824730	-1.6	0.0015	13	7.0	mbPb-846553	-1.1	0.0015	-	-	soPt-832113	0.8	0.0028	-	-	mbPt-868185	-0.5	0.0016
	8	39.9	mbPt-847829	1.3	0.0020	-	-	mbPt-849064	-1.0	0.0020	16	27.5	mbPt-868723	0.8	0.0038	-	-	soPb-855952	-0.5	0.0021
	4	15.2	soPb-831980	-0.8	0.0025	-	-	mbPt-868312	1.0	0.0025	-	-	mbPt-877288	0.8	0.0047	13	24.5	mbPt-848703	0.5	0.0026
	4	13.5	mbPt-848909	0.8	0.0030	-	-	mbPt-876985	0.9	0.0030	17	28.9	mbPb-870410	0.8	0.0056	7	17.2	mbPt-867849	-0.5	0.0032
	3	103.7	soPt-854190	-0.7	0.0035	11	36.5	mbPt-846471	-0.8	0.0035	11	12.4	mbPt-849021	-0.6	0.0066	-	-	soPt-854310	-0.4	0.0037
	7	4.5	mbPt-869433	-0.6	0.0040	11	36.3	mbPt-846260	-0.8	0.0040	3	104.6	mbPt-848435	-0.6	0.0075	7	0.6	mbPb-868009	-0.4	0.0042
	-	-	mbPb-871838	0.6	0.0045	11	36.3	mbPt-867926	-0.6	0.0045	3	1.1	mbPt-848259	-0.6	0.0085	7	0.0	mbPb-869293	-0.4	0.0048
	11	46.7	soPb-825660	-0.6	0.0050	-	-	soPb-831636	0.6	0.0050	-	-	mbPb-868032	-0.5	0.0094	-	-	mbPt-848943	-0.4	0.0053
	8	20.7	mbPt-846832	0.6	0.0055	19	4.9	mbPb-870677	0.6	0.0055	16	25.8	mbPt-868172	0.4	0.0104	-	-	mbPb-868185	-0.3	0.0058
	4	13.6	mbPt-868654	0.6	0.0060	13	6.7	mbPb-847440	-0.6	0.0060						-	-	soPt-854803	-0.3	0.0064
	11	45.6	soPb-824700	-0.5	0.0065	8	33.7	mbPt-847771	-0.5	0.0065						-	-	soPb-855174	-0.1	0.0069
	-	-	mbPt-868663	0.5	0.0070	3	31.9	mbPt-870568	0.5	0.0070						-	-	mbPb-847794	-0.1	0.0074
	-	-	mbPb-847393	-0.4	0.0075	-	-	soPb-824735	-0.4	0.0075						-	-	mbPb-847286	0.1	0.0079
	11	50.9	mbPb-871281	-0.4	0.0079	8	24.1	mbPt-846792	0.3	0.0080						7	17.7	mbPt-871328	-0.1	0.0085
	4	34.3	mbPt-846915	0.4	0.0084	8	24.1	mbPt-849009	0.2	0.0085						-	-	mbPb-877252	-0.1	0.0090
	-	-	soPt-853543	0.3	0.0089	17	38.8	mbPt-849118	-0.2	0.0090						-	-	mbPt-847794	-0.1	0.0095
	-	-	mbPt-871318	-0.3	0.0094	13	2.2	mbPb-867852	-0.2	0.0095						-	-	mbPb-867742	-0.1	0.0101
	4	33.9	mbPt-846869	0.3	0.0099	8	23.9	mbPb-846792	0.2	0.0100										
	4	16.0	soPb-831980/ mbPt-848196	-22.7		1	28.0	mbPt-867902/ mbPt-869470 †	10.7											
	8	39.0	mbPb-847809/ mbPb-877288	18.0		8	18.0	mbPt-868384/ mbPb-847535 †	19.5											
						8	26.0	mbPt-868381/ mbPt-847654 †	-30.3											
						11	48.0	mbPb-848513/ mbPt-849182 †	-20.7											
						17	40.0	mbPt-849118/ mbPt-848472 †	-12.0											
	-	-	soPb-824730	-2.1	0.0000	-	-	mbPb-868147	-2.8	0.0000	11	12.4	mbPt-849021	-5.6	0.0000			soPb-832155	3.8	0.0000
Leaf petiole colour	-	-	mbPt-869240	1.8	0.0005	-	-	soPb-832055	-2.2	0.0005			mbPb-847817	3.5	0.0009			mbPb-847286	2.2	0.0005

			1xB					1xK					87xB					87xK		
Leaf petiole colour (cont…)	8	39.7	mbPb-877288	1.4	0.0010	-	-	soPt-853484	2.2	0.0010			mbPb-848128	-3.5	0.0019			soPt-832155	1.6	0.0011
	3	75.8	mbPt-867849	-1.2	0.0015	-	-	mbPt-846376	-0.8	0.0015	9	33.3	mbPt-876817	2.7	0.0028	8	10.4	soPb-824879	0.9	0.0016
	11	46.7	soPb-824755	-1.1	0.0020	-	-	mbPt-871111	0.5	0.0020			mbPb-869293	-2.3	0.0038			mbPt-846680	0.8	0.0021
	11	46.7	soPb-825660	-0.8	0.0025	16	51.5	mbPb-848665	-0.4	0.0025	17	28.9	mbPb-870410	2.2	0.0047	12	62.6	mbPb-848657	0.6	0.0026
	8	39.9	mbPt-877288	0.8	0.0030	-	-	soPb-825361	0.4	0.0030	14	21.9	mbPt-849166	2.1	0.0056	8	12.1	mbPb-877305	0.5	0.0032
	-	-	mbPt-869316	0.6	0.0035	-	-	soPt-824432	0.3	0.0035	16	28.8	mbPt-846634	1.9	0.0066	4	13.6	mbPt-849009	0.4	0.0037
	-	-	soPt-853177	0.6	0.0040	-	-	mbPt-849005	-0.3	0.0040	-	-	mbPt-847817	1.3	0.0075	1	16.0	soPt-856925	0.4	0.0042
	1	27.2	soPt-854614	-0.6	0.0045	-	-	soPt-855536	0.3	0.0045	13	13.1	mbPt-869039	-1.3	0.0085	-	-	soPt-825485	-0.3	0.0048
	8	39.9	mbPt-847829	0.5	0.0050	11	36.4	mbPb-868763	0.2	0.0050	17	48.5	mbPt-848673	-1.1	0.0094	-	-	mbPb-847654	0.3	0.0053
	-	-	mbPt-849156	-0.5	0.0055	-	-	mbPt-849064	-0.2	0.0055	16	27.5	mbPt-868634	0.8	0.0104	9	1.9	mbPt-848220	-0.2	0.0058
	4	13.5	soPt-831980	-0.5	0.0060	11	37.3	mbPb-877269	0.2	0.0060						8	15.0	mbPb-868706	0.2	0.0064
	-	-	mbPb-871609	-0.5	0.0065	-	-	mbPt-869348	-0.2	0.0065						13	0.0	mbPb-847032	0.2	0.0069
	3	50.4	mbPb-849156	-0.5	0.0070	-	-	mbPt-876467	0.2	0.0070						-	-	soPt-856369	-0.2	0.0074
	3	103.7	soPt-854190	-0.4	0.0075	5	24.9	mbPb-846551	-0.2	0.0075						-	-	mbPb-870844	-0.1	0.0079
	4	15.2	soPb-831980	-0.4	0.0079	11	36.5	mbPt-846471	-0.1	0.0080						15	25.4	mbPb-877325	0.1	0.0085
	3	56.7	mbPb-868592	0.4	0.0084	6	9.8	mbPt-869216	0.1	0.0085						-	-	mbPt-846748	0.1	0.0090
	14	2.1	mbPt-846990	-0.4	0.0089	3	31.9	mbPt-870568	0.1	0.0090						4	12.7	mbPb-849009	0.1	0.0095
	-	-	soPt-853601	0.3	0.0094	-	-	soPt-853440	0.1	0.0095						-	-	mbPt-871307	-0.1	0.0101
	7	4.5	mbPt-869433	-0.3	0.0099	-	-	soPb-823939	0.1	0.0100										
	1	63.0	mbPt-869213/ mbPt-868339 †	-10.2		3	32.0	mbPt-870568/ mbPt-868868 †	27.0											
	3	50.0	mbPb-868582/ mbPb-849156 †	10.3		3	46.0	soPb-831873/ mbPb-871848 †	-12.6											
	4	15.0	soPb-825932/ soPb-831980 †	-19.6		7	18.0	soPt-855854/ mbPb-847372 †	11.4											
						9	7.0	mbPt-847916/ mbPt-847896 †	-13.6											
Plant hair density	4	6.8	mbPb-846685	-0.9	0.0000	16	48.3	mbPt-870410	3.4	0.0000	12	9.5	mbPb-877486	-1.2	0.0000	16	49.5	mbPt-869376	2.9	0.0000
	6	52.9	mbPt-877453	-0.6	0.0005	-	-	mbPt-871155	-2.2	0.0005	-	-	soPt-825889	1.1	0.0009	-	-	mbPt-846445	-2.5	0.0005
	7	32.8	mbPt-846835	0.6	0.0010	-	-	soPb-824602	1.9	0.0010	3	84.9	mbPt-868483	-0.9	0.0019	-	-	mbPb-847947	-2.3	0.0011
	6	8.0	mbPb-869464	-0.6	0.0015	16	65.0	mbPt-868711	-1.8	0.0015	12	8.3	mbPb-877453	-0.7	0.0028	13	22.9	mbPt-876897	1.7	0.0016
	-	-	soPt-854882	0.5	0.0020	18	20.2	mbPt-869376	1.7	0.0020	-	-	mbPb-867894	0.7	0.0038	14	40.8	mbPt-867952	1.5	0.0021
	4	6.8	mbPb-867995	-0.5	0.0025	-	-	mbPt-871300	-1.3	0.0025	9	29.6	mbPb-868828	0.6	0.0047	16	35.1	mbPb-848907	1.5	0.0026
	1	47.7	mbPt-847735	0.5	0.0030	19	4.9	mbPb-870677	1.3	0.0030	-	-	mbPt-846256	0.5	0.0056	-	-	mbPt-849037	-1.0	0.0032
	6	52.8	mbPt-877486	-0.4	0.0035	18	21.5	mbPt-848907	1.2	0.0035	-	-	mbPt-847829	0.5	0.0066	-	-	soPt-855343	-1.0	0.0037
	6	52.9	mbPb-877486	-0.4	0.0040	-	-	mbPt-876892	-1.1	0.0040	-	-	mbPt-869412	0.5	0.0075	-	-	mbPt-876494	0.9	0.0042
	6	52.8	mbPb-877453	-0.4	0.0045	11	10.7	mbPt-848641	-1.0	0.0045	-	-	mbPb-868533	0.5	0.0085	-	-	mbPt-870744	-0.8	0.0048
	-	-	mbPt-847342	-0.4	0.0050	-	-	mbPt-876644	-0.9	0.0050	-	-	mbPb-849044	0.5	0.0094	-	-	mbPb-870918	0.6	0.0053
	4	6.5	mbPt-849092	-0.4	0.0055	-	-	mbPb-870742	-0.8	0.0055	1	16.3	mbPt-847057	0.4	0.0104	-	-	mbPt-871643	0.5	0.0058
	-	-	mbPt-867976	0.3	0.0060	-	-	mbPt-877475	0.7	0.0060						14	43.0	mbPb-876653	0.4	0.0064
	3	37.9	mbPt-849062	-0.3	0.0065	18	29.6	mbPt-867765	-0.7	0.0065						14	43.0	mbPb-867988	0.4	0.0069
	-	-	soPt-855840	-0.3	0.0070	11	10.6	mbPb-848586	-0.7	0.0070						-	-	mbPt-876693	0.4	0.0074
	4	6.8	mbPt-877063	-0.3	0.0075	16	41.1	mbPt-848216	-0.6	0.0075						-	-	mbPt-871830	-0.4	0.0079
	1	47.5	mbPt-847641	0.2	0.0079	-	-	mbPt-849132	-0.6	0.0080						-	-	mbPb-871539	0.2	0.0085
	3	37.9	mbPt-847968	-0.2	0.0084	16	51.5	mbPt-848665	0.6	0.0085						14	43.7	mbPt-848322	0.2	0.0090
	1	45.4	mbPt-846664	0.2	0.0089	-	-	soPt-855980	0.6	0.0090						13	23.7	mbPt-877087	0.2	0.0095
	-	-	mbPt-877328	-0.2	0.0094	-	-	soPt-855374	-0.5	0.0095						-	-	mbPt-847488	-0.2	0.0101
	4	6.8	mbPt-846736	-0.2	0.0099	-	-	mbPt-868670	-0.5	0.0100										
	3	37.0	mbPt-867694/ mbPb-868464 †	-12.7		16	48.0	mbPt-846915/ mbPt-870410	19.7							16	32.0	mbPt-867765/ mbPb-848907	30.1	

			1xB																	
Plant hair density (cont…)	4	9.0	mbPb-847658/ soPt-855555 †	20.4																
	7	33.0	mbPt-846835/ soPt-855837 †	17.1																
	7	42.0	soPt-854555/ mbPt-871572 †	-14.0																
	12	65.0	mbPb-876762/ mbPb-846334 †	-10.6																
Plant hair colour	8	19.5	mbPb-876991	-1.0	0.0000	-	-	mbPt-869245	3.3	0.0000			mbPb-848400	-1.1	0.0000	3	34.9	mbPt-847829	-1.5	0.0000
	8	14.5	soPb-855550	0.9	0.0005	-	-	mbPt-867794	-2.6	0.0005			mbPt-876534	0.7	0.0009			soPb-854119	-0.7	0.0005
	11	51.5	mbPb-877325	-0.8	0.0010	-	-	soPb-856981	-2.3	0.0010			mbPb-847055	0.6	0.0019			mbPb-869222	-0.7	0.0011
	3	0.0	mbPt-849095	0.7	0.0015	7	19.5	mbPb-877288	-2.1	0.0015	1	27.1	mbPt-848349	0.6	0.0028	12	15.0	mbPt-848152	0.7	0.0016
	12	60.0	mbPt-846334	-0.7	0.0020	-	-	mbPt-847583	-2.1	0.0020			mbPt-877288	-0.6	0.0038			mbPt-848089	0.6	0.0021
	-	-	mbPb-848692	0.7	0.0025	18	7.3	soPb-853232	-1.7	0.0025	-	-	mbPt-876550	-0.6	0.0047	-	-	soPb-825259	0.6	0.0026
	-	-	mbPt-876675	-0.6	0.0030	-	-	mbPb-870338	-1.3	0.0030	-	-	mbPb-847817	0.5	0.0056	-	-	mbPb-847952	-0.6	0.0032
	3	0.1	mbPt-867792	0.6	0.0035	-	-	mbPb-870710	1.3	0.0035	-	-	mbPb-877332	0.5	0.0066	11	0.0	mbPb-849156	-0.6	0.0037
	8	27.9	soPt-825803	-0.6	0.0040	16	70.4	mbPt-847627	-1.3	0.0040	4	4.5	mbPb-848808	-0.5	0.0075	-	-	mbPt-848055	-0.6	0.0042
	-	-	soPt-854139	0.5	0.0045	-	-	soPb-856595	1.1	0.0045	-	-	soPt-855714	0.5	0.0085	3	35.8	mbPt-877288	-0.5	0.0048
	15	18.9	mbPb-868706	0.5	0.0050	-	-	mbPt-870974	-0.9	0.0050	6	7.2	mbPb-868542	-0.3	0.0094	3	34.9	mbPb-847829	-0.5	0.0053
	-	-	soPt-856348	0.5	0.0055	-	-	mbPt-848673	-0.8	0.0055			mbPb-867780	-0.3	0.0104	-	-	soPb-824460	0.4	0.0058
	8	19.6	mbPt-847520	-0.5	0.0060	-	-	mbPb-868205	-0.6	0.0060						8	0.0	mbPb-848543	-0.4	0.0064
	-	-	mbPb-847947	0.5	0.0065	-	-	mbPb-876729	-0.6	0.0065						-	-	mbPb-848982	0.4	0.0069
	-	-	soPt-855550	0.4	0.0070	16	77.7	mbPb-849092	-0.5	0.0070						-	-	soPb-854593	-0.3	0.0074
	15	20.5	mbPb-877316	0.4	0.0075	-	-	mbPt-846390	-0.5	0.0075						3	36.2	mbPb-877288	-0.3	0.0079
			mbPt-876656	-0.3	0.0079	7	19.6	mbPt-877288	-0.4	0.0080						11	5.8	mbPt-848406	0.2	0.0085
	8	26.6	mbPt-867887	0.3	0.0084	-	-	mbPt-846978	-0.4	0.0085						-	-	mbPt-871465	0.2	0.0090
	8	19.6	mbPb-847520	-0.3	0.0089	-	-	mbPt-868370	-0.3	0.0090						14	59.9	mbPb-849104	0.2	0.0095
	-	-	mbPt-876671	0.3	0.0094	3	5.2	mbPt-870337	-0.2	0.0095						-	-	mbPt-847698	-0.2	0.0101
	-	-	mbPt-867907	0.2	0.0099	7	19.6	mbPb-847829	-0.2	0.0100						3	30.0	mbPt-847626/ soPb-857559 †	11.7	
	3	0.0	mbPt-849095/ mbPt-867792 †	32.8		16	70.0	mbPt-877344/ mbPt-847627	-18.3							3	35.0	mbPt-847829/ mbPt-877288 †	-15.1	
	3	8.0	mbPb-846396/ mbPt-876637 †	-21.4																
	11	51.0	mbPb-871281/ mbPb-877325 †	-13.0																
	12	59.0	mbPt-848460/ mbPt-846334 †	11.5																
Growth habit	11	42.1	mbPt-868763	2.4	0.0000	-	-	mbPt-871661	6.6	0.0000			soPb-855834	-3.7	0.0000			mbPt-848943	6.6	0.0000
	11	51.5	mbPb-877325	2.0	0.0005	-	-	mbPt-871497	4.1	0.0005	3	40.6	mbPt-870630	2.9	0.0009	3	32.9	mbPt-848400	5.9	0.0005
	8	47.2	soPt-824253	1.8	0.0010	11	46.8	mbPt-868828	3.8	0.0010	16	36.7	mbPb-868581	2.1	0.0019	3	33.0	mbPb-848400	2.7	0.0011
	-	-	mbPt-869537	1.5	0.0015	7	19.5	mbPb-877288	3.7	0.0015			mbPt-871474	-1.3	0.0028			mbPt-868050	1.8	0.0016
	15	16.0	mbPb-876838	1.3	0.0020	-	-	soPt-823980	-3.5	0.0020	3	125.7	mbPt-848859	0.8	0.0038			mbPt-871774	1.6	0.0021
	8	46.1	soPt-855179	1.3	0.0025	-	-	mbPt-876592	2.4	0.0025	15	13.7	mbPb-848346	0.8	0.0047			mbPb-849188	1.6	0.0026
	-	-	soPt-857531	-0.7	0.0030	-	-	mbPt-876952	2.1	0.0030	-	-	soPt-831748	0.8	0.0056	-	-	mbPb-847947	-1.5	0.0032
	8	50.3	soPb-855179	0.7	0.0035	-	-	mbPt-871431	1.5	0.0035	1	31.3	mbPt-847664	0.7	0.0066	-	-	mbPt-847794	1.5	0.0037
	9	17.7	mbPt-868185	0.7	0.0040	8	22.8	mbPb-867653	1.3	0.0040	-	-	mbPt-867700	-0.5	0.0075	3	34.9	mbPt-847829	1.4	0.0042
	5	3.8	mbPt-867879	-0.6	0.0045	-	-	mbPb-869184	1.2	0.0045	11	1.7	mbPt-868026	-0.5	0.0085	-	-	mbPt-870337	-1.4	0.0048
	9	16.6	mbPb-847506	-0.5	0.0050	7	19.6	mbPt-877288	1.0	0.0050	8	30.3	mbPb-870385	0.4	0.0094	-	-	mbPt-849188	1.2	0.0053
	15	18.0	mbPt-876838	0.5	0.0055	11	46.8	mbPb-868828	0.8	0.0055	3	84.9	mbPt-868483	0.4	0.0104	8	15.0	mbPb-868706	1.2	0.0058
	11	65.9	mbPb-868263	-0.5	0.0060	11	60.4	mbPt-847510	-0.8	0.0060	11	3.0	mbPt-846602/ mbPt-867887	19.6		-	-	mbPb-876842	-1.2	0.0064
	8	39.9	mbPt-877288	0.5	0.0065	7	19.6	mbPb-847829	0.4	0.0065						-	-	mbPt-876465	1.1	0.0069

	1xB					1xK					87xB					87xK				
Growth habit (cont…)	5	4.0	mbPt-877553	-0.4	0.0070	-	-	mbPt-869245	-0.4	0.0070						8	12.1	mbPb-877305	1.1	0.0074
	-	-	mbPb-848563	-0.4	0.0075	-	-	soPt-825848	0.4	0.0075						-	-	mbPt-871563	-1.1	0.0079
	-	-	mbPt-847294	0.3	0.0079	7	19.6	mbPt-847829	0.3	0.0080						-	-	mbPb-870918	1.1	0.0085
	11	42.1	mbPb-868763	0.3	0.0084	17	8.1	mbPt-847929	0.2	0.0085						4	30.4	soPb-824525	-1.1	0.0090
	-	-	mbPt-846456	-0.3	0.0089	-	-	soPt-856420	-0.2	0.0090						-	-	soPt-855006	-0.9	0.0095
	3	62.4	mbPt-848088	0.3	0.0094	-	-	soPb-825355	0.1	0.0095						4	31.2	mbPt-848184	0.8	0.0101
	-	-	mbPb-871614	-0.3	0.0099	-	-	mbPt-849044	0.1	0.0100										
	3	62.0	mbPt-846669/ mbPb-868471	13.5		1	21.0	mbPt-848946/ mbPt-848646	12.0							3	32.0	mbPb-847621/ mbPt-848400	20.3	
	8	39.0	mbPb-847809/ mbPb-877288	13.4																
	11	51.0	mbPb-871281/ mbPb-877325	16.0																
Twining	6	25.1	mbPt-868384	-1.2	0.0000	-	-	soPt-824253	4.6	0.0000	-	-	mbPb-877550	5.2	0.0000	-	-	mbPt-870337	-3.0	0.0000
	11	65.9	mbPb-868263	-0.6	0.0005	-	-	soPt-857318	-2.3	0.0005	17	0.0	soPb-856544	4.6	0.0009	-	-	mbPb-848706	-2.4	0.0005
	-	-	mbPb-847393	-0.5	0.0010	-	-	soPb-825355	2.0	0.0010	15	13.7	mbPb-848346	2.3	0.0019	-	-	mbPt-848885	-1.6	0.0011
	-	-	mbPt-876970	0.5	0.0015	-	-	mbPt-846811	-1.9	0.0015	-	-	soPb-857635	1.9	0.0028	-	-	soPb-855048	-1.5	0.0016
	6	26.1	mbPb-848613	0.4	0.0020	19	34.4	mbPb-871193	-1.5	0.0020	16	12.4	mbPt-847782	1.7	0.0038	10	26.2	mbPt-847739	0.9	0.0021
	2	20.4	mbPt-868678	0.3	0.0025	-	-	soPb-853734	1.5	0.0025	17	54.3	mbPb-846311	0.9	0.0047	-	-	soPt-856422	-0.9	0.0026
	6	24.1	mbPt-846949	0.3	0.0030	-	-	mbPt-871346	1.4	0.0030	-	-	mbPb-849024	-0.8	0.0056	-	-	mbPt-870744	0.8	0.0032
	9	10.1	mbPt-870681	-0.2	0.0035	1	17.3	soPt-853573	-1.3	0.0035	1	0.0	mbPt-869037	-0.6	0.0066	-	-	mbPb-847506	-0.7	0.0037
	-	-	mbPt-867890	0.2	0.0040	8	25.7	mbPt-868381	-1.0	0.0040	16	15.6	mbPt-847390	0.4	0.0075	4	12.7	mbPb-849009	-0.4	0.0042
	-	-	soPt-855633	-0.2	0.0045	8	23.9	mbPb-846792	-1.0	0.0045	-	-	soPb-856391	0.4	0.0085	-	-	mbPt-871830	0.4	0.0048
	6	23.3	mbPb-847535	0.2	0.0050	8	23.9	mbPb-848781	0.8	0.0050	11	23.2	mbPb-847372	0.3	0.0094	-	-	mbPt-870460	0.4	0.0053
	8	20.7	mbPb-846687	0.2	0.0055	-	-	mbPb-867685	-0.5	0.0055	11	1.7	mbPt-868026	-0.3	0.0104	-	-	mbPt-876763	-0.3	0.0058
	-	-	mbPt-846671	-0.2	0.0060	-	-	mbPt-871177	0.5	0.0060	14	19.0	mbPb-849009	21.1		-	-	mbPt-868047	0.3	0.0064
	-	-	mbPt-876952	0.2	0.0065	8	0.0	mbPb-847400	0.3	0.0065			mbPt-849166			-	-	mbPb-868571	-0.2	0.0069
	11	68.3	mbPt-848641	-0.1	0.0070	8	24.1	mbPt-846792	-0.2	0.0070						-	-	soPt-856027	-0.2	0.0074
	6	24.1	mbPb-848781	0.1	0.0075	8	24.5	mbPb-848500	0.2	0.0075						4	16.4	mbPt-868381	-0.2	0.0079
	9	3.7	mbPt-848838	0.1	0.0079	-	-	mbPt-849188	0.2	0.0080						8	12.2	mbPt-877316	0.2	0.0085
	-	-	mbPb-870924	0.1	0.0084	-	-	mbPt-871107	-0.2	0.0085						-	-	soPt-824253	0.1	0.0090
	9	14.1	mbPt-848943	0.1	0.0089	18	7.3	soPb-853232	-0.2	0.0090						-	-	mbPt-847294	-0.1	0.0095
	9	17.7	mbPt-868185	0.1	0.0094	-	-	soPt-855179	0.2	0.0095						-	-	mbPt-869411	-0.1	0.0101
	-	-	soPt-856433	0.1	0.0099	8	24.1	mbPt-849009	-0.2	0.0100										
	6	24.0	mbPt-847535/ mbPb-846949	48.3		1	21.0	mbPt-848946/ mbPt-848646	17.3							3	13.0	mbPt-849021/ mbPt-867887	15.6	
	9	10.0	mbPt-876465/ mbPt-870681	10.2												4	15.0	mbPt-848613/ mbPb-848500	20.1	
Leaflet lobing	11	68.3	mbPt-848641	-1.4	0.0000	11	10.8	mbPt-848586	-6.6	0.0000	-	-	mbPb-848641	15.5	0.0000	15	9.7	mbPt-848641	-5.6	0.0000
	-	-	mbPt-849022	1.1	0.0005	11	10.6	mbPb-848586	-5.1	0.0005	-	-	mbPb-848586	-8.7	0.0009	15	9.7	mbPb-867674	4.9	0.0005
	11	68.3	mbPt-848586	-1.1	0.0010	11	14.3	mbPb-868263	-3.3	0.0010	-	-	mbPb-867674	-3.7	0.0019	15	9.7	mbPb-848641	-4.5	0.0011
	11	62.9	soPb-854393	1.0	0.0015	11	10.7	mbPb-867674	-3.2	0.0015	-	-	mbPb-868263	-2.9	0.0028	15	9.8	mbPt-848586	-3.5	0.0016
	11	63.1	soPb-855762	0.8	0.0020	11	10.7	mbPt-848641	2.3	0.0020	9	43.6	mbPb-877325	2.8	0.0038	15	10.0	soPt-832053	3.3	0.0021
	10	12.2	mbPt-871421	0.7	0.0025	-	-	soPb-855180	2.2	0.0025	12	0.5	soPb-857372	-1.8	0.0047	15	6.6	mbPt-876514	-2.3	0.0026
	1	0.0	mbPt-846226	0.5	0.0030	11	10.7	mbPt-876514	-2.0	0.0030	-	-	mbPb-877485	-1.7	0.0056	15	11.2	soPb-825492	2.0	0.0032
	11	63.1	soPb-825492	0.5	0.0035	11	22.9	mbPb-877325	1.7	0.0035	-	-	mbPb-870825	-1.4	0.0066	15	22.7	mbPb-871281	-1.6	0.0037
	11	68.4	mbPb-848641	-0.4	0.0040	-	-	mbPt-846892	1.2	0.0040	13	0.0	mbPt-848648	-1.3	0.0075	15	13.7	soPb-853645	1.6	0.0042
	11	59.9	soPt-854557	0.4	0.0045	-	-	soPt-855980	0.9	0.0045	11	1.2	mbPb-848710	-1.2	0.0085	15	7.4	soPb-832053	1.5	0.0048
	7	17.0	soPt-855993	0.3	0.0050	10	9.8	mbPt-848723	-0.9	0.0050	9	11.7	soPt-853944	1.2	0.0094	15	10.4	soPt-832240	1.4	0.0053
	11	63.1	soPb-853645	0.3	0.0055	11	6.0	soPb-853216	0.6	0.0055	-	-	mbPb-870577	-1.2	0.0104	15	9.9	soPb-853806	1.4	0.0058

			1xB					1xK					87xB					87xK		
	-	-	mbPt-871318	0.3	0.0060	-	-	soPb-854394	0.6	0.0060	9	11.0	soPb-853806/ soPt-853944	33.6		15	25.4	mbPb-877325	1.3	0.0064
Leaflet lobing	-	-	mbPt-869084	-0.2	0.0065	-	-	soPb-855071	0.6	0.0065						15	10.4	soPt-824256	0.7	0.0069
(cont…)	11	62.4	soPt-855762	0.2	0.0070	-	-	soPb-825492	0.4	0.0070	12	0.0	soPb-831551/ soPb-857372	11.0		15	11.0	soPb-832240	0.7	0.0074
	5	4.2	soPt-824927	-0.2	0.0075	-	-	soPb-832053	0.4	0.0075						15	9.3	soPb-854393	0.7	0.0079
	11	62.7	soPt-853575	0.1	0.0079	11	5.0	soPb-853944	0.4	0.0080						15	10.2	soPt-854393	0.7	0.0085
	11	67.4	mbPb-848586	-0.1	0.0084	-	-	mbPb-848227	0.4	0.0085						13	35.3	mbPt-867926	-0.7	0.0090
	15	30.2	mbPb-847272	0.1	0.0089	-	-	mbPb-867712	-0.4	0.0090						15	11.8	soPb-855071	0.6	0.0095
	11	63.1	soPb-824256	0.1	0.0094	11	4.6	soPb-855762	0.3	0.0095						15	16.9	mbPb-868263	-0.6	0.0101
	-	-	soPt-857521	0.1	0.0099	-	-	mbPb-868885	0.2	0.0100						11	13.0	mbPb-849000/ mbPb-868592 †	14.3	
	11	63.0	soPb-854393/ soPb-855762	14.8		11	9.0	soPb-853216/ mbPb-848586	66.2							15	14.0	soPb-853645/ mbPb-870853 †	15.4	
	11	68.0	mbPb-848586/ mbPt-876514	23.4																
	11	68.4	mbPb-848641	-1.4	0.0000	-	-	soPt-854823	1.0	0.0000	9	10.3	soPb-853575	6.3	0.0000	9	48.3	mbPt-868815	0.9	0.0000
Flower colour	11	68.5	mbPb-867674	-1.4	0.0005	-	-	mbPt-849064	0.7	0.0005	6	14.7	mbPb-847184	4.0	0.0009	-	-	soPt-854506	0.9	0.0005
	11	67.4	mbPb-848586	-1.3	0.0010	-	-	soPt-854770	0.4	0.0010	7	22.2	mbPt-877371	-2.3	0.0019	-	-	soPt-825659	0.8	0.0011
	11	64.6	soPb-853944	1.2	0.0015	-	-	mbPt-868630	-0.3	0.0015	-	-	mbPb-868285	1.4	0.0028	3	5.6	soPt-854844	-0.8	0.0016
	11	68.3	mbPt-876514	-0.9	0.0020	18	21.5	mbPt-848907	0.2	0.0020	5	12.4	mbPt-848061	1.1	0.0038	-	-	mbPb-848706	0.8	0.0021
	-	-	mbPb-877071	-0.9	0.0025	-	-	mbPt-848944	0.2	0.0025	-	-	mbPb-848128	-0.7	0.0047	-	-	soPt-853238	-0.7	0.0026
	11	68.3	mbPt-848641	-0.8	0.0030	1	18.1	soPt-855905	-0.2	0.0030	6	18.0	mbPb-876656	0.7	0.0056	9	52.3	mbPb-876762	0.7	0.0032
	11	63.4	soPb-832053	0.7	0.0035	-	-	soPb-855825	-0.1	0.0035	-	-	mbPb-870577	-0.7	0.0066	-	-	soPt-854454	0.6	0.0037
	-	-	soPt-854494	0.5	0.0040	18	7.3	soPb-853232	-0.1	0.0040	-	-	mbPb-876726	-0.6	0.0075	-	-	soPt-854310	0.5	0.0042
	11	63.1	soPb-855180	0.5	0.0045	-	-	soPt-853554	0.1	0.0045	5	0.0	mbPb-876709	-0.6	0.0085	-	-	soPt-853997	0.5	0.0048
	11	63.1	soPb-854394	0.5	0.0050	-	-	mbPt-877017	-0.1	0.0050	-	-	mbPb-849156	0.6	0.0094	10	37.5	mbPb-870614	0.4	0.0053
	11	63.1	soPb-824256	0.5	0.0055	-	-	soPt-856847	-0.1	0.0055	6	0.0	mbPt-848296	-0.5	0.0104	-	-	soPt-856856	-0.4	0.0058
	11	63.4	soPb-832240	0.5	0.0060	-	-	soPt-853321	0.1	0.0060	9	2.0	mbPb-846264/ soPt-832240	41.8		-	-	soPt-856491	0.4	0.0064
	11	59.0	mbPb-877485	-0.5	0.0065	-	-	mbPb-848446	-0.1	0.0065						3	5.9	soPt-854547	-0.3	0.0069
	3	56.5	mbPb-848786	-0.4	0.0070	-	-	soPb-824288	-0.1	0.0070						-	-	mbPb-877471	0.3	0.0074
	-	-	mbPt-846770	-0.4	0.0075	19	0.0	mbPb-848812	0.1	0.0075						15	10.5	soPt-854557	0.3	0.0079
	11	68.3	mbPt-848586	-0.4	0.0079	13	0.6	mbPb-847695	0.1	0.0080						15	10.2	soPt-825492	0.3	0.0085
	-	-	mbPb-870577	-0.3	0.0084	6	0.0	mbPt-847507	-0.1	0.0085						9	47.7	mbPt-877054	0.2	0.0090
	-	-	mbPt-849047	-0.3	0.0089	-	-	mbPb-871068	0.1	0.0090						-	-	mbPt-848135	-0.2	0.0095
	-	-	mbPb-846275	0.3	0.0094	11	0.0	mbPb-876726	-0.1	0.0095						9	7.7	mbPt-849047	-0.2	0.0101
	11	59.0	mbPb-870853	-0.3	0.0099	11	10.8	mbPt-848586	-0.1	0.0100										
	11	68.0	mbPb-848586/ mbPt-876514	23.4		11	0.0	mbPb-876726/ mbPb-877485	17.2											
	6	25.1	mbPt-868384	1.3	0.0000	-	-	mbPb-848919	1.3	0.0000	-	-	mbPb-868285	-1.6	0.0000	10	15.9	mbPb-876675	-1.6	0.0000
Inflorescence structure	11	59.0	mbPb-870853	0.6	0.0005	-	-	mbPb-868631	-1.0	0.0005	14	11.2	mbPt-848172	-1.6	0.0009	4	0.0	mbPb-847400	-1.3	0.0005
	-	-	mbPt-876725	0.5	0.0010	-	-	mbPt-876766	-0.9	0.0010	14	12.4	mbPb-848781	-1.4	0.0019	4	20.3	mbPb-868023	1.1	0.0011
	2	6.8	mbPt-870563	-0.3	0.0015	-	-	soPt-857002	-0.5	0.0015	14	10.8	mbPt-848781	-1.1	0.0028	10	17.2	mbPb-846522	-0.9	0.0016
	-	-	mbPt-877196	-0.3	0.0020	-	-	soPb-853734	-0.3	0.0020	17	0.0	soPb-856544	-1.0	0.0038	-	-	mbPt-848653	0.8	0.0021
	9	14.1	mbPt-848943	-0.2	0.0025	13	7.1	mbPb-867898	-0.2	0.0025	-	-	mbPb-868626	-0.9	0.0047	-	-	mbPt-867932	-0.8	0.0026
	-	-	mbPb-848601	0.2	0.0030	8	25.7	mbPb-868381	0.2	0.0030	14	12.7	mbPb-848500	-0.9	0.0056	4	16.9	mbPb-846792	0.8	0.0032
	6	26.1	mbPb-848613	-0.1	0.0035	-	-	mbPt-876467	-0.2	0.0035	14	10.0	mbPt-846949	-0.9	0.0066	4	16.0	mbPt-848781	-0.6	0.0037
	6	24.1	mbPt-848781	-0.1	0.0040	8	24.1	mbPt-846792	0.1	0.0040	14	12.7	mbPb-848172	-0.9	0.0075	-	-	mbPt-871111	-0.6	0.0042
	-	-	soPt-824752	-0.1	0.0045	8	11.0	mbPt-868384	0.1	0.0045	-	-	mbPt-847718	-0.8	0.0085	-	-	mbPb-846946	-0.6	0.0048
	-	-	mbPt-846671	0.1	0.0050	-	-	mbPt-849005	0.1	0.0050	14	12.2	mbPb-846949	-0.7	0.0094	7	0.6	mbPb-868009	-0.6	0.0053
	6	22.3	mbPb-849009	0.1	0.0055	-	-	mbPt-848710	0.1	0.0055	-	-	mbPb-868160	-0.6	0.0104	10	16.8	mbPb-868723	-0.6	0.0058
	-	-	mbPt-867658	-0.1	0.0060	-	-	soPt-857318	0.1	0.0060						7	0.0	mbPb-869293	-0.5	0.0064
	8	39.7	mbPb-877288	-0.1	0.0065	17	8.1	mbPt-847929	-0.1	0.0065						4	20.0	mbPb-847109	0.5	0.0069

	1xB					1xK					87xB					87xK				
Inflorescence structure (cont…)	-	-	mbPt-868675	-0.1	0.0070	-	-	soPt-853209	0.1	0.0070						-	-	soPt-855509	0.5	0.0074
	-	-	mbPt-847531	0.1	0.0075	-	-	mbPt-847775	-0.1	0.0075						-	-	mbPb-869222	0.5	0.0079
	4	34.1	mbPt-877415	-0.1	0.0079	7	55.1	mbPt-848023	0.1	0.0080						-	-	mbPb-847286	0.4	0.0085
	3	73.1	mbPb-868160	-0.1	0.0084	-	-	mbPt-877269	0.1	0.0085						4	24.3	mbPb-868573	-0.4	0.0090
	-	-	soPt-824197	0.1	0.0089	-	-	soPt-853440	-0.1	0.0090						-	-	mbPt-869411	0.3	0.0095
	6	40.0	mbPb-847872	-0.1	0.0094	8	24.5	mbPb-848500	-0.1	0.0095						4	5.1	mbPt-868384	0.3	0.0101
	11	77.1	soPt-854998	0.1	0.0099	-	-	soPt-824253	-0.1	0.0100						4	2.0	mbPb-847400/ mbPb-868384	-21.8	
	6	24.0	mbPt-847535/ mbPb-846949	-40.0		7	55.0	mbPt-847642/ mbPt-848023	-16.8											
Dry pod colour	3	73.1	mbPb-868160	2.4	0.0000	16	19.1	mbPt-846885	1.7	0.0000	15	19.0	mbPb-868706	3.7	0.0000	-	-	mbPt-876498	12.8	0.0000
	3	57.2	mbPt-868642	1.6	0.0005	16	18.4	mbPt-849070	1.3	0.0005	15	13.7	mbPb-848346	3.2	0.0009	-	-	mbPb-847045	-5.0	0.0005
	3	67.7	mbPb-846221	-1.4	0.0010	16	18.3	mbPt-848870	1.1	0.0010	14	31.7	mbPt-848184	2.6	0.0019	-	-	soPb-856883	-4.4	0.0011
	7	108.6	soPt-853076	0.9	0.0015	16	18.4	mbPt-848679	1.0	0.0015	4	2.9	mbPb-867942	2.2	0.0028	-	-	soPb-855157	4.3	0.0016
	-	-	mbPb-848278	-0.9	0.0020	-	-	mbPb-848355	-0.9	0.0020	8	25.5	mbPb-847226	2.0	0.0038	8	15.0	mbPb-868706	2.8	0.0021
	-	-	mbPt-867920	-0.9	0.0025	16	18.4	mbPt-848982	0.8	0.0025	3	119.2	mbPt-848800	-1.6	0.0047	8	12.1	mbPb-877305	0.7	0.0026
	3	57.3	mbPb-848482	0.8	0.0030	18	9.6	mbPt-876498	0.8	0.0030	-	-	mbPb-848630	-1.4	0.0056	-	-	soPt-824872	-0.7	0.0032
	-	-	soPt-853236	0.8	0.0035	18	9.4	mbPt-848780	0.8	0.0035	-	-	mbPb-847295	-1.2	0.0066	16	16.4	soPb-857372	-0.6	0.0037
	3	53.5	mbPb-868529	-0.5	0.0040	4	11.7	mbPt-877115	-0.7	0.0040	8	10.8	mbPt-848152	-1.0	0.0075	-	-	soPt-856226	0.6	0.0042
	3	55.8	mbPt-868592	0.5	0.0045	18	34.3	mbPb-847443	0.6	0.0045	16	25.8	mbPt-868172	-0.9	0.0085	-	-	mbPt-870337	-0.4	0.0048
	-	-	mbPt-847629	-0.5	0.0050	18	33.1	mbPb-877453	0.6	0.0050	1	25.4	mbPt-847731	-0.9	0.0094	-	-	soPb-854385	0.3	0.0053
	3	57.2	mbPt-868369	0.5	0.0055	16	18.4	mbPt-848909	0.5	0.0055	-	-	mbPb-847269	0.7	0.0104	-	-	mbPb-869135	0.3	0.0058
	3	92.0	mbPt-846554	-0.5	0.0060	4	13.3	mbPb-846857	-0.5	0.0060	15	21.0	mbPb-868706/ mbPt-869539	31.6		3	20.1	mbPb-868537	0.3	0.0064
	3	57.3	mbPt-848482	0.5	0.0065	16	18.4	mbPt-868654	0.5	0.0065						3	20.0	mbPb-876963	0.2	0.0069
	-	-	mbPb-868056	-0.4	0.0070	-	-	soPb-831636	-0.4	0.0070						3	34.9	mbPb-847829	0.2	0.0074
	1	40.5	mbPt-848397	0.4	0.0075	8	40.1	mbPt-848184	0.4	0.0075						-	-	mbPt-877264	-0.2	0.0079
	-	-	mbPt-846221	-0.3	0.0079	4	7.2	mbPb-846165	-0.3	0.0080						3	20.0	mbPb-876614	0.1	0.0085
	-	-	mbPb-868143	-0.3	0.0084	16	18.4	mbPt-848221	0.3	0.0085						-	-	mbPt-877422	-0.1	0.0090
	3	49.9	mbPb-868582	0.3	0.0089	-	-	soPt-855434	-0.2	0.0090						13	38.7	mbPt-871632	-0.1	0.0095
	-	-	mbPb-867920	-0.3	0.0094	4	11.7	mbPt-846905	-0.2	0.0095						-	-	soPt-857270	0.1	0.0101
	3	52.9	mbPb-849000	0.3	0.0099	16	27.1	mbPt-848196	0.2	0.0100						2	12.0	mbPt-869016/ soPt-857021 †	45.2	
	3	73.0	mbPt-848800/ mbPb-868160	22.8		16	19.0	mbPt-848221/ mbPt-846885	20.2							13	38.0	mbPt-867926/ mbPt-871632 †	18.1	
																14	42.0	mbPt-848177/ mbPb-849151 †	12.5	
																16	14.0	soPt-853267/ soPb-857372 †	10.7	
Pod dehiscence	-	-	mbPt-846215	-2.4	0.0000	18	19.8	mbPt-847817	1.6	0.0000	-	-	mbPt-848285	-1.2	0.0000	-	-	mbPt-870744	1.4	0.0000
	-	-	soPt-853635	1.9	0.0005	18	19.8	mbPb-847817	1.5	0.0005	14	10.0	mbPt-846949	1.1	0.0009	8	18.7	mbPb-871035	-1.3	0.0005
	-	-	mbPt-877231	-1.6	0.0010	11	36.8	soPb-824700	1.0	0.0010	16	39.7	mbPt-847739	1.1	0.0019	16	16.4	soPb-857372	-1.3	0.0011
	6	40.6	soPb-853232	-1.5	0.0015	-	-	soPt-854823	-0.7	0.0015	-	-	mbPt-846613	-1.1	0.0028	16	19.7	mbPb-877155	-1.1	0.0016
	-	-	mbPb-849025	-1.5	0.0020	-	-	mbPt-871759	0.6	0.0020	16	41.3	mbPb-847739	1.0	0.0038	16	19.3	mbPb-868747	-1.1	0.0021
	-	-	mbPt-868548	-1.3	0.0025	18	20.2	mbPt-869376	-0.6	0.0025	3	113.8	mbPt-848441	-0.9	0.0047	14	35.4	mbPb-847271	1.1	0.0026
	11	59.0	mbPb-870853	-0.8	0.0030			soPb-831636	0.6	0.0030	3	28.1	mbPt-849068	-0.8	0.0056	-	-	soPb-832155	1.1	0.0032
	3	67.5	mbPt-868056	-0.7	0.0035	18	0.0	mbPt-869085	-0.4	0.0035	16	28.8	mbPt-846634	0.8	0.0066	16	18.9	mbPb-868071	-0.9	0.0037
	-	-	soPt-857197	0.3	0.0040	18	31.3	mbPb-877486	0.3	0.0040	8	47.8	mbPb-846816	-0.7	0.0075	-	-	mbPt-877134	0.8	0.0042
	-	-	mbPb-849138	-0.3	0.0045	-	-	mbPt-870978	0.3	0.0045	3	23.8	mbPt-848508	-0.7	0.0085	-	-	soPt-832155	0.6	0.0048
	6	24.1	mbPt-846949	0.3	0.0050	7	54.5	mbPt-847809	-0.2	0.0050	3	21.7	mbPt-846696	-0.7	0.0094	14	35.7	mbPb-867780	0.6	0.0053
	-	-	soPt-857531	-0.2	0.0055	16	65.0	mbPt-868711	0.2	0.0055	14	0.0	mbPb-847400	0.6	0.0104	14	36.1	mbPb-868016	0.6	0.0058

			1xB					1xK					87xB					87xK		
	-	-	mbPt-868832	-0.2	0.0060	17	7.4	mbPb-847343	0.2	0.0060	14	10.0	mbPt-846949/ mbPt-848613	15.6		-	-	soPb-854315	-0.6	0.0064
	3	67.7	mbPb-846221	-0.2	0.0065	-	-	mbPb-848774	-0.2	0.0065						9	48.3	mbPt-868815	0.6	0.0069
Pod dehiscence (cont…)	-	-	mbPt-876551	0.2	0.0070	-	-	mbPt-876574	-0.1	0.0070						8	8.0	mbPb-869539	-0.6	0.0074
	11	59.0	mbPb-877485	-0.1	0.0075	7	30.8	soPb-824035	-0.1	0.0075						-	-	mbPt-849188	0.5	0.0079
	6	24.0	mbPt-847535	0.1	0.0079	18	19.4	mbPt-847350	-0.1	0.0080						8	10.4	soPb-824879	0.5	0.0085
	2	31.2	mbPt-877301	0.1	0.0084	-	-	mbPt-871608	0.1	0.0085						-	-	mbPb-847267	-0.5	0.0090
	-	-	mbPt-870942	0.1	0.0089	6	7.6	mbPt-848066	-0.1	0.0090						-	-	mbPt-846445	0.4	0.0095
	-	-	mbPt-868397	-0.1	0.0094	11	35.2	soPb-825660	0.1	0.0095						8	11.7	soPb-825667	0.4	0.0101
	-	-	soPt-824112	-0.1	0.0099	-	-	soPb-832238	-0.1	0.0100						8	18.0	mbPb-868706/ mbPb-871035	20.7	
	1	10.0	mbPt-846139/ mbPt-876878 †	-19.2		7	39.0	mbPt-868516/ mbPt-846324 †	18.7											
	6	41.0	soPb-853232/ mbPt-868047 †	10.4																
	11	59.0	mbPt-848587/ mbPb-870853 †	14.6																
	-	-	mbPt-848368	2.5	0.0000	-	-	mbPt-877076	-1.0	0.0000	7	10.9	mbPt-871507	-4.0	0.0000	-	-	soPt-824890	4.8	0.0000
Powdery mildew	5	46.8	mbPb-847671	-2.4	0.0005	4	29.2	mbPt-848599	0.9	0.0005	17	23.4	mbPt-848216	3.5	0.0009	-	-	mbPb-869240	-4.6	0.0005
	-	-	mbPt-876596	2.2	0.0010	-	-	mbPt-871365	0.9	0.0010	13	40.5	mbPb-846131	-1.8	0.0019	13	19.2	mbPb-877264	4.3	0.0011
	11	2.7	mbPt-847132	2.1	0.0015	7	35.4	mbPt-876544	0.8	0.0015	-	-	mbPt-870924	1.6	0.0028	-	-	soPb-856301	-3.8	0.0016
	11	8.7	mbPt-867688	2.0	0.0020	-	-	mbPb-868595	-0.8	0.0020	17	24.1	mbPt-848057	1.5	0.0038	13	19.6	mbPb-848513	3.8	0.0021
	-	-	mbPt-849188	-1.9	0.0025	-	-	mbPt-877140	-0.6	0.0025	13	45.2	mbPt-868067	-1.3	0.0047	-	-	soPb-854293	-2.7	0.0026
	6	17.4	mbPb-846398	-1.8	0.0030	6	2.2	mbPt-846276	-0.6	0.0030	7	0.0	mbPb-848050	1.3	0.0056	-	-	mbPb-846816	2.3	0.0032
	11	33.2	mbPb-868828	-1.7	0.0035	11	58.3	mbPb-848590	-0.5	0.0035	7	13.8	soPt-825810	-1.2	0.0066	12	63.6	soPt-831671	-2.2	0.0037
	8	32.8	mbPb-870338	-1.5	0.0040	2	24.6	mbPt-867849	0.4	0.0040	17	23.7	mbPt-848087	1.1	0.0075	-	-	soPb-855237	1.8	0.0042
	-	-	mbPt-868133	1.5	0.0045	-	-	mbPb-846924	-0.4	0.0045	13	45.1	mbPt-868647	-1.0	0.0085	-	-	soPt-855509	1.4	0.0048
	11	41.6	mbPb-877269	-1.3	0.0050	11	52.3	mbPb-877052	-0.4	0.0050	-	-	mbPt-847835	-0.9	0.0094	6	29.0	mbPt-848493	-1.3	0.0053
	-	-	mbPb-849188	-1.3	0.0055	4	29.3	mbPt-847773	0.3	0.0055	9	5.7	soPt-832240	-0.9	0.0104	5	9.9	mbPt-876749	1.1	0.0058
	-	-	soPt-856857	1.2	0.0060	-	-	mbPb-876686	0.3	0.0060	3	106.0	mbPt-848435/ mbPt-848441	15.7		4	11.3	mbPt-877442	-0.8	0.0064
	12	39.9	mbPt-868108	-1.1	0.0065	4	21.1	mbPb-871533	0.3	0.0065						5	16.3	mbPb-876749	0.8	0.0069
	3	31.8	mbPt-876847	1.0	0.0070	11	53.2	mbPt-846681	-0.3	0.0070	7	12.0	mbPt-871507/ soPt-825810	-24.0		4	10.9	mbPt-849032	-0.7	0.0074
	6	10.2	mbPb-876807	1.0	0.0075	11	53.2	mbPt-847356	-0.3	0.0075								mbPb-849188	-0.6	0.0079
	5	46.8	mbPt-847671	-0.9	0.0079	-	-	mbPt-871608	-0.3	0.0080	17	23.0	mbPt-846869/ mbPt-848216	14.5		8	12.1	mbPb-877316	-0.4	0.0085
	5	50.8	soPt-853688	0.8	0.0084	11	36.0	soPb-824755	0.3	0.0085						4	11.1	mbPt-849131	-0.4	0.0090
	5	44.4	mbPb-867966	-0.7	0.0089	1	35.3	mbPt-876503	-0.3	0.0090						4	9.1	mbPt-847718	-0.4	0.0095
	-	-	mbPt-848254	0.7	0.0094	4	22.9	mbPt-868741	0.3	0.0095						16	25.6	mbPt-867765	0.3	0.0101
	11	42.1	mbPt-868763	-0.7	0.0099	11	50.8	mbPt-867894	-0.3	0.0100						13	22.0	mbPb-848100/ mbPb-846991	23.2	
	5	46.0	mbPb-867966/ mbPb-847671	10.7		7	40.0	mbPt-846324/ mbPt-868542	-19.0											
	6	13.0	mbPt-869281/ mbPt-876816	-16.6																
	8	32.0	mbPt-876991/ mbPb-870338	-17.4																
	11	0.0	mbPt-847209/ mbPt-847132	-9.5																
	11	42.0	mbPb-877269/ mbPt-868763	-8.9																
Thrips	11	22.6	mbPt-868656	3.1	0.0000	-	-	mbPt-868952	1.5	0.0000	-	-	mbPb-848630	3.1	0.0000	13	24.7	mbPb-877602	2.4	0.0000
	11	22.6	mbPt-846155	2.5	0.0005	4	11.7	mbPt-876847	1.4	0.0005	-	-	mbPt-868763	-2.4	0.0009	13	24.8	mbPb-876477	1.1	0.0005
	11	22.6	mbPt-876612	1.5	0.0010	15	19.9	mbPb-867920	1.1	0.0010	-	-	mbPt-848472	1.9	0.0019	13	24.8	mbPb-876817	1.1	0.0011
	11	25.4	mbPt-868408	1.2	0.0015	-	-	soPt-825988	-0.9	0.0015	-	-	mbPt-876762	1.8	0.0028	13	24.7	mbPb-846155	1.0	0.0016
	-	-	mbPt-847726	1.0	0.0020	-	-	mbPt-871365	0.8	0.0020	-	-	mbPb-846924	-1.7	0.0038	3	34.9	mbPt-847829	-0.6	0.0021

			1xB					1xK					87xB					87xK		
	-	-	soPb-825015	0.9	0.0025	-	-	mbPt-867794	0.7	0.0025	8	49.4	mbPt-867668	-1.7	0.0047	13	25.3	mbPb-868656	0.6	0.0026
	14	4.7	mbPt-867654	-0.7	0.0030	-	-	mbPt-870460	0.7	0.0030	-	-	mbPb-868118	1.4	0.0056	-	-	mbPb-869222	-0.5	0.0032
	-	-	soPt-824256	-0.6	0.0035	6	22.8	mbPb-867966	-0.6	0.0035	12	1.4	mbPb-868747	1.4	0.0066	-	-	mbPb-846406	-0.5	0.0037
Thrips	-	-	soPt-853145	-0.6	0.0040	-	-	soPb-857527	-0.6	0.0040	-	-	mbPt-876498	-1.3	0.0075	13	24.2	mbPb-877087	0.5	0.0042
(cont…)	7	0.0	soPt-855815	0.4	0.0045	-	-	mbPb-867652	0.6	0.0045	9	6.4	soPt-855180	-1.2	0.0085	-	-	mbPt-871798	-0.5	0.0048
	-	-	soPt-855951	0.3	0.0050	-	-	mbPt-867874	-0.5	0.0050	-	-	mbPb-871443	-1.1	0.0094	-	-	soPb-853733	0.4	0.0053
	2	7.1	soPt-857598	-0.3	0.0055	9	8.8	mbPb-847457	-0.4	0.0055	-	-	mbPb-847817	-1.0	0.0104	5	0.0	soPt-856842	0.4	0.0058
	-	-	mbPt-876551	0.3	0.0060	-	-	mbPb-868003	0.4	0.0060	12	1.0	soPb-857372/ mbPb-868823	-16.4		-	-	soPb-853425	0.4	0.0064
	-	-	mbPt-877396	0.3	0.0065	-	-	soPt-855537	0.3	0.0065						-	-	soPb-854477	0.2	0.0069
	-	-	mbPt-846581	0.3	0.0070	4	11.3	mbPb-846182	0.3	0.0070						9	47.7	mbPt-877054	0.2	0.0074
	11	62.4	soPt-855762	-0.2	0.0075	4	11.7	mbPt-846797	0.3	0.0075						13	22.5	mbPb-846991	-0.2	0.0079
	11	23.0	mbPt-868118	0.2	0.0079	-	-	soPt-854576	0.3	0.0080						-	-	mbPt-877422	0.1	0.0085
	-	-	mbPt-848296	0.2	0.0084	9	7.2	mbPt-847896	-0.2	0.0085						9	48.3	mbPt-868815	0.1	0.0090
	-	-	soPt-855180	-0.1	0.0089	-	-	mbPt-846824	-0.2	0.0090						-	-	mbPt-877264	0.1	0.0095
	11	62.3	soPt-854394	-0.1	0.0094	-	-	soPt-855675	-0.2	0.0095						13	19.0	mbPb-877422	0.1	0.0101
	3	18.8	mbPb-867820	-0.1	0.0099	4	11.7	mbPb-868485	0.2	0.0100						3	34.0	mbPb-848400/ mbPt-847829	-18.0	
	11	23.0	mbPt-877602/ mbPt-868118	-22.3		4	5.0	mbPb-867668/ mbPb-877185 †	16.9											
						15	20.0	mbPb-867920/ soPb-855725 †	74.4											
Perenniality			Not applicable					Not applicable			15	19.0	mbPb-868706	5.8	0.0000	3	33.0	mbPb-848400	1.9	0.0000
											8	28.6	mbPb-868592	1.5	0.0009	3	32.9	mbPt-848400	1.9	0.0005
											15	10.9	mbPt-846174	-1.5	0.0019	8	15.0	mbPb-868706	1.8	0.0011
											13	45.2	mbPt-868067	1.3	0.0028	12	81.9	mbPb-870893	-1.6	0.0016
											14	4.1	mbPt-849009	-1.3	0.0038	8	18.7	mbPb-871035	-1.3	0.0021
											13	45.1	mbPt-868647	1.3	0.0047	12	67.5	soPt-824363	1.0	0.0026
											-	-	mbPt-848145	-1.2	0.0056	10	16.6	mbPb-846634	1.0	0.0032
											17	28.9	mbPb-870410	1.1	0.0066	-	-	soPb-825303	0.9	0.0037
											-	-	mbPb-847272	-1.0	0.0075	-	-	mbPt-877544	0.9	0.0042
											-	-	mbPt-871137	-1.0	0.0085	-	-	soPt-824253	0.8	0.0048
											-	-	mbPb-847817	0.8	0.0094	-	-	mbPb-847947	-0.8	0.0053
											2	12.8	soPt-855244	-0.8	0.0104	-	-	mbPt-876644	-0.7	0.0058
											17	18.0	mbPt-870410/ mbPt-877415 †	-22.6		8	12.2	mbPt-877316	0.6	0.0064
																10	16.8	mbPb-868723	0.5	0.0069
																8	12.1	mbPb-877316	0.5	0.0074
																12	74.4	mbPb-868056	0.5	0.0079
																3	39.1	mbPb-870338	0.4	0.0085
																-	-	mbPt-849188	0.4	0.0090
																3	36.2	mbPb-877288	0.4	0.0095
																10	16.7	mbPt-868634	0.3	0.0101
																8	18.0	mbPb-868706 mbPb-871035	17.5	
																8	19.0	mbPb-871035/ mbPb-847338	17.4	
	-	-	mbPb-868147	40.8	0.0000	-	-	mbPb-868147	59.6	0.0000	-	-	mbPb-868147	79.6	0.0000	-	-	mbPt-868032	70.6	0.0000
Testa colour	-	-	mbPt-868147	14.5	0.0005	11	42.3	mbPb-847032	-4.1	0.0005	-	-	mbPb-868032	3.6	0.0009	-	-	soPb-854233	-1.9	0.0005
	11	14.5	mbPt-846225	5.5	0.0010	-	-	mbPt-868810	-1.7	0.0010	-	-	mbPb-847295	-1.7	0.0019	-	-	mbPb-868032	1.9	0.0011
	-	-	mbPb-846466	-2.3	0.0015	9	0.0	mbPt-870769	-1.7	0.0015	-	-	mbPt-868047	0.8	0.0028	-	-	mbPt-869395	1.8	0.0016
	11	8.7	mbPt-867688	-2.1	0.0020	-	-	mbPb-848954	-1.5	0.0020	-	-	mbPt-876614	-0.7	0.0038	-	-	mbPt-868147	1.6	0.0021
	-	-	mbPt-877014	-1.7	0.0025	11	63.4	mbPt-877174	-1.1	0.0025	-	-	mbPt-868032	0.7	0.0047	-	-	mbPb-871078	-1.2	0.0026
	11	0.0	mbPt-847209	-1.2	0.0030	-	-	mbPb-870721	-1.1	0.0030	2	14.3	soPt-831652	0.4	0.0056	14	41.5	mbPt-848177	0.6	0.0032

			1xB					1xK					87xB					87xK		
	-	-	mbPt-848612	0.7	0.0035	-	-	soPb-855332	-1.1	0.0035	-	-	mbPt-848472	-0.2	0.0066	8	14.3	mbPt-848110	0.5	0.0037
Testa colour	-	-	soPb-857252	-0.5	0.0040	11	46.8	mbPt-877264	-0.4	0.0040	-	-	mbPb-846225	0.2	0.0075	-	-	soPb-854293	-0.5	0.0042
(cont…)	-	-	soPt-824297	0.4	0.0045	-	-	mbPt-877138	-0.3	0.0045	8	3.9	mbPt-849095	0.1	0.0085	-	-	soPb-853425	-0.2	0.0048
	5	20.1	mbPb-848547	-0.3	0.0050	11	47.4	mbPb-848513	-0.2	0.0050	-	-	mbPt-847718	0.1	0.0094	-	-	mbPt-877477	0.1	0.0053
	4	12.9	mbPb-846677	0.2	0.0055	-	-	soPt-855980	-0.1	0.0055	-	-	mbPb-877052	0.0	0.0104	-	-	soPt-856013	0.1	0.0058
	1	45.7	mbPt-868577	0.1	0.0060	-	-	mbPb-848629	-0.1	0.0060						-	-	soPt-855671	0.1	0.0064
	4	12.9	mbPt-846677	0.1	0.0065	11	46.8	mbPt-848513	-0.1	0.0065						1	17.4	soPt-853185	-0.1	0.0069
	4	13.6	mbPb-846803	0.1	0.0070	7	60.7	mbPt-848296	-0.1	0.0070						-	-	mbPt-876465	0.1	0.0074
	4	13.6	mbPb-847608	0.1	0.0075	-	-	mbPt-868267	-0.1	0.0075						-	-	soPb-856686	0.0	0.0079
	11	63.1	soPb-855071	0.1	0.0079	-	-	mbPb-868885	-0.1	0.0080						-	-	mbPb-870992	0.0	0.0085
	4	13.5	mbPb-877447	0.1	0.0084	11	46.8	mbPt-877422	-0.1	0.0085						-	-	soPt-831962	0.0	0.0090
	-	-	mbPt-876932	0.0	0.0089	-	-	mbPt-847794	-0.1	0.0090						13	24.9	mbPb-876897	0.0	0.0095
	-	-	mbPt-869447	0.0	0.0094	-	-	mbPt-871187	0.1	0.0095						4	31.2	mbPt-848184	0.0	0.0101
	-	-	soPb-855739	0.0	0.0099	13	3.5	mbPb-846132	0.1	0.0100										
	11	14.0	mbPt-867688/ mbPt-846225	45.6		11	43.0	mbPt-847032/ mbPb-847470	29.1							13	6.0	mbPb-847032/ mbPt-848579	24.2	
Seed mottling	-	-	mbPb-868147	55.9	0.0000	-	-	mbPb-868147	64.7	0.0000	-	-	mbPb-868147	76.8	0.0000	-	-	mbPt-868032	52.1	0.0000
	-	-	mbPt-868147	9.2	0.0005	11	42.3	mbPb-847032	-2.7	0.0005	-	-	mbPb-868032	4.3	0.0009	-	-	mbPt-868147	7.1	0.0005
	11	14.5	mbPt-846225	3.9	0.0010	-	-	mbPb-870721	-1.1	0.0010	-	-	mbPb-847295	-1.3	0.0019	-	-	mbPb-868032	5.9	0.0011
	-	-	mbPb-846466	-2.5	0.0015	-	-	mbPt-868810	-1.0	0.0015	-	-	mbPt-868047	1.0	0.0028	-	-	soPb-854233	-1.9	0.0016
	11	8.7	mbPt-867688	-1.5	0.0020	-	-	mbPb-848954	-0.7	0.0020	-	-	mbPt-876614	-0.5	0.0038	-	-	mbPt-869395	1.7	0.0021
	-	-	mbPt-848612	1.0	0.0025	11	63.4	mbPt-877174	-0.5	0.0025	-	-	mbPt-847817	0.2	0.0047	14	41.5	mbPt-848177	1.2	0.0026
	11	0.0	mbPt-847209	-0.7	0.0030	-	-	mbPt-847794	-0.4	0.0030	-	-	mbPt-848472	-0.1	0.0056	8	14.3	mbPt-848110	1.1	0.0032
	-	-	soPb-857252	-0.4	0.0035	-	-	soPb-855332	-0.2	0.0035	8	16.0	mbPb-877356	0.1	0.0066	-	-	mbPb-871078	-0.9	0.0037
	-	-	mbPt-877014	-0.3	0.0040	-	-	mbPt-877138	-0.2	0.0040	-	-	mbPb-877052	0.1	0.0075	-	-	soPb-853425	-0.7	0.0042
	5	20.1	mbPb-848547	-0.1	0.0045	11	46.8	mbPt-877264	-0.2	0.0045	-	-	mbPt-868032	0.1	0.0085	-	-	soPb-854293	-0.6	0.0048
	-	-	soPt-824297	0.1	0.0050	-	-	soPb-854413	-0.1	0.0050	-	-	mbPt-847271	0.0	0.0094	13	0.0	mbPb-847032	-0.4	0.0053
	-	-	mbPt-876932	0.1	0.0055	11	47.4	mbPb-848513	-0.1	0.0055	-	-	mbPt-871115	0.0	0.0104	13	24.9	mbPb-876897	-0.1	0.0058
	-	-	mbPt-846576	-0.1	0.0060	13	3.5	mbPb-846132	0.1	0.0060						-	-	mbPt-876465	0.1	0.0064
	-	-	soPb-855739	0.0	0.0065	-	-	mbPb-870441	0.1	0.0065						-	-	soPt-855671	0.1	0.0069
	-	-	mbPb-846448	0.0	0.0070	-	-	soPt-855736	-0.1	0.0070						-	-	mbPt-877477	0.1	0.0074
	4	13.5	mbPb-877447	0.0	0.0075	9	0.0	mbPt-870769	-0.1	0.0075						-	-	soPt-853688	0.1	0.0079
	11	63.1	soPb-855071	0.0	0.0079	-	-	mbPb-871068	0.1	0.0080						-	-	mbPt-848202	0.0	0.0085
	4	13.6	mbPb-847608	0.0	0.0084	-	-	mbPt-868267	-0.1	0.0085						8	7.3	mbPt-869539	0.0	0.0090
	-	-	mbPt-847586	0.0	0.0089	-	-	soPt-856015	-0.1	0.0090								soPt-825332	0.0	0.0095
	4	13.6	mbPb-846803	0.0	0.0094	7	60.7	mbPt-848296	-0.1	0.0095						13	25.3	mbPb-868118	0.0	0.0101
	4	12.9	mbPb-846677	0.0	0.0099	-	-	mbPb-876686	0.1	0.0100										
	11	13.0	mbPt-867688/ mbPt-846225	54.7		11	42.0	soPb-832041/ mbPb-847032	33.6							13	7.0	mbPt-847032/ mbPt-848579	22.4	
	11	33.0	mbPt-877422/ mbPt-868828	-18.6																
Seed coat ridging			None					None			-	-	mbPb-868763	18.0	0.0000	-	-	mbPt-847646	-4.0	0.0000
											-	-	mbPt-868763	6.7	0.0009	13	16.7	mbPb-877269	3.3	0.0005
											11	18.0	soPt-854844	-1.9	0.0019	15	25.4	mbPb-877325	1.1	0.0011
											11	21.3	mbPt-848696	1.4	0.0028	-	-	mbPb-868047	0.4	0.0016
											-	-	soPb-825238	1.4	0.0038	15	21.5	mbPb-876726	-0.4	0.0021
											9	21.2	mbPt-867926	-1.0	0.0047	-	-	mbPt-849118	0.3	0.0026
											8	56.2	mbPt-846816	-0.9	0.0056	13	40.4	mbPt-870753	-0.2	0.0032
											-	-	mbPb-877332	0.9	0.0066	8	19.8	mbPb-847338	-0.2	0.0037
											-	-	mbPb-868494	-0.7	0.0075	16	19.3	mbPb-868747	0.2	0.0042
											8	20.3	mbPb-868347	0.6	0.0085	-	-	mbPb-847655	0.2	0.0048

	1xB							1xK						87xB					87xK		
Seed coat ridging (cont…)														-	-	mbPt-868260	0.6	0.0094	13	16.7	mbPb-868763	0.1	0.0053
														-	-	soPb-855834	-0.4	0.0104	-	-	mbPb-871763	-0.1	0.0058
														9	23.0	soPb-824730 soPb-825660 †	26.2		13	47.9	soPb-824730	-0.1	0.0064
																			-	-	mbPb-869385	0.1	0.0069
																			13	33.1	mbPt-877498	0.1	0.0074
																			11	0.0	mbPb-849156	0.1	0.0079
																			-	-	soPt-824435	0.1	0.0085
																			-	-	mbPb-848045	-0.1	0.0090
																			15	15.2	mbPb-877485	-0.1	0.0095
																			15	9.7	mbPb-848641	-0.1	0.0101
																			13	33.0	mbPt-868260/ mbPt-877498	17.4	
Lustre	None							None						11	18.0	soPt-854844	3.8	0.0000	4	7.5	mbPb-848613	-5.3	0.0000
														11	18.5	soPt-854547	3.7	0.0009	-	-	soPb-825303	-3.7	0.0005
														13	13.1	mbPt-869039	1.9	0.0019	-	-	mbPb-847655	-2.4	0.0011
														16	46.1	soPt-832155	1.6	0.0028	-	-	mbPt-868026	2.0	0.0016
														-	-	mbPb-848400	1.4	0.0038	-	-	mbPt-876498	1.6	0.0021
														-	-	mbPb-877332	-1.3	0.0047	9	9.4	mbPb-848643	-1.2	0.0026
														-	-	mbPt-847372	-1.1	0.0056	-	-	mbPb-848618	-0.9	0.0032
														-	-	mbPb-868506	-1.1	0.0066	4	15.7	mbPt-848172	-0.5	0.0037
														13	13.2	mbPt-876746	1.1	0.0075	10	16.7	mbPt-868634	0.5	0.0042
														-	-	mbPt-876498	-1.0	0.0085	-	-	mbPb-868626	-0.5	0.0048
														-	-	mbPb-876817	1.0	0.0094	-	-	soPb-855733	0.5	0.0053
														-	-	mbPb-868118	0.8	0.0104	-	-	mbPb-849005	0.5	0.0058
																			9	1.9	mbPt-848220	-0.5	0.0064
																			-	-	mbPt-871830	-0.4	0.0069
																			16	19.3	mbPb-868747	-0.4	0.0074
																			-	-	mbPt-849118	-0.3	0.0079
																			10	19.5	mbPt-868172	0.3	0.0085
																			-	-	soPb-856133	-0.3	0.0090
																			4	15.8	mbPb-846949	-0.3	0.0095
																			-	-	soPt-854615	-0.2	0.0101
Texture layer depth	-	-	mbPt-877004	-1.5	0.0000	11	34.4	soPb-824730	-2.7	0.0000	-	-	mbPb-868763	13.5	0.0000	13	16.7	mbPb-877269	3.5	0.0000			
	11	51.5	mbPb-877325	0.9	0.0005	11	35.2-	mbPb-847088	-1.7	0.0010	-	-	mbPt-868763	3.3	0.0009	13	25.2	mbPb-876592	1.3	0.0005			
	3	103.7	soPt-854190	0.7	0.0010	11	36.4	mbPt-868763	1.7	0.0015	9	28.1	soPt-824812	2.4	0.0019	15	25.4	mbPb-877325	1.0	0.0011			
	11	42.1	mbPt-868763	0.4	0.0015	11	36.5	mbPt-870753	-1.1	0.0020	9	23.2	soPb-825660	-1.6	0.0028	13	26.3	soPt-832041	0.8	0.0016			
	8	0.0	mbPt-848267	-0.4	0.0020	-	-	mbPt-849156	-1.0	0.0025	9	29.6	mbPb-868828	1.4	0.0038	13	33.1	mbPt-877498	0.8	0.0021			
	11	47.7	soPb-831975	-0.4	0.0025	11	36.5	mbPt-846471	-1.0	0.0030	9	43.6	mbPb-877325	1.3	0.0047	13	16.7	mbPb-868763	0.8	0.0026			
	-	-	soPt-854051	-0.3	0.0030	3	10.2	mbPb-868143	-0.8	0.0035	11	18.0	soPt-854844	-1.3	0.0056	9	9.7	mbPb-847269	0.7	0.0032			
	-	-	soPt-855259	0.3	0.0035	2	10.6	mbPb-868529	-0.8	0.0040	-	-	mbPb-846543	-1.2	0.0066	-	-	mbPb-868626	0.7	0.0037			
	-	-	mbPt-876932	0.3	0.0040	11	36.3	mbPt-846260	-0.7	0.0045	-	-	soPb-855834	-1.1	0.0075	4	15.7	mbPt-848172	0.6	0.0042			
	11	41.6	mbPb-877269	0.2	0.0045	-	-	soPt-855675	-0.7	0.0050	13	13.1	mbPt-869039	-1.0	0.0085	15	15.5	mbPb-870577	-0.6	0.0048			
	7	9.9	mbPt-847065	0.2	0.0050	11	36.3	mbPt-867926	-0.6	0.0055	-	-	mbPb-876817	-0.9	0.0094	13	32.6	mbPt-868260	0.5	0.0053			
	11	52.4	mbPt-871281	-0.2	0.0055	2	9.0	mbPb-849156	-0.6	0.0060	11	18.5	soPt-854547	-0.9	0.0104	-	-	mbPb-848736	-0.5	0.0058			
	-	-	mbPb-848563	-0.2	0.0060	-	-	mbPt-869348	-0.6	0.0065	9	25.0	soPb-825660/ mbPt-868828	46.3		9	11.3	mbPt-849035	0.4	0.0064			
	-	-	mbPb-846275	-0.1	0.0065	-	-	soPb-855332	-0.6	0.0070						13	47.9	soPb-824730	-0.4	0.0069			
	11	65.9	mbPb-868263	-0.1	0.0070	-	-	mbPt-867756	-0.5	0.0075						-	-	mbPb-847655	0.3	0.0074			
	-	-	soPt-854882	0.1	0.0075	-	-	mbPt-869349	-0.5	0.0080						13	38.7	mbPt-871632	-0.3	0.0079			
	-	-	soPb-854091	0.1	0.0079	11	37.3	mbPb-877269	0.4	0.0085						12	38.2	mbPb-868464	0.3	0.0085			
	3	1.6	mbPb-846396	0.1	0.0084	-	-	mbPt-870377	-0.4	0.0090						4	15.2	mbPb-848500	0.3	0.0090			
	11	52.0	mbPt-847882	-0.1	0.0089	-	-	mbPt-847588	-0.4	0.0095						11	0.0	mbPb-849156	0.3	0.0095			

	1xB					1xK					87xB					87xK				
Texture layer	3	57.3	mbPb-848482	0.1	0.0094	7	30.8	soPb-824035	0.3	0.0100						15	15.2	mbPb-877485	-0.3	0.0101
depth	-	-	mbPt-846350	0.1	0.0099	11	34.0	mbPb-877325/	22.5							13	33.0	mbPt-868260/	20.4	
(cont…)	-	-	mbPt-847583	0.1	0.0099			soPb-824730										mbPt-877498		
	11	41.6	mbPb-877269	50.7	0.0000	11	36.4	mbPb-868763	53.7	0.0000	-	-	mbPb-868763	36.6	0.0000	13	16.7	mbPb-877269	55.5	0.0000
Hilum colour	11	42.1	mbPt-868763	14.5	0.0005	11	36.4	mbPt-868763	13.0	0.0005	-	-	mbPt-868763	7.1	0.0009	13	16.7	mbPb-868763	6.7	0.0005
	11	42.1	mbPb-868763	3.9	0.0010	11	37.3	mbPb-877269	2.7	0.0010	9	51.0	mbPt-877498	3.5	0.0019	13	32.6	mbPt-868260	3.2	0.0011
	11	43.9	mbPt-846471	-1.7	0.0015	11	35.2	mbPb-847088	-2.1	0.0015	-	-	mbPt-868260	2.0	0.0028	13	33.1	mbPt-877498	2.9	0.0016
	-	-	mbPb-870689	-1.7	0.0020	-	-	soPb-824042	-1.6	0.0020	14	4.1	mbPt-849009	1.7	0.0038	13	35.3	mbPt-867926	-2.0	0.0021
	-	-	soPb-856436	0.7	0.0025	11	63.3	mbPb-846129	-1.5	0.0025	-	-	soPb-854239	1.6	0.0047	15	25.4	mbPb-877325	1.3	0.0026
	11	46.7	soPb-825660	-0.7	0.0030	-	-	mbPb-868365	1.0	0.0030	-	-	mbPt-847817	1.6	0.0056	13	30.7	mbPt-868828	0.9	0.0032
	11	46.7	soPb-824755	-0.7	0.0035	-	-	mbPb-871090	0.9	0.0035	5	3.8	mbPb-876509	1.2	0.0066	-	-	soPb-854329	-0.9	0.0037
	11	45.6	soPb-824700	-0.7	0.0040	11	35.6	soPb-831975	-0.6	0.0040	9	13.5	mbPt-871632	-1.0	0.0075	13	30.1	mbPb-868828	0.9	0.0042
	11	33.2	mbPb-868828	0.6	0.0045	-	-	mbPb-848079	-0.6	0.0045	-	-	mbPt-849035	0.9	0.0085	13	47.9	soPb-824730	-0.6	0.0048
	11	33.0	mbPt-868828	0.5	0.0050	11	22.9	mbPb-877325	0.3	0.0050	9	23.2	soPb-825660	-0.8	0.0094	-	-	mbPt-849175	0.6	0.0053
	11	44.1	mbPt-870753	-0.4	0.0055	-	-	mbPb-848816	-0.2	0.0055	9	22.1	soPb-824730	-0.6	0.0104	-	-	soPb-856273	-0.5	0.0058
	-	-	mbPt-846205	-0.3	0.0060	-	-	soPb-824094	-0.2	0.0060	9	23.0	soPb-824730/	16.1		-	-	soPb-856712	-0.3	0.0064
	-	-	mbPb-846466	-0.2	0.0065	-	-	mbPt-869348	-0.1	0.0065			soPb-825660			13	40.4	mbPt-870753	-0.2	0.0069
	-	-	soPt-855764	0.2	0.0070	-	-	soPt-853749	-0.1	0.0070						-	-	soPb-824755	-0.1	0.0074
	11	43.5	mbPt-846260	-0.2	0.0075	-	-	mbPt-848253	0.1	0.0075						16	16.4	soPb-857372	0.1	0.0079
	11	51.5	mbPb-877325	0.2	0.0079	11	34.4	soPb-824730	-0.1	0.0080						-	-	soPb-854608	0.0	0.0085
	11	44.2	mbPt-871632	-0.2	0.0084	11	36.7	mbPt-871632	-0.1	0.0085						16	19.3	mbPb-868747	0.0	0.0090
	-	-	mbPb-848548	0.1	0.0089	11	36.5	mbPt-846471	-0.1	0.0090						-	-	mbPb-849044	0.0	0.0095
	11	47.7	soPb-831975	-0.1	0.0094	6	26.1	mbPt-848226	0.1	0.0095						-	-	mbPt-849168	0.0	0.0101
	-	-	mbPt-848843	0.0	0.0099			soPb-832074	-0.1	0.0100						13	16.0	mbPt-848579/	29.7	
																		mbPb-868763		
																13	32.0	mbPt-868828/	37.7	
																		mbPt-868260		
	11	41.6	mbPb-877269	54.9	0.0000	11	36.4	mbPb-868763	99.0	0.0000	-	-	mbPb-868763	50.6	0.0000	13	16.7	mbPb-877269	67.9	0.0000
Texture layer colour	11	42.1	mbPt-868763	14.9	0.0005	11	46.8	mbPt-868828	0.1	0.0005	-	-	mbPt-868763	10.8	0.0009	15	25.4	mbPb-877325	3.7	0.0005
	11	42.1	mbPb-868763	3.8	0.0010						-	-	mbPt-868260	1.6	0.0019	13	47.9	soPb-824730	-1.9	0.0011
	11	33.0	mbPt-868828	1.4	0.0015						9	43.6	mbPb-877325	1.5	0.0028	-	-	mbPt-847646	-1.7	0.0016
	11	43.9	mbPt-846471	-1.3	0.0020						9	28.1	soPt-824812	1.2	0.0038	13	33.1	mbPt-877498	1.2	0.0021
	11	46.7	soPb-825660	-1.3	0.0025						9	21.2	mbPt-867926	-1.0	0.0047	13	16.7	mbPb-868763	0.8	0.0026
	11	46.7	soPb-824755	-0.8	0.0030						1	33.2	mbPt-867825	0.9	0.0056	-	-	soPb-856712	-0.8	0.0032
	-	-	soPb-856436	0.8	0.0035						16	15.6	mbPt-847390	0.8	0.0066	13	32.6	mbPt-868260	0.7	0.0037
	11	33.2	mbPb-868828	0.5	0.0040						9	23.2	soPb-825660	-0.8	0.0075	16	19.3	mbPb-868747	0.5	0.0042
	-	-	soPb-857306	0.5	0.0045						-	-	mbPb-849028	-0.7	0.0085	-	-	mbPb-868047	0.4	0.0048
	11	47.7	soPb-831975	-0.4	0.0050						9	22.1	soPb-824730	-0.5	0.0094	15	21.5	mbPb-876726	-0.3	0.0053
	11	51.5	mbPb-877325	0.3	0.0055						11	21.3	mbPt-848696	0.5	0.0104	13	40.4	mbPt-870753	-0.3	0.0058
	-	-	soPb-824730	-0.3	0.0060						9	24.0	soPb-825660/	27.0		13	25.2	mbPb-876592	0.3	0.0064
	11	44.2	mbPt-871632	-0.2	0.0065								mbPt-868828			-	-	soPt-855995	-0.3	0.0069
	-	-	mbPb-870689	-0.2	0.0070						9	42.0	mbPb-876592/	21.9		13	30.1	mbPb-868828	0.2	0.0074
	12	39.9	mbPt-868108	0.2	0.0075								mbPb-877325			-	-	mbPt-868032	-0.1	0.0079
	11	43.5	mbPt-846260	-0.2	0.0079											13	26.3	soPt-832041	0.1	0.0085
	11	44.1	mbPt-870753	-0.2	0.0084											-	-	soPb-856273	-0.1	0.0090
	11	14.5	mbPt-846225	0.1	0.0089											-	-	soPt-855040	0.1	0.0095
	-	-	mbPt-876932	0.1	0.0094											-	-	mbPt-868147	-0.1	0.0101
	-	-	soPt-854051	-0.1	0.0099											13	32.0	mbPt-868828/	37.3	
	11	42.0	mbPb-877269/	92.3														mbPt-868260		
			mbPt-868763													15	25.0	mbPb-871281/	13.2	
																		mbPb-877325		

	1xB					1xK					87xB					87xK				
Overall visual seed traits	-	-	mbPb-868147	30.8	0.0000	-	-	mbPb-868147	55.3	0.0000	-	-	mbPb-868147	39.2	0.0000	-	-	mbPt-868032	33.7	0.0000
	11	14.5	mbPt-846225	10.2	0.0005	11	36.4	mbPb-868763	18.3	0.0005	-	-	mbPb-868763	20.5	0.0009	-	-	mbPb-868032	4.0	0.0005
	-	-	mbPt-868147	10.0	0.0010	11	42.3	mbPb-847032	-2.0	0.0010	-	-	mbPb-868032	7.8	0.0019	-	-	mbPt-868147	3.5	0.0011
	11	42.1	mbPt-868763	5.9	0.0015	-	-	soPb-855332	-2.0	0.0015	-	-	mbPb-847295	-4.4	0.0028	13	16.7	mbPb-877269	3.3	0.0016
	-	-	mbPb-846466	-1.6	0.0020	9	0.0	mbPt-870769	-0.8	0.0020	9	23.2	soPb-825660	-2.5	0.0038	13	47.9	soPb-824730	-2.5	0.0021
	11	8.7	mbPt-867688	-1.5	0.0025	11	36.4	mbPt-868763	0.7	0.0025	-	-	mbPt-868763	1.4	0.0047	-	-	soPb-854233	-1.1	0.0026
	11	41.6	mbPb-877269	1.3	0.0030	-	-	soPt-855736	-0.5	0.0030	9	28.1	soPt-824812	0.9	0.0056	-	-	soPb-856273	-1.0	0.0032
	-	-	mbPt-876932	1.1	0.0035	-	-	soPt-855374	0.5	0.0035			mbPt-847718	0.7	0.0066	-	-	mbPt-869395	0.9	0.0037
	-	-	soPb-856436	1.0	0.0040	11	63.4	mbPt-877174	-0.5	0.0040	9	29.6	mbPb-868828	0.6	0.0075	13	30.1	mbPb-868828	0.9	0.0042
	11	42.1	mbPb-868763	1.0	0.0045	-	-	mbPt-877138	-0.4	0.0045	-	-	mbPt-868047	0.6	0.0085	-	-	soPb-856712	-0.8	0.0048
	-	-	soPt-824297	0.7	0.0050	11	46.8	mbPt-877264	-0.3	0.0050	9	43.6	mbPb-877325	0.6	0.0094	-	-	soPt-855509	0.7	0.0053
	11	44.2	mbPt-871632	-0.7	0.0055	-	-	soPt-856015	-0.2	0.0055	-	-	mbPb-848400	-0.6	0.0104	13	32.6	mbPt-868260	0.6	0.0058
	11	0.0	mbPt-847209	-0.5	0.0060	-	-	mbPb-870924	-0.2	0.0060	9	28.0	mbPt-868828/ soPt-824812	30.8		-	-	mbPt-849118	0.5	0.0064
	-	-	soPb-857252	-0.5	0.0065	1	15.8	soPt-831941	-0.1	0.0065						-	-	soPt-856013	0.4	0.0069
	-	-	soPb-855739	-0.5	0.0070	-	-	soPt-856984	0.1	0.0070						-	-	soPt-855605	0.4	0.0074
	1	45.7	mbPt-868577	0.2	0.0075	13	3.5	mbPb-846132	0.1	0.0075						-	-	soPb-824460	-0.3	0.0079
	11	63.1	soPb-855071	0.2	0.0079	-	-	mbPb-870721	-0.1	0.0080						13	25.3	mbPb-868118	-0.3	0.0085
	11	63.1	soPb-855180	0.1	0.0084	4	29.3	mbPt-847773	0.1	0.0085						-	-	mbPb-868595	0.3	0.0090
	11	62.0	soPt-832053	0.1	0.0089	-	-	soPb-854653	0.1	0.0090						13	33.1	mbPt-877498	0.2	0.0095
	4	29.1	mbPt-848216	0.1	0.0094	-	-	mbPb-871068	0.1	0.0095						13	30.7	mbPt-868828	0.2	0.0101
	11	46.7	soPb-825660	-0.1	0.0099	-	-	soPt-824333	0.0	0.0100						13	24.9	mbPb-876897/ mbPb-876592	41.7	
	11	12.0	mbPt-867688/ mbPt-846225	67.7		11	38.0	mbPb-877269/ mbPt-847428	12.1											
	11	42.0	mbPb-877269/ mbPt-868763	13.8		11	43.0	mbPb-847032/ mbPb-847470	23.2											
Time to flowering	8	32.8	mbPb-870338	15.7	0.0000	-	-	mbPb-870338	18.5	0.0000	-	-	mbPb-871145	2.8	0.0000	13	30.7	mbPt-868828	1.0	0.0000
	8	39.7	mbPb-877288	2.5	0.0005	-	-	mbPb-876778	-3.3	0.0005	-	-	mbPt-847829	1.3	0.0009	3	36.2	mbPb-877288	0.9	0.0005
	3	85.1	soPt-855371	-2.0	0.0010	11	35.2	soPb-825660	-3.0	0.0010	17	46.5	mbPt-847488	1.3	0.0019	-	-	soPb-853363	0.4	0.0011
	7	53.0	mbPt-848839	-1.5	0.0015	11	36.3	mbPt-846260	-2.7	0.0015	16	12.4	mbPt-847782	1.1	0.0028	13	38.7	mbPt-871632	-0.4	0.0016
	-	-	soPt-855304	1.3	0.0020	-	-	mbPt-847947	-2.0	0.0020	16	0.0	mbPt-870769	1.0	0.0038	13	27.9	soPt-824456	0.3	0.0021
	-	-	mbPt-869240	1.2	0.0025	-	-	soPt-854765	1.3	0.0025	5	7.9	mbPt-876620	-0.8	0.0047	-	-	mbPb-847556	0.3	0.0026
	11	41.6	mbPb-877269	1.1	0.0030	-	-	soPb-855926	-1.2	0.0030	9	31.2	mbPt-876592	0.8	0.0056	14	13.8	mbPb-867966	0.3	0.0032
	-	-	mbPt-868133	-1.1	0.0035	-	-	mbPt-848820	1.2	0.0035	-	-	mbPt-847671	0.7	0.0066	-	-	mbPb-848982	-0.3	0.0037
	8	39.9	mbPt-877288	1.0	0.0040	-	-	mbPb-847947	-1.0	0.0040	9	49.5	mbPt-848587	-0.5	0.0075	13	30.1	mbPb-868828	0.2	0.0042
	-	-	mbPt-877314	0.9	0.0045	11	36.3	mbPt-867926	-0.7	0.0045	-	-	mbPt-848177	0.5	0.0085	14	0.0	mbPt-848226	-0.2	0.0048
	-	-	mbPt-868938	0.7	0.0050	11	36.8	soPb-824700	-0.6	0.0050	8	14.0	mbPt-867904	-0.5	0.0094			mbPt-868670	0.2	0.0053
	-	-	mbPt-867812	-0.7	0.0055	-	-	soPb-825426	0.5	0.0055	-	-	mbPb-876726	-0.4	0.0104	13	35.3	mbPt-867926	-0.2	0.0058
	11	52.0	mbPt-847882	-0.7	0.0060	-	-	mbPb-870888	0.4	0.0060	9	23.0	soPb-824730/ soPb-825660	21.0		-	-	soPt-824315	-0.2	0.0064
	-	-	soPb-824730	-0.6	0.0065	11	46.8	mbPb-868828	0.4	0.0065						-	-	mbPt-867904	-0.2	0.0069
	-	-	mbPt-877196	0.5	0.0070	11	35.6	soPb-831975	-0.4	0.0070	16	15.0	mbPt-847782/ mbPt-847390	11.2		13	47.9	soPb-824730	-0.2	0.0074
	-	-	soPb-832041	0.5	0.0075	11	36.0	soPb-824755	-0.4	0.0075						-	-	mbPt-876498	0.2	0.0079
	11	44.1	mbPt-870753	-0.5	0.0079	7	39.8	mbPt-846324	-0.3	0.0080						-	-	mbPt-847788	0.2	0.0085
	11	46.7	soPb-825660	-0.5	0.0084	-	-	mbPt-848688	-0.3	0.0085						-	-	mbPt-867977	0.1	0.0090
	8	39.9	mbPt-847829	0.4	0.0089	4	4.2	mbPb-867668	-0.2	0.0090						-	-	mbPb-868047	0.1	0.0095
	-	-	soPt-855878	-0.4	0.0094	11	36.5	mbPt-846471	-0.2	0.0095						-	-	soPt-856491	-0.1	0.0101
	-	-	mbPt-848934	-0.3	0.0099	11	36.5	mbPt-870753	-0.2	0.0100						4	1.0	mbPb-847400/ mbPt-868384	11.6	
						7	40.0	mbPt-846324/ mbPt-868542	27.2											
						11	36.0	soPb-824755/ mbPt-867926	28.6							13	31.0	mbPt-868828/ mbPt-868260	29.9	
						19	27.0	mbPb-846990/ soPb-832109	-8.1											

	1xB					1xK					87xB					87xK				
	8	32.8	mbPb-870338	1.7	0.0000	-	-	mbPb-870338	6.6	0.0000	16	36.7	mbPb-868581	5.2	0.0000	8	12.1	mbPb-877316	4.7	0.0000
Duration of flowering	8	12.7	mbPb-848296	-1.1	0.0005	-	-	mbPb-848079	2.5	0.0005	-	-	mbPt-848376	2.8	0.0009	14	17.1	mbPt-847297	-3.0	0.0005
	-	-	mbPb-846581	-1.1	0.0010	-	-	soPb-854003	1.5	0.0010	12	10.5	mbPb-847443	1.5	0.0019	8	11.8	mbPt-868778	-2.6	0.0011
	13	31.4	soPb-824241	1.0	0.0015	-	-	mbPt-868063	1.4	0.0015	2	0.0	mbPt-867968	-1.3	0.0028	-	-	soPt-856487	2.4	0.0016
	-	-	mbPt-846581	-1.0	0.0020	-	-	soPt-855179	1.2	0.0020	-	-	mbPt-871474	-1.3	0.0038	-	-	soPt-824253	2.3	0.0021
	13	31.1	soPb-824633	0.9	0.0025	-	-	mbPt-846341	-1.2	0.0025	8	21.5	mbPt-849029	-0.9	0.0047	3	33.0	mbPb-848400	1.9	0.0026
	-	-	mbPt-849110	-0.9	0.0030	11	35.2	soPb-825660	-1.1	0.0030	16	36.1	mbPt-847457	-0.8	0.0056	-	-	soPt-854615	1.8	0.0032
	6	43.9	mbPt-847817	0.8	0.0035	-	-	mbPt-848688	-1.1	0.0035	1	6.3	mbPt-869315	-0.8	0.0066	-	-	soPb-854385	1.4	0.0037
	-	-	mbPt-849144	-0.7	0.0040	7	19.6	mbPt-877288	1.1	0.0040	-	-	mbPb-876762	-0.6	0.0075	-	-	soPt-824733	-1.2	0.0042
	11	34.2	mbPt-847428	-0.7	0.0045	-	-	mbPb-848634	1.1	0.0045	9	22.1	soPb-824730	-0.5	0.0085	-	-	soPb-856686	-1.0	0.0048
	-	-	soPb-832238	0.6	0.0050	7	39.8	mbPt-846324	-0.9	0.0050	-	-	soPt-857622	0.5	0.0094	3	34.9	mbPt-847829	0.9	0.0053
	-	-	soPb-824730	-0.6	0.0055	-	-	soPt-857002	0.9	0.0055	-	-	mbPt-868719	0.5	0.0104	-	-	soPt-853812	0.9	0.0058
	-	-	mbPt-846602	-0.6	0.0060	-	-	mbPt-869245	-0.8	0.0060	1	6.0	mbPt-869143/	-66.1		-	-	mbPt-847794	0.8	0.0064
	4	10.0	mbPb-847445	-0.6	0.0065	7	19.5	mbPb-877288	0.8	0.0065			mbPt-869315			-	-	mbPt-868580	-0.8	0.0069
	11	28.0	mbPb-877056	-0.6	0.0070	-	-	soPb-825440	-0.8	0.0070	1	14.0	mbPt-868911/	32.4		-	-	mbPb-849005	-0.7	0.0074
	-	-	mbPt-876763	-0.5	0.0075	-	-	mbPt-846390	0.7	0.0075			mbPt-869413			3	32.9	mbPt-848400	0.6	0.0079
	-	-	soPt-856063	0.5	0.0079	8	25.2	mbPt-867653	0.7	0.0080						14	22.8	mbPt-869216	-0.5	0.0085
	6	40.6	soPb-853232	-0.5	0.0084	11	36.8	soPb-824700	-0.6	0.0085						-	-	mbPt-876644	-0.3	0.0090
	13	38.1	mbPb-867891	0.5	0.0089	-	-	soPt-824253	0.6	0.0090						-	-	soPt-825838	-0.3	0.0095
	-	-	mbPt-848296	-0.5	0.0094	-	-	mbPt-867756	-0.6	0.0095						8	19.8	mbPb-847338	-0.3	0.0101
	6	43.5	mbPt-869376	-0.4	0.0099	-	-	mbPb-870815	0.6	0.0100						14	17.0	mbPb-867966/	12.5	
						7	39.0	mbPt-868516/ mbPt-846324	29.4									mbPt-847297		
	-	-	mbPt-877231	9.6	0.0000	11	35.2	soPb-825660	-2.1	0.0000	-	-	mbPt-848177	3.8	0.0000	-	-	soPb-855048	-0.9	0.0000
Pod growth duration	-	-	mbPt-868938	2.7	0.0005	-	-	mbPb-870338	1.9	0.0005	-	-	mbPb-867891	-2.1	0.0009	-	-	soPb-855733	0.8	0.0005
	1	35.5	mbPt-876485	-2.7	0.0010	-	-	mbPt-847947	-1.8	0.0010	-	-	mbPt-870886	1.7	0.0019	4	0.0	mbPb-847400	0.8	0.0011
	8	32.8	mbPb-870338	2.6	0.0015	11	36.3	mbPt-846260	-1.5	0.0015	4	0.0	mbPt-846518	-1.7	0.0028	-	-	soPb-831947	-0.8	0.0016
	-	-	mbPt-870975	-1.7	0.0020	11	36.8	soPb-824700	-1.4	0.0020	9	9.4	soPt-854394	1.3	0.0038	16	36.7	mbPt-847817	-0.6	0.0021
	-	-	mbPt-846172	1.6	0.0025	-	-	mbPb-847947	-1.3	0.0025	-	-	mbPt-848579	1.3	0.0047	5	13.5	mbPt-848809	0.5	0.0026
	11	40.4	soPb-824843	1.5	0.0030	-	-	mbPt-848688	-1.3	0.0030	4	4.3	mbPt-848547	-1.3	0.0056	-	-	soPt-853732	0.5	0.0032
	-	-	mbPt-871774	1.3	0.0035	11	36.3	mbPt-867926	-1.1	0.0035	4	3.7	mbPt-867942	-1.2	0.0066	-	-	mbPb-849113	-0.5	0.0037
	-	-	soPb-832041	1.2	0.0040	-	-	mbPb-876778	-1.1	0.0040	7	25.9	mbPt-870337	1.2	0.0075	-	-	mbPb-847419	0.4	0.0042
	11	46.7	soPb-824755	-1.1	0.0045	19	26.3	mbPb-846990	-1.1	0.0045	9	5.7	soPt-832240	0.9	0.0085	-	-	soPt-824315	-0.4	0.0048
	-	-	mbPt-846676	1.0	0.0050	-	-	mbPt-847899	-1.0	0.0050	4	5.2	mbPt-848322	-0.9	0.0094	8	7.3	mbPt-869539	0.3	0.0053
	-	-	mbPt-868133	-0.9	0.0055	11	36.0	soPb-824755	-1.0	0.0055	8	43.9	mbPt-847968	-0.9	0.0104	5	19.8	mbPt-848620	-0.2	0.0058
	11	46.7	soPb-825660	-0.8	0.0060	7	39.8	soPt-856272	-0.8	0.0060	4	1.0	mbPt-846518/	-19.8		9	35.6	mbPb-847174	-0.2	0.0064
	-	-	mbPt-847529	0.6	0.0065	-	-	soPb-855926	-0.7	0.0065			mbPt-871643			4	22.3	mbPt-848008	-0.1	0.0069
	-	-	mbPt-848202	-0.5	0.0070	7	39.8	mbPb-849130	-0.7	0.0070						12	78.5	mbPt-870843	0.1	0.0074
	-	-	mbPt-877233	0.4	0.0075	11	35.6	soPb-831975	-0.6	0.0075						-	-	soPt-823934	-0.1	0.0079
	-	-	mbPt-848504	0.3	0.0079	4	18.7	mbPb-849024	-0.6	0.0080						-	-	soPb-854329	0.1	0.0085
	-	-	mbPt-847015	0.3	0.0084	4	7.2	mbPb-846165	0.6	0.0085						-	-	soPb-826030	-0.1	0.0090
	2	20.4	mbPt-868678	-0.3	0.0089	-	-	mbPt-867991	-0.6	0.0090						12	74.4	mbPb-846221	-0.1	0.0095
	13	15.4	soPb-856458	0.2	0.0094	-	-	mbPt-849100	-0.5	0.0095						-	-	soPt-856013	-0.1	0.0101
	-	-	soPt-855453	0.2	0.0099	11	36.5	mbPt-846471	-0.5	0.0100										
						7	40.0	mbPt-846324/ mbPt-868542	16.7											
						11	37.0	soPb-824700/ mbPb-877269	14.9											
						19	25.0	soPb-825612/ mbPb-846990	-14.0											

			1xB					1xK					87xB					87xK		
	11	59.0	mbPb-868500	-4.1	0.0000	-	-	soPt-853630	2.0	0.0000	16	36.7	mbPt-868581	7.8	0.0000	8	15.0	mbPt-868706	3.3	0.0000
Growth duration	11	59.0	mbPb-877485	-3.4	0.0005	-	-	soPb-824080	-1.3	0.0005	15	19.0	mbPb-868706	6.6	0.0009	8	12.1	mbPt-877305	2.7	0.0005
	11	46.7	soPb-825660	-2.7	0.0010	11	34.4	soPb-824730	-1.1	0.0010	-	-	mbPt-868023	3.1	0.0019	8	12.2	mbPt-877316	1.6	0.0011
	-	-	soPb-857598	2.6	0.0015	11	36.4	mbPb-868763	1.1	0.0015	9	6.7	soPt-832053	2.7	0.0028	3	32.9	mbPt-848400	1.5	0.0016
	-	-	soPb-824437	2.3	0.0020	11	67.5	mbPt-868487	-0.8	0.0020	15	13.7	mbPb-848346	2.5	0.0038	16	18.3	soPb-831551	-1.4	0.0021
	2	7.1	soPt-857598	2.0	0.0025	-	-	soPb-824345	0.8	0.0025	-	-	mbPt-871474	-2.2	0.0047	3	34.9	mbPb-847829	1.4	0.0026
	11	50.9	mbPb-871281	-2.0	0.0030	11	36.4	mbPt-868763	0.8	0.0030	14	31.7	mbPt-848184	1.9	0.0056	16	19.7	mbPb-877155	-1.3	0.0032
	-	-	mbPb-870577	-1.9	0.0035	-	-	mbPt-848688	-0.8	0.0035	-	-	soPt-857280	-1.7	0.0066	16	18.9	mbPb-868071	-1.2	0.0037
	-	-	mbPt-877196	1.8	0.0040	-	-	mbPt-877076	0.7	0.0040	8	49.4	mbPt-867668	1.6	0.0075	3	34.9	mbPt-847829	1.1	0.0042
	-	-	mbPb-847271	-1.2	0.0045	11	35.6	soPb-831975	-0.5	0.0045	11	2.3	mbPt-846602	-1.6	0.0085	8	19.8	mbPb-847338	-1.1	0.0048
	-	-	mbPt-870532	-0.9	0.0050	-	-	soPt-854770	0.5	0.0050	8	28.6	mbPb-868592	1.1	0.0094	8	12.1	mbPb-877316	1.1	0.0053
	3	27.5	mbPt-867738	-0.8	0.0055	-	-	mbPb-871825	-0.5	0.0055	10	11.3	mbPb-868000	1.1	0.0104	8	21.2	mbPb-847272	-1.0	0.0058
	-	-	mbPt-846541	-0.8	0.0060	-	-	mbPb-870924	-0.4	0.0060	15	19.0	mbPb-868706/ mbPt-869539	18.2		16	16.4	soPb-857372	-1.0	0.0064
	-	-	mbPt-847471	-0.7	0.0065	7	39.8	mbPt-846324	-0.3	0.0065						3	35.8	mbPt-877288	0.9	0.0069
	-	-	mbPb-876498	0.7	0.0070	-	-	soPt-825308	-0.3	0.0070	16	37.0	mbPb-868581/ mbPt-847739	15.8		4	26.4	mbPt-847571	-0.9	0.0074
	11	59.0	mbPb-870853	-0.7	0.0075	-	-	mbPb-846298	-0.3	0.0075						8	8.0	mbPb-869539	-0.9	0.0079
	11	46.7	soPb-824755	-0.7	0.0079	-	-	mbPt-869327	-0.2	0.0080						3	33.0	mbPb-848400	0.7	0.0085
	11	40.4	soPb-824843	0.7	0.0084	12	27.1	soPb-824356	-0.2	0.0085						8	14.3	mbPt-848110	0.7	0.0090
	-	-	mbPb-867780	-0.4	0.0089			mbPt-848036	-0.2	0.0090						-	-	mbPt-847338	-0.7	0.0095
	-	-	soPt-855259	0.4	0.0094	11	36.8	soPb-824700	-0.2	0.0095						-	-	mbPt-876498	0.7	0.0101
	-	-	mbPt-876544	-0.4	0.0099	-	-	soPt-856732	-0.1	0.0100						3	35.0	mbPt-847829/ mbPt-877288	11.4	
	3	55.0	mbPb-868786/ mbPt-868786 †	-21.0		1	27.0	mbPt-877351/ mbPt-867902 †	10.5							8	16.0	mbPb-868706/ mbPb-871035	25.4	
						2	3.0	mbPt-868143/ mbPb-846816 †	-12.9							16	9.0	soPt-853267/ soPb-857372	21.5	
						7	35.0	mbPt-867887/ soPb-825779 †	-11.4											
						7	40.0	mbPt-846324/ mbPt-868542 †	33.9											
	8	39.9	mbPt-877288	-3.7	0.0000	11	14.3	mbPb-868263	-1.3	0.0000	17	15.7	mbPt-870410	3.2	0.0000	14	11.4	mbPt-847671	2.8	0.0000
Leaflet length	3	71.3	mbPt-847660	3.5	0.0005	2	28.9	mbPb-848179	1.2	0.0005	-	-	mbPb-868763	2.7	0.0009	-	-	mbPb-846816	-2.0	0.0005
	11	68.5	mbPb-867674	-3.1	0.0010	-	-	soPt-853360	1.1	0.0010	17	28.9	mbPb-870410	2.2	0.0019	-	-	soPt-855342	1.2	0.0011
	11	68.3	mbPt-848641	-2.2	0.0015	-	-	mbPt-877530	-1.0	0.0015	-	-	mbPt-848472	-1.9	0.0028	-	-	soPt-825030	0.9	0.0016
	6	50.3	mbPb-847703	1.9	0.0020	-	-	mbPt-871360	-1.0	0.0020	-	-	soPt-825889	1.3	0.0038	-	-	soPb-831947	0.9	0.0021
	3	81.2	mbPb-869293	1.9	0.0025	11	10.7	mbPb-867674	-1.0	0.0025	-	-	mbPb-877332	-1.2	0.0047	-	-	mbPb-846235	-0.7	0.0026
	11	68.3	mbPt-876514	-1.9	0.0030	2	28.0	mbPt-848179	0.7	0.0030	-	-	mbPb-847817	1.2	0.0056	8	21.2	mbPb-847272	-0.6	0.0032
	8	39.9	mbPt-847829	-1.8	0.0035	-	-	mbPb-867860	0.7	0.0035	9	6.4	soPt-855180	1.1	0.0066	9	14.9	mbPt-868489	0.6	0.0037
	5	35.3	mbPb-868679	1.1	0.0040	12	40.4	soPt-853239	0.7	0.0040	-	-	soPb-855834	-1.0	0.0075	-	-	soPb-854416	-0.4	0.0042
	-	-	soPt-853635	1.1	0.0045	6	2.2	mbPt-846276	0.7	0.0045	8	10.9	mbPb-868719	1.0	0.0085	16	22.9	mbPb-877486	0.4	0.0048
	-	-	soPb-854511	-1.0	0.0050	-	-	soPb-831604	-0.6	0.0050	17	41.5	mbPt-848650	-0.9	0.0094	16	0.0	soPt-853267	0.4	0.0053
	-	-	mbPt-877004	-1.0	0.0055	-	-	mbPt-847020	-0.6	0.0055	2	10.3	soPt-854122	-0.9	0.0104	-	-	soPt-856532	0.3	0.0058
	12	39.1	soPb-825518	1.0	0.0060	-	-	mbPb-867770	0.6	0.0060	17	16.0	mbPt-870410/ mbPt-877415	-18.6		-	-	soPt-857270	0.2	0.0064
	8	39.7	mbPb-877288	-0.9	0.0065	-	-	soPb-857384	0.5	0.0065						-	-	mbPt-868119	0.2	0.0069
	11	68.4	mbPb-848641	-0.8	0.0070	-	-	mbPt-847947	0.5	0.0070						9	7.7	mbPt-849047	0.2	0.0074
	11	67.4	mbPb-848586	-0.7	0.0075	11	10.7	mbPt-876514	-0.4	0.0075						5	18.6	mbPt-867706	-0.2	0.0079
	11	68.3	mbPt-848586	-0.7	0.0079	-	-	mbPb-876656	-0.4	0.0080						16	36.7	mbPt-847817	0.2	0.0085
	3	75.7	mbPt-848179	0.6	0.0084	14	29.5	mbPb-871157	0.4	0.0085						-	-	mbPb-846554	-0.2	0.0090
			soPb-857252	0.6	0.0089	2	29.1	mbPt-848616	0.3	0.0090						3	20.0	mbPb-876963	0.2	0.0095
	3	75.1	mbPt-848616	0.6	0.0094	-	-	mbPb-868456	-0.2	0.0095						-	-	soPb-854070	-0.1	0.0101
	3	81.2	mbPb-868009	0.5	0.0099	-	-	soPb-831794	-0.2	0.0100										

	1xB					1xK				87xB					87xK				
Leaflet length (cont…)	1	32.0	mbPt-870422/ mbPt-847689 †	21.4		6	0.0	mbPt-847507/ mbPt-846276 †	-12.3										
						7	19.0	mbPb-847372/ mbPb-877288 †	-16.7										
						11	58.0	mbPb-877020/ mbPb-848590 †	-26.2										
						12	40.0	soPt-825518/ soPt-853239 †	-10.2										
Leaflet width	5	35.3	mbPb-868679	2.4	0.0000	-	-	mbPt-846299	2.9	0.0000	15	19.0	mbPb-868706	2.1	0.0000	14	11.4	mbPt-847671	1.6	0.0000
	6	43.9	mbPt-847817	-1.3	0.0005	-	-	mbPt-871360	-2.5	0.0005	15	12.2	mbPb-877305	1.7	0.0009	-	-	mbPt-870331	-1.1	0.0005
	6	43.8	mbPt-846295	1.2	0.0010	-	-	soPt-855586	2.0	0.0010	15	12.6	mbPt-847964	1.5	0.0019	3	34.9	mbPb-847829	0.9	0.0011
	6	43.5	mbPt-869376	1.2	0.0015	11	58.3	mbPb-848590	-1.8	0.0015	15	12.3	mbPt-848346	1.2	0.0028	-	-	mbPb-868161	0.9	0.0016
	6	52.8	mbPt-877486	-0.9	0.0020	-	-	mbPb-867860	1.7	0.0020	15	12.8	mbPt-877316	1.2	0.0038	-	-	mbPt-847535	0.7	0.0021
	6	52.9	mbPb-877486	-0.9	0.0025	15	17.1	mbPb-868056	-1.4	0.0025	-	-	soPt-825889	1.0	0.0047	-	-	mbPb-848781	0.6	0.0026
	6	52.9	mbPt-877453	-0.8	0.0030	7	13.3	mbPb-871067	-1.3	0.0030	17	21.9	mbPt-846869	0.8	0.0056	3	34.9	mbPt-847829	0.6	0.0032
	12	32.4	mbPt-876746	0.8	0.0035	-	-	mbPt-846977	1.2	0.0035	15	10.9	mbPt-846174	-0.6	0.0066	3	35.8	mbPt-877288	0.5	0.0037
	6	52.8	mbPb-877453	-0.7	0.0040	-	-	mbPt-848212	0.8	0.0040	17	21.3	mbPt-877415	0.6	0.0075	5	12.7	mbPt-877042	0.5	0.0042
	5	16.8	mbPt-848177	0.6	0.0045	11	35.2	mbPb-847088	0.8	0.0045	17	28.9	mbPb-870410	0.6	0.0085	14	12.5	mbPb-847671	0.4	0.0048
	5	62.5	mbPt-867687	0.6	0.0050	18	20.2	mbPt-869376	0.7	0.0050	-	-	mbPt-848472	-0.5	0.0094	14	13.8	mbPb-867966	0.4	0.0053
	12	32.4	mbPt-869039	0.6	0.0055	18	19.8	mbPb-847817	-0.6	0.0055	17	24.1	mbPt-848057	-0.5	0.0104	-	-	mbPb-847644	0.4	0.0058
	5	32.3	mbPt-877276	0.6	0.0060	18	56.3	soPt-853267	-0.6	0.0060	15	19.0	mbPb-868706/ mbPt-869539	24.4		-	-	soPt-855342	0.4	0.0064
	6	50.3	mbPb-847703	0.6	0.0065	-	-	mbPb-877150	0.4	0.0065						3	20.0	mbPb-876963	0.4	0.0069
	-	-	mbPt-869234	-0.6	0.0070	18	19.4	mbPt-847350	0.4	0.0070						4	16.9	mbPb-846792	-0.3	0.0074
	5	59.8	mbPb-868713	0.5	0.0075	-	-	mbPt-867787	0.4	0.0075						4	16.4	mbPt-868381	-0.3	0.0079
	6	55.1	mbPb-847443	-0.5	0.0079	12	6.7	mbPt-846913	0.3	0.0080						-	-	soPt-824460	0.3	0.0085
	6	42.9	mbPt-847350	0.5	0.0084	-	-	soPt-856336	0.2	0.0085						4	16.0	mbPt-848781	0.3	0.0090
	-	-	mbPb-868147	-0.4	0.0089	-	-	mbPt-876993	-0.2	0.0090						3	32.9	mbPt-848400	0.2	0.0095
	-	-	mbPt-876952	-0.4	0.0094	-	-	soPb-831604	-0.2	0.0095						4	10.9	mbPt-849032	0.2	0.0101
	6	53.2	mbPb-868534	0.4	0.0099	15	18.0	mbPt-868056	-0.2	0.0100						14	11.0	mbPt-848226/ mbPt-847671	-15.1	
						7	13.0	mbPt-848629/ mbPb-871067 †	-10.5										
						7	42.0	mbPt-868161/ mbPt-848267 †	-10.5										
						12	24.0	mbPt-877400/ soPt-857221 †	-10.3										
Leaflet ratio	-	-	mbPt-868675	1.4	0.0000	-	-	mbPt-846229	-2.3	0.0000	16	18.4	mbPt-868581	1.9	0.0000	-	-	mbPt-848943	1.9	0.0000
	-	-	mbPt-876728	-0.8	0.0005	-	-	mbPt-869395	-1.6	0.0005	-	-	mbPb-848641	-1.8	0.0009	8	12.1	mbPb-877305	0.9	0.0005
	-	-	soPt-857628	0.7	0.0010	11	36.4	mbPb-868763	1.6	0.0010	16	12.4	mbPt-847782	1.8	0.0019	-	-	mbPt-868185	0.8	0.0011
	11	28.5	mbPt-846262	-0.5	0.0015	18	19.4	mbPt-847350	-1.4	0.0015	4	4.9	mbPb-876653	1.5	0.0028	12	74.2	mbPb-867920	0.8	0.0016
	6	46.8	soPb-855913	-0.3	0.0020	11	36.8	soPb-824700	-1.4	0.0020	-	-	mbPt-847541	1.4	0.0038	-	-	mbPt-847794	0.7	0.0021
	11	64.6	soPb-853944	0.2	0.0025	-	-	mbPt-871497	-1.2	0.0025	4	5.5	mbPb-847087	1.1	0.0047	8	10.4	soPb-824879	0.7	0.0026
	11	67.4	mbPb-848586	-0.1	0.0030	11	10.7	mbPt-848641	-1.2	0.0030	15	12.8	mbPt-877316	-1.1	0.0056	-	-	mbPt-876465	0.6	0.0032
	-	-	soPt-853635	0.1	0.0035	11	14.3	mbPb-868263	-1.1	0.0035	-	-	mbPt-848808	1.1	0.0066	14	0.0	mbPt-848226	0.6	0.0037
	-	-	mbPt-847507	-0.1	0.0040	11	10.7	mbPt-876514	-1.0	0.0040	-	-	mbPt-847835	1.0	0.0075	-	-	mbPb-870918	0.6	0.0042
	-	-	mbPt-868152	0.1	0.0045	18	56.3	soPt-853267	0.9	0.0045	-	-	mbPb-848586	-0.9	0.0085	-	-	soPt-831980	-0.5	0.0048
	6	42.9	mbPt-847350	-0.1	0.0050	-	-	soPt-832133	-0.9	0.0050	-	-	mbPb-868602	0.8	0.0094	-	-	mbPb-871067	0.5	0.0053
	-	-	mbPt-868137	-0.1	0.0055	11	35.2	mbPb-847088	-0.8	0.0055	-	-	mbPb-867674	-0.8	0.0104	8	12.2	mbPt-877316	0.5	0.0058
	11	62.1	soPt-855071	0.1	0.0060	-	-	soPt-853194	-0.8	0.0060	4	5.0	mbPb-876653/ mbPt-847087 †	19.9		8	15.0	mbPb-868706	0.5	0.0064
	6	43.5	mbPt-869376	-0.1	0.0065	11	36.4	mbPt-868763	0.7	0.0065						-	-	mbPt-871563	-0.4	0.0069
	11	62.4	soPt-855762	0.1	0.0070	-	-	mbPb-870742	0.6	0.0070						-	-	soPt-855627	0.4	0.0074
	-	-	soPt-824160	-0.1	0.0075	11	36.3	mbPt-846260	-0.6	0.0075						4	30.4	soPb-824525	-0.4	0.0079

	1xB					1xK				87xB						87xK		
	-	-	soPt-855171	-0.1	0.0079	11	10.8	mbPt-848586	-0.5	0.0080						-	-	soPt-831540	-0.4	0.0085
Leaflet ratio (cont…)	6	40.6	soPb-853232	-0.1	0.0084	11	35.2	soPb-825660	-0.5	0.0085						3	33.0	mbPb-848400	0.3	0.0090
			soPt-856094	0.1	0.0089	11	36.3	mbPt-867926	-0.5	0.0090						4	31.2	mbPt-848184	0.3	0.0095
			mbPt-849156	0.1	0.0094			mbPt-876900	-0.4	0.0095						3	32.9	mbPt-848400	0.3	0.0101
			mbPt-846201	-0.1	0.0099	11	36.7	mbPt-871632	-0.3	0.0100						4	0.0	mbPb-847400/ mbPt-868384	-11.4	
																10	15.0	mbPt-847457/ mbPb-876675	38.9	
	6	52.9	mbPt-877453	-3.8	0.0000	-	-	mbPt-869465	4.9	0.0000	-	-	mbPt-868763	3.7	0.0000	-	-	mbPb-847045	-3.3	0.0000
Petiole length	-	-	soPb-857598	2.0	0.0005	-	-	soPt-855537	-3.4	0.0005	17	21.9	mbPt-846869	2.8	0.0009	10	45.7	mbPt-848723	1.7	0.0005
	7	51.1	soPt-853126	1.4	0.0010	-	-	soPb-857306	1.5	0.0010	-	-	mbPb-868763	2.4	0.0019	14	11.4	mbPt-847671	1.6	0.0011
	7	82.7	mbPt-877349	-1.3	0.0015	8	42.5	mbPb-869464	-1.0	0.0015	-	-	soPt-825889	2.2	0.0028	-	-	soPb-856613	-1.3	0.0016
	7	45.5	mbPt-876658	1.2	0.0020	-	-	soPb-856203	-0.6	0.0020	17	28.9	mbPb-870410	1.5	0.0038	-	-	mbPt-867776	-1.1	0.0021
	6	52.8	mbPb-877453	-1.1	0.0025	10	26.6	mbPb-877550	-0.6	0.0025	16	41.3	mbPt-847739	1.4	0.0047	-	-	soPt-856697	-1.0	0.0026
	1	23.8	mbPt-847785	-1.0	0.0030	6	0.0	mbPt-847507	0.4	0.0030	-	-	mbPb-877332	-1.3	0.0056	-	-	soPt-825030	0.9	0.0032
	-	-	mbPt-869368	1.0	0.0035	18	19.8	mbPb-847817	-0.3	0.0035	-	-	mbPt-876762	-1.1	0.0066	-	-	soPt-856532	0.7	0.0037
	12	21.8	mbPt-846408	-1.0	0.0040	-	-	mbPt-869348	0.3	0.0040	8	22.3	mbPb-868642	-0.8	0.0075	-	-	mbPt-871465	-0.6	0.0042
	-	-	soPt-855247	-0.9	0.0045	6	9.9	mbPt-846941	0.3	0.0045	9	23.2	soPb-825660	-0.8	0.0085	3	34.9	mbPb-847829	0.5	0.0048
	11	63.1	soPb-853645	0.8	0.0050	15	17.1	mbPb-868056	-0.3	0.0050	-	-	mbPt-848472	-0.7	0.0094	-	-	mbPb-868161	0.5	0.0053
	11	62.4	soPt-855762	0.6	0.0055	12	6.5	mbPt-868815	0.2	0.0055	14	1.9	mbPt-871418	0.7	0.0104	3	34.9	mbPt-847829	0.5	0.0058
	11	63.1	soPb-853575	0.5	0.0060	16	51.5	mbPb-848665	0.2	0.0060	9	25.0	soPb-825660/ mbPt-868828 †	16.3		-	-	soPt-853261	0.5	0.0064
	6	52.8	mbPt-877486	-0.5	0.0065	15	30.0	mbPb-876683	-0.2	0.0065						-	-	soPb-824017	-0.4	0.0069
	-	-	mbPt-849047	0.4	0.0070	-	-	soPb-857369	-0.2	0.0070						11	3.9	mbPb-848151	-0.4	0.0074
	11	63.4	soPb-832053	0.3	0.0075	12	6.6	mbPt-877054	0.2	0.0075						-	-	mbPb-847922	-0.4	0.0079
	12	14.4	mbPt-848236	0.2	0.0079	-	-	soPb-853886	-0.2	0.0080						3	20.1	mbPb-868537	0.3	0.0085
	6	52.9	mbPb-877486	-0.2	0.0084	15	29.5	mbPb-868039	-0.2	0.0085						3	19.9	mbPt-868537	0.3	0.0090
	7	41.0	soPt-854555	0.2	0.0089	-	-	soPt-855586	0.1	0.0090						-	-	soPb-825087	0.2	0.0095
	-	-	mbPb-847117	-0.2	0.0094	-	-	mbPb-846748	0.1	0.0095						-	-	mbPt-848089	-0.2	0.0101
	1	25.1	mbPt-847260	-0.2	0.0099	8	40.1	mbPt-848184	-0.1	0.0100										
						15	29.0	soPt-831671/ mbPb-868039 †	-15.8											
	1	27.4	mbPt-847027	1.6	0.0000	6	10.0	mbPt-876555	1.7	0.0000	17	21.9	mbPt-846869	6.9	0.0000	13	16.7	mbPb-868763	4.7	0.0000
Stem diameter	5	50.8	soPt-853688	-1.2	0.0005	-	-	mbPb-847947	-1.4	0.0005	-	-	mbPb-847272	-2.0	0.0009	-	-	soPt-824253	2.5	0.0005
	-	-	soPb-854886	0.8	0.0010	-	-	mbPt-848688	-1.2	0.0010	11	2.3	mbPt-846602	-2.0	0.0019	-	-	soPt-854310	-2.3	0.0011
	5	16.9	mbPb-848405	0.7	0.0015	11	36.8	soPb-824700	-1.2	0.0015	17	28.9	mbPb-870410	1.8	0.0028	3	34.9	mbPb-847829	2.0	0.0016
	-	-	soPt-855453	0.7	0.0020	-	-	mbPt-847947	-1.1	0.0020	-	-	mbPb-868506	1.3	0.0038	10	0.0	mbPt-848229	-1.5	0.0021
	1	27.0	mbPt-870961	0.6	0.0025	1	13.3	mbPt-868454	-1.1	0.0025	9	23.2	soPb-825660	-1.3	0.0047	3	34.9	mbPt-847829	1.5	0.0026
	13	27.4	mbPb-868602	-0.5	0.0030	17	12.2	soPb-825667	-1.0	0.0030	9	22.1	soPb-824730	-1.0	0.0056	-	-	mbPb-871763	-1.3	0.0032
	-	-	mbPt-877033	0.5	0.0035	-	-	mbPb-867630	-0.9	0.0035	3	28.1	mbPt-849068	0.9	0.0066	3	35.8	mbPt-877288	1.2	0.0037
	11	63.1	soPb-853575	0.5	0.0040	-	-	soPb-857423	0.9	0.0040	8	17.9	mbPt-849000	-0.9	0.0075	-	-	soPt-823938	1.2	0.0042
	1	26.2	mbPt-870407	0.4	0.0045	6	9.9	mbPt-846941	0.8	0.0045	4	5.3	mbPb-846428	-0.8	0.0085	15	25.4	mbPb-877325	1.1	0.0048
	13	31.4	soPb-824241	-0.4	0.0050	6	9.9	mbPt-846657	0.7	0.0050	-	-	mbPt-868763	0.8	0.0094	13	35.3	mbPt-867926	-1.1	0.0053
	-	-	mbPt-877231	0.4	0.0055	11	35.2	soPb-825660	-0.6	0.0055	6	18.2	mbPt-848266	0.7	0.0104	-	-	soPt-856697	-1.0	0.0058
	6	43.9	mbPt-847817	-0.4	0.0060	6	9.9	mbPt-846877	0.6	0.0060	9	22.0	mbPt-867926/ soPb-824730	22.3		14	11.4	mbPt-847671	1.0	0.0064
	8	46.1	soPt-855179	0.3	0.0065	-	-	soPb-857384	0.5	0.0065						14	12.5	mbPb-847671	0.9	0.0069
	1	27.4	mbPt-877268	0.3	0.0070	7	39.8	mbPb-849130	-0.5	0.0070	17	22.0	mbPb-846869/ mbPt-848216	-14.3		-	-	mbPt-876498	0.6	0.0074
	8	39.7	mbPb-877288	0.3	0.0075	6	9.9	soPt-854178	0.5	0.0075						-	-	soPt-856532	0.5	0.0079
	5	16.9	mbPt-848405	0.3	0.0079	-	-	mbPt-869465	0.4	0.0080						14	13.8	mbPb-867966	0.4	0.0085
	-	-	mbPt-869474	0.2	0.0084	7	39.8	soPt-856272	-0.4	0.0085						-	-	mbPb-871843	0.4	0.0090
	-	-	soPb-832238	0.2	0.0089	-	-	soPt-853797	-0.4	0.0090						3	32.9	mbPt-848400	0.3	0.0095
	-	-	mbPb-847271	-0.2	0.0094	6	9.9	mbPt-871512	0.4	0.0095						-	-	soPb-853425	-0.3	0.0101

			1xB					1xK					87xB						87xK		
Stem diameter (cont…)	5	62.5	mbPt-867687	0.2	0.0099	11	36.3	mbPt-846260	-0.3	0.0100						13	17.0	mbPb-868763/ mbPt-848513	22.8	
						6	10.0	mbPt-876555/ mbPt-847297 †	-13.9											
						7	39.0	mbPt-868516/ mbPt-846324 †	38.0											
						16	86.0	mbPt-849105/ mbPt-846677 †	-12.1											
Internode length	11	33.2	mbPb-868828	-2.1	0.0000	7	19.6	mbPt-877288	-2.7	0.0000	-	-	soPt-853358	3.8	0.0000	-	-	mbPb-847703	-1.3	0.0000
	7	47.3	soPt-855771	1.6	0.0005	-	-	mbPb-870338	-2.5	0.0005	-	-	mbPt-870886	-3.6	0.0009	-	-	mbPb-846789	-0.8	0.0005
	-	-	mbPt-869240	-1.5	0.0010	-	-	mbPb-847947	2.2	0.0010	-	-	mbPb-848128	-2.7	0.0019	-	-	soPb-857163	0.8	0.0011
	0	2.3	mbPt-846403	-1.1	0.0015	7	19.6	mbPb-847829	-1.5	0.0015	1	14.7	mbPt-847237	2.6	0.0028	16	37.1	mbPb-869376	0.7	0.0016
	2	13.5	soPt-855873	1.1	0.0020	-	-	mbPt-847192	1.4	0.0020	10	1.2	mbPt-846221	2.4	0.0038	-	-	soPt-825810	0.7	0.0021
	12	39.9	mbPt-868108	-1.0	0.0025	-	-	soPb-857576	-1.3	0.0025	9	32.0	mbPt-846709	2.3	0.0047	-	-	soPb-855733	-0.6	0.0026
	8	32.8	mbPb-870338	-0.8	0.0030	7	19.5	mbPb-877288	-1.3	0.0030	16	12.4	mbPt-847782	-1.2	0.0056	15	11.2	soPb-825492	0.6	0.0032
	12	40.0	mbPt-848161	-0.4	0.0035	-	-	mbPt-847947	1.1	0.0035	-	-	mbPb-846225	-1.1	0.0066	9	26.9	mbPb-846954	0.5	0.0037
	-	-	soPt-853236	0.3	0.0040	7	19.6	mbPt-847829	-1.0	0.0040	9	31.9	mbPt-867723	1.1	0.0075	-	-	mbPb-869135	-0.4	0.0042
	4	22.7	mbPb-877583	-0.3	0.0045	18	34.3	mbPb-847443	0.9	0.0045	13	30.6	mbPb-846791	-1.0	0.0085	-	-	mbPt-849188	-0.3	0.0048
	-	-	mbPt-870953	-0.3	0.0050	-	-	mbPt-869485	0.9	0.0050	-	-	mbPt-877288	-1.0	0.0094	-	-	mbPt-871350	0.3	0.0053
	5	1.7	mbPt-848758	0.3	0.0055	-	-	mbPt-848848	0.9	0.0055	14	17.8	mbPt-847771	-0.9	0.0104	-	-	soPb-824436	0.3	0.0058
	-	-	soPt-853601	-0.3	0.0060	-	-	mbPb-846236	-0.7	0.0060						-	-	soPb-831821	0.2	0.0064
	-	-	mbPt-871122	-0.2	0.0065	-	-	soPt-855225	-0.5	0.0065						12	52.5	mbPb-868719	0.2	0.0069
	-	-	mbPt-877550	-0.2	0.0070	-	-	soPb-856203	-0.5	0.0070						-	-	soPt-857270	0.2	0.0074
	-	-	soPt-853177	-0.2	0.0075	-	-	soPt-855840	0.5	0.0075						-	-	mbPt-870942	0.1	0.0079
	3	81.3	mbPt-847588	-0.2	0.0079	-	-	mbPt-848123	0.5	0.0080						-	-	mbPt-868732	-0.1	0.0085
	-	-	soPt-823875	0.2	0.0084	-	-	soPt-853554	0.3	0.0085						6	13.3	mbPt-848649	-0.1	0.0090
	8	17.7	mbPb-876656	-0.2	0.0089	-	-	mbPt-871187	0.2	0.0090						-	-	mbPt-868050	-0.1	0.0095
	-	-	soPt-857112	0.2	0.0094	-	-	soPb-857423	-0.2	0.0095						12	47.6	mbPb-868741	-0.1	0.0101
	-	-	soPt-855951	0.1	0.0099	18	28.0	mbPb-867765	0.2	0.0100										
						7	19.0	mbPb-847372/ mbPb-877288	-20.7											
Floral standard width	8	9.3	soPb-856272	5.3	0.0000	9	3.8	mbPb-868634	2.8	0.0000	8	12.8	mbPb-877140	-5.9	0.0000	-	-	mbPt-870769	1.3	0.0000
	-	-	mbPb-870769	-1.6	0.0005	-	-	mbPb-876778	1.7	0.0005	8	35.6	mbPb-868412	4.0	0.0009	14	13.8	mbPb-867966	1.3	0.0005
	-	-	mbPt-868682	-1.2	0.0010	4	21.2	mbPt-871533	0.9	0.0010	-	-	mbPt-868260	3.7	0.0019	-	-	mbPt-848216	0.9	0.0011
	-	-	mbPt-869368	-0.8	0.0015	-	-	mbPb-868802	0.7	0.0015	-	-	soPb-825238	2.3	0.0028	14	12.5	mbPb-847671	0.8	0.0016
	-	-	mbPt-868961	0.8	0.0020	-	-	soPt-825848	-0.7	0.0020	8	15.3	mbPb-876985	-2.2	0.0038	7	18.1	mbPt-848800	0.7	0.0021
	-	-	mbPt-848934	0.8	0.0025	-	-	mbPb-871479	0.5	0.0025	-	-	soPt-826006	-1.7	0.0047	11	3.9	mbPb-848151	-0.7	0.0026
	6	52.9	mbPb-877486	-0.7	0.0030	-	-	soPt-823891	0.5	0.0030	-	-	mbPb-867966	1.7	0.0056	4	15.2	mbPb-848500	0.6	0.0032
	8	32.8	mbPb-870338	-0.6	0.0035	18	29.6	mbPt-877453	-0.4	0.0035	10	14.3	mbPt-867997	-1.6	0.0066	14	17.1	mbPt-847297	0.5	0.0037
	6	52.9	mbPt-867765	-0.6	0.0040	-	-	mbPb-876490	0.4	0.0040	17	28.9	mbPb-870410	-1.4	0.0075	-	-	mbPt-848057	0.5	0.0042
	8	0.0	mbPt-848267	0.5	0.0045	4	29.2	mbPt-848599	0.3	0.0045	-	-	soPt-855001	1.3	0.0085	11	5.8	mbPt-848151	-0.5	0.0048
	2	9.5	soPt-857002	-0.4	0.0050	-	-	mbPb-870338	-0.3	0.0050	8	47.8	mbPb-846816	1.2	0.0094	-	-	mbPt-867629	-0.5	0.0053
	7	0.0	soPt-855815	0.4	0.0055	4	24.1	mbPt-868438	0.3	0.0055	12	0.5	soPb-857372	1.0	0.0104	4	15.7	mbPt-848172	0.5	0.0058
	10	10.0	mbPt-871017	0.4	0.0060	-	-	soPb-856519	-0.2	0.0060						11	5.8	mbPb-848089	-0.5	0.0064
	2	7.9	mbPt-870443	-0.4	0.0065	7	39.8	mbPb-849130	0.2	0.0065						4	13.0	mbPb-867653	0.5	0.0069
	2	0.0	mbPt-870856	-0.4	0.0070	11	34.4	soPb-824730	0.2	0.0070						14	11.4	mbPt-847671	0.4	0.0074
	-	-	mbPb-868143	0.3	0.0075	-	-	soPb-825426	-0.2	0.0075						-	-	soPt-857485	0.4	0.0079
	-	-	mbPb-848860	-0.2	0.0079	16	37.9	mbPt-871350	0.2	0.0080						13	38.7	mbPt-871632	-0.4	0.0085
	-	-	mbPb-849130	0.2	0.0084	4	17.8	mbPt-867694	0.1	0.0085						11	5.7	mbPb-848406	-0.4	0.0090
	13	28.6	mbPb-877600	-0.2	0.0089	-	-	mbPt-848036	0.1	0.0090						14	22.8	mbPt-869216	0.3	0.0095
	11	8.7	mbPt-867688	0.2	0.0094	18	29.6	mbPt-877486	-0.1	0.0095						-	-	mbPt-848087	0.3	0.0101

	1xB					1xK				87xB					87xK				
Floral standard width (cont…)	11	0.0	mbPt-847209	0.1	0.0099	-	-	mbPt-877193	0.1	0.0100						14	14.0	mbPb-867966/ mbPt-847297	-17.2	
						4	18.0	mbPt-867694/ mbPb-849024	-23.1											
						7	43.0	mbPt-848267/ mbPt-876614	-10.7											
						11	37.0	soPb-824700/ mbPb-877269	-11.4											
Stem length	6	24.0	mbPt-848172	3.8	0.0000	8	0.0	mbPb-847400	2.1	0.0000	14	12.4	mbPb-848781	2.0	0.0000	15	22.7	mbPb-871281	-1.9	0.0000
	-	-	mbPt-876952	2.7	0.0005	8	23.9	mbPb-848781	2.1	0.0005	14	18.2	mbPb-849009	-1.9	0.0009	15	9.7	mbPb-848641	-1.8	0.0005
	6	24.0	mbPb-848172	2.0	0.0010	8	24.5	mbPb-848500	2.0	0.0010	-	-	mbPb-848641	-1.9	0.0019	15	9.7	mbPb-867674	-1.6	0.0011
	6	24.1	mbPb-848781	1.8	0.0015	-	-	mbPt-876952	1.8	0.0015	14	12.7	mbPb-848500	1.6	0.0028	-	-	mbPb-847045	-1.6	0.0016
	6	25.1	mbPt-868384	-1.5	0.0020	-	-	mbPt-868631	1.7	0.0020	14	12.2	mbPb-846949	1.4	0.0038	-	-	soPt-853461	1.6	0.0021
	6	24.1	mbPt-846949	1.5	0.0025	8	11.0	mbPt-868384	-1.4	0.0025	-	-	mbPb-867674	-1.4	0.0047	4	24.0	mbPb-876816	1.4	0.0026
	6	24.0	mbPt-848500	1.4	0.0030	8	7.1	mbPt-847673	1.4	0.0030	14	7.1	mbPb-848613	1.2	0.0056	13	35.3	mbPt-867926	-1.4	0.0032
	6	22.9	mbPt-848025	-1.2	0.0035	-	-	mbPb-877125	1.1	0.0035	9	14.8	soPb-853944	1.0	0.0066	-	-	mbPt-870744	1.1	0.0037
	6	24.0	mbPt-848781	1.2	0.0040	8	24.4	mbPt-847535	0.9	0.0040	14	12.7	mbPb-848172	1.0	0.0075	-	-	mbPb-869135	-1.0	0.0042
	6	23.6	mbPb-847768	1.2	0.0045	-	-	mbPt-876728	-0.9	0.0045	-	-	mbPb-877485	-0.9	0.0085	-	-	mbPb-877244	-0.9	0.0048
	6	23.3	mbPb-847535	1.1	0.0050	5	12.0	mbPt-867879	-0.8	0.0050	-	-	mbPb-867966	0.9	0.0094	-	-	mbPt-877134	0.9	0.0053
	6	25.3	mbPt-846792	-0.9	0.0055	2	0.0	mbPb-848786	0.8	0.0055	-	-	mbPb-868160	0.9	0.0104	15	9.7	mbPt-848641	-0.9	0.0058
	6	23.6	mbPt-847768	0.9	0.0060	-	-	soPt-824974	-0.7	0.0060	14	19.0	mbPb-849009/ mbPt-849166	30.5		-	-	mbPt-871830	0.6	0.0064
	6	40.0	mbPb-847872	0.9	0.0065	-	-	mbPt-847775	0.7	0.0065						-	-	mbPb-847718	0.5	0.0069
	-	-	mbPb-847400	0.8	0.0070	8	23.9	mbPb-846792	-0.7	0.0070						-	-	soPt-854310	-0.5	0.0074
	6	27.8	mbPt-847654	-0.7	0.0075	8	24.6	mbPt-848781	0.6	0.0075						12	8.0	mbPb-846396	-0.5	0.0079
	6	24.4	mbPb-848500	0.7	0.0079	-	-	soPb-855502	-0.6	0.0080						-	-	mbPt-847794	0.5	0.0085
	6	21.4	mbPb-867653	0.7	0.0084	-	-	mbPb-868284	0.6	0.0085						4	24.3	mbPb-868573	0.4	0.0090
	6	24.0	mbPt-847535	0.6	0.0089	8	5.0	mbPt-867977	0.5	0.0090						-	-	soPt-824264	0.4	0.0095
	6	25.1	mbPb-846792	-0.6	0.0094	18	56.3	soPt-853267	0.5	0.0095						15	9.5	mbPt-867674	-0.3	0.0101
	6	23.4	mbPt-868381	-0.5	0.0099	8	24.4	mbPt-848613	0.5	0.0100						3	35.0	mbPt-847829/ mbPt-877288	9.7	
						8	0.0	mbPb-847400/ mbPb-868626	16.6							4	24.0	mbPb-876816/ mbPb-846828	15.3	
						11	23.0	mbPb-877325/ soPb-824730	11.4											
Branch length	6	24.0	mbPt-848172	2.7	0.0000	-	-	mbPt-868063	11.2	0.0000	-	-	mbPt-848196	-1.9	0.0000	-	-	soPb-854416	-3.4	0.0000
	6	24.0	mbPb-848172	2.0	0.0005	8	7.1	mbPt-847673	3.5	0.0005	-	-	mbPb-867966	1.3	0.0009	3	34.9	mbPt-847829	3.1	0.0005
	6	24.1	mbPb-848781	1.6	0.0010	11	46.8-	mbPt-868828	3.3	0.0010	14	18.2	mbPb-849009	-1.1	0.0019	-	-	soPb-854205	2.3	0.0011
	6	25.1	mbPt-868384	-1.6	0.0015	8	24.5	mbPb-848500	2.0	0.0015	-	-	mbPb-847272	-1.0	0.0028	15	9.5	mbPt-867674	-1.7	0.0016
	6	24.0	mbPt-848500	1.5	0.0020	8	0.0	mbPb-847400	2.0	0.0020	-	-	soPt-824917	-0.9	0.0038	-	-	soPt-832155	1.6	0.0021
	6	24.0	mbPt-848781	1.2	0.0025	-	-	mbPb-877125	1.9	0.0025	16	41.3	mbPb-847739	0.8	0.0047	3	34.9	mbPb-847829	1.3	0.0026
	6	24.0	mbPt-847535	1.1	0.0030	-	-	mbPt-876952	1.8	0.0030	-	-	mbPt-877288	0.7	0.0056	-	-	mbPb-849188	1.3	0.0032
	6	23.6	mbPb-847768	1.0	0.0035	8	23.9	mbPb-848781	1.7	0.0035	-	-	soPb-857635	0.7	0.0066	15	25.4	mbPb-877325	1.2	0.0037
	11	65.9	mbPb-868263	-0.9	0.0040	-	-	mbPb-846236	1.7	0.0040	-	-	mbPb-876726	-0.7	0.0075	-	-	mbPt-847794	1.2	0.0042
	6	23.9	mbPt-848613	0.9	0.0045	11	35.6	soPb-831975	-1.4	0.0045	12	1.2	mbPb-868071	-0.6	0.0085	-	-	mbPt-868185	1.0	0.0048
	6	22.3	mbPb-849009	-0.9	0.0050	11	46.8	mbPb-868828	1.1	0.0050	-	-	mbPt-848405	-0.4	0.0094	-	-	mbPb-846946	0.9	0.0053
	-	-	mbPb-847400	0.9	0.0055	-	-	mbPb-876550	-0.9	0.0055	15	11.3	mbPb-877316	0.4	0.0104	3	32.9	mbPt-848400	0.8	0.0058
	-	-	mbPt-869240	0.8	0.0060	-	-	mbPb-847760	0.9	0.0060						-	-	soPt-854803	-0.7	0.0064
	6	24.1	mbPt-846949	0.8	0.0065	-	-	soPb-824664	0.9	0.0065						-	-	mbPt-870744	0.7	0.0069
	-	-	soPb-825015	-0.7	0.0070	-	-	mbPb-870338	0.8	0.0070						-	-	mbPb-847947	-0.5	0.0074
	11	50.9	mbPb-871281	-0.7	0.0075	-	-	mbPt-868631	0.7	0.0075						4	15.7	mbPt-848172	0.4	0.0079
	6	23.3	mbPb-847535	0.7	0.0079	8	11.0	mbPt-868384	-0.7	0.0080						3	36.2	mbPb-877288	0.4	0.0085
	6	23.6	mbPt-847768	0.7	0.0084	-	-	mbPt-847234	0.6	0.0085						3	35.8	mbPt-877288	0.4	0.0090
	11	53.8	mbPt-848587	-0.7	0.0089	-	-	mbPt-877438	-0.5	0.0090						-	-	mbPb-846543	-0.3	0.0095

			1xB					1xK					87xB					87xK		
	-	-	soPb-824730	-0.7	0.0094	-	-	soPb-855502	-0.5	0.0095						-	-	mbPb-868185	0.3	0.0101
Branch length (cont…)	11	51.5	mbPb-877325	0.6	0.0099	-	-	mbPt-871502	-0.5	0.0100						3	32.0	mbPb-847621/ mbPt-848400	32.4	
	5	0.0	mbPt-846370/ soPt-824786 †	27.1		1	30.0	mbPt-848952/ mbPt-848611	18.7							4	16.0	mbPb-846949/ mbPt-848781	16.6	
	5	10.0	mbPb-846370/ mbPt-876620 †	-49.2																
	11	46.7	soPb-825660	2.3	0.0000	11	36.5	mbPt-870753	6.4	0.0000	13	40.5	mbPb-846131	1.2	0.0000	3	20.8	mbPb-868715	1.2	0.0000
Peduncle length	11	44.1	mbPt-867926	2.1	0.0005	11	36.3	mbPt-846260	3.0	0.0005	-	-	mbPb-876762	-1.2	0.0009	5	13.5	mbPt-848809	-1.0	0.0005
	11	46.7	soPb-824755	1.9	0.0010	-	-	mbPt-871187	2.0	0.0010	-	-	mbPt-871820	0.8	0.0019	14	11.4	mbPt-847671	0.7	0.0011
	11	53.8	mbPt-848587	1.5	0.0015	11	36.3	mbPt-867926	1.7	0.0015	-	-	mbPt-846959	-0.6	0.0028	10	0.0	mbPt-848229	-0.6	0.0016
	5	16.3	mbPt-848322	-1.2	0.0020	6	22.8	mbPb-867966	1.6	0.0020	13	42.1	mbPt-871427	0.6	0.0038	4	13.0	mbPb-867653	0.4	0.0021
	5	16.3	mbPt-867942	-1.0	0.0025	11	36.7	mbPt-871632	1.5	0.0025	-	-	mbPb-847817	0.5	0.0047	4	19.1	mbPb-867807	-0.4	0.0026
	11	44.1	mbPt-870753	0.9	0.0030	11	36.5	mbPt-846471	1.5	0.0030	-	-	mbPb-847295	-0.5	0.0056	-	-	mbPt-848190	0.4	0.0032
	11	43.9	mbPt-846471	0.9	0.0035	11	34.4	soPb-824730	1.3	0.0035	-	-	mbPt-868260	0.5	0.0066	3	20.5	mbPb-876757	0.2	0.0037
	-	-	soPb-824730	0.8	0.0040	-	-	mbPt-847947	1.1	0.0040	-	-	soPt-825889	0.5	0.0075	14	12.5	mbPb-847671	0.2	0.0042
	15	30.2	mbPb-847272	-0.7	0.0045	17	38.8	mbPt-849118	1.0	0.0045	9	23.2	soPb-825660	-0.5	0.0085	5	12.7	mbPt-877042	0.2	0.0048
	11	44.2	mbPt-871632	0.7	0.0050	11	37.3	mbPb-877269	-1.0	0.0050	-	-	mbPt-877054	-0.5	0.0094	5	11.6	mbPb-877063	-0.2	0.0053
	11	51.5	mbPb-877325	-0.7	0.0055	15	18.0	mbPt-868056	-0.8	0.0055	-	-	mbPb-868147	0.5	0.0104	-	-	mbPt-868715	0.2	0.0058
	11	43.5	mbPt-846260	0.6	0.0060	-	-	mbPb-847898	-0.8	0.0060						5	13.2	mbPb-847658	0.2	0.0064
	15	30.2	mbPt-847338	-0.6	0.0065	16	81.4	mbPt-868511	-0.6	0.0065						3	20.0	mbPb-876991	0.2	0.0069
	11	41.6	mbPb-877269	-0.5	0.0070	-	-	mbPb-868534	-0.5	0.0070						-	-	soPt-856697	-0.2	0.0074
	11	42.1	mbPt-868763	-0.5	0.0075	-	-	mbPt-869485	0.4	0.0075						-	-	mbPt-876757	0.2	0.0079
	11	50.9	mbPb-871281	0.4	0.0079	11	36.0	soPb-824755	0.3	0.0080						-	-	soPt-857314	0.2	0.0085
	11	42.1	mbPb-868763	-0.4	0.0084	-	-	mbPb-847947	0.2	0.0085						4	25.9	mbPt-847571	-0.1	0.0090
	11	52.0	mbPt-847882	0.4	0.0089	11	41.7	soPb-832041	-0.2	0.0090						4	16.4	mbPt-868381	-0.1	0.0095
	11	47.7	soPb-831975	0.4	0.0094	16	37.9	mbPt-871350	0.1	0.0095						15	21.5	mbPb-876726	-0.1	0.0101
	11	52.4	mbPt-871281	0.3	0.0099	-	-	mbPt-876467	0.1	0.0100										
	12	40.0	mbPt-868108/ mbPt-848161	-15.7																
	-	-	mbPb-847271	-2.3	0.0000	7	19.6	mbPt-877288	4.4	0.0000	-	-	soPb-855834	-4.0	0.0000	3	33.0	mbPb-848400	4.6	0.0000
No. of branches per plant	-	-	mbPb-848278	1.3	0.0005	-	-	soPb-857384	0.7	0.0005	16	36.7	mbPb-868581	3.8	0.0009	-	-	mbPt-876498	3.7	0.0005
	-	-	mbPt-869359	1.2	0.0010	-	-	soPt-855225	0.6	0.0010	-	-	mbPb-876726	-3.3	0.0019	-	-	mbPb-847947	-2.8	0.0011
	-	-	mbPt-869395	1.0	0.0015	-	-	mbPt-870460	-0.4	0.0015	5	7.9	mbPt-876620	-2.2	0.0028	3	34.9	mbPt-847829	2.4	0.0016
	8	32.8	mbPb-870338	0.6	0.0020	4	4.2	mbPb-867668	-0.3	0.0020	-	-	mbPt-847829	2.1	0.0038	12	67.7	mbPt-847239	-2.4	0.0021
	-	-	mbPt-846508	-0.2	0.0025	18	28.0	mbPb-867765	-0.3	0.0025	8	12.8	mbPb-877140	1.9	0.0047	12	67.6	mbPb-876938	-2.3	0.0026
	5	16.8	mbPt-848547	0.2	0.0030	11	34.4	soPb-824730	-0.2	0.0030	-	-	mbPt-877288	1.5	0.0056	13	32.6	mbPt-868260	2.3	0.0032
	-	-	mbPt-876993	0.2	0.0035	-	-	mbPt-848191	-0.2	0.0035	3	47.5	mbPt-848330	1.5	0.0066	3	34.9	mbPb-847829	2.2	0.0037
	-	-	soPt-853236	-0.2	0.0040	11	35.6	soPb-831975	-0.2	0.0040	-	-	mbPb-871145	1.4	0.0075	13	12.8	mbPt-848579	-2.1	0.0042
	-	-	mbPt-871122	0.2	0.0045	7	19.5	mbPb-877288	0.2	0.0045	-	-	mbPt-869482	-1.2	0.0085	13	0.0	mbPb-847032	-2.1	0.0048
	-	-	mbPt-848934	-0.2	0.0050	-	-	mbPt-870886	0.2	0.0050	-	-	mbPb-867894	1.0	0.0094	-	-	mbPt-870337	-2.0	0.0053
	11	53.8	mbPt-848587	-0.2	0.0055	6	10.0	mbPt-876555	0.2	0.0055	-	-	mbPt-847138	1.0	0.0104	13	35.3	mbPt-867926	-1.4	0.0058
	11	46.7	soPb-825660	-0.2	0.0060	-	-	mbPt-847947	-0.1	0.0060						3	36.2	mbPb-877288	1.4	0.0064
	11	50.9	mbPb-871281	-0.2	0.0065	-	-	mbPb-847947	-0.1	0.0065						-	-	mbPt-871563	-1.3	0.0069
	2	7.1	soPt-857598	0.2	0.0070	-	-	mbPb-870338	0.1	0.0070						4	24.0	mbPb-876816	1.1	0.0074
	-	-	mbPt-849080	-0.1	0.0075	11	36.4	mbPb-868763	0.1	0.0075						3	32.9	mbPt-848400	1.1	0.0079
	8	39.9	mbPt-877288	0.1	0.0079	-	-	soPt-853173	0.1	0.0080						-	-	mbPb-870918	1.0	0.0085
	7	53.0	mbPt-848839	-0.1	0.0084	11	46.8	mbPt-868828	0.1	0.0085						3	35.8	mbPt-877288	0.9	0.0090
	12	40.0	mbPt-848161	0.1	0.0089	11	36.3	mbPt-846260	-0.1	0.0090						13	33.1	mbPt-877498	0.7	0.0095
	9	10.5	mbPb-871067	0.1	0.0094	-	-	mbPt-869245	-0.1	0.0095						-	-	mbPt-877417	0.7	0.0101
	8	12.7	mbPb-848296	-0.1	0.0099	11	36.0	soPb-824755	-0.1	0.0100										

			1xB					1xK					87xB					87xK		
	-	-	mbPt-871774	2.8	0.0000	11	36.3	mbPt-846260	-2.7	0.0000	9	22.1	soPb-824730	-0.9	0.0000	3	33.0	mbPb-848400	3.0	0.0000
No. of leaves on stem	8	32.8	mbPb-870338	2.0	0.0005	-	-	mbPb-870338	1.8	0.0005	9	23.2	soPb-825660	-0.9	0.0009	-	-	mbPt-871843	2.7	0.0005
	-	-	mbPt-867812	-0.7	0.0010	-	-	mbPt-871187	-1.8	0.0010	-	-	mbPb-868595	0.8	0.0019	3	32.9	mbPt-848400	2.1	0.0011
	-	-	mbPb-847271	-0.7	0.0015	7	39.8	mbPb-849130	-1.5	0.0015	-	-	mbPb-868506	0.7	0.0028	-	-	mbPt-868993	1.7	0.0016
	-	-	soPb-824730	-0.5	0.0020	11	35.2	soPb-825660	-1.5	0.0020	-	-	soPb-855834	-0.7	0.0038	3	34.9	mbPb-847829	1.6	0.0021
	8	39.9	mbPt-877288	0.5	0.0025	4	29.3	mbPt-847773	-1.2	0.0025	-	-	mbPt-848756	-0.6	0.0047	3	34.9	mbPt-847829	1.5	0.0026
	-	-	mbPt-868133	-0.5	0.0030	12	41.2	mbPt-868108	1.1	0.0030	-	-	mbPt-848579	0.6	0.0056	-	-	mbPt-876498	1.5	0.0032
	11	0.0	mbPt-847209	-0.3	0.0035	-	-	mbPt-847947	-0.9	0.0035	-	-	mbPb-846983	-0.5	0.0066	-	-	soPt-857232	1.2	0.0037
	-	-	mbPb-847126	0.3	0.0040	7	39.8	soPt-856272	-0.8	0.0040	17	25.5	mbPt-871350	-0.5	0.0075	-	-	soPb-825259	-1.1	0.0042
	-	-	mbPb-847280	0.2	0.0045	11	36.3	mbPt-867926	-0.7	0.0045	16	36.7	mbPb-868581	0.4	0.0085	4	30.4	soPb-824525	-1.0	0.0048
	-	-	mbPt-848798	-0.2	0.0050	-	-	mbPb-847947	-0.5	0.0050	-	-	mbPb-870825	-0.4	0.0094	3	20.0	mbPb-876963	1.0	0.0053
	1	35.5	mbPt-876485	-0.2	0.0055	11	69.9	mbPt-848192	-0.5	0.0055	-	-	mbPb-846991	0.4	0.0104	-	-	soPt-824315	-1.0	0.0058
	15	34.2	mbPt-868234	0.1	0.0060	11	36.5	mbPt-846471	-0.5	0.0060	9	24.0	soPb-825660/	18.3		-	-	soPb-855100	-0.8	0.0064
	8	36.7	mbPb-847809	-0.1	0.0065	11	36.8	soPb-824700	-0.5	0.0065			mbPt-868828 †			3	35.8	mbPt-877288	0.8	0.0069
	-	-	mbPb-870924	-0.1	0.0070	11	35.6	soPb-831975	-0.5	0.0070	13	50.0	mbPt-868067/	16.6		-	-	mbPt-849188	0.7	0.0074
	7	25.0	mbPt-869384	0.1	0.0075	-	-	soPt-853797	-0.4	0.0075			mbPt-867692 †			-	-	soPt-824253	0.6	0.0079
	-	-	mbPt-868938	0.1	0.0079	12	36.7	mbPb-847953	0.3	0.0080						12	29.0	mbPb-846351	-0.6	0.0085
	-	-	mbPt-877196	0.1	0.0084	-	-	soPb-824818	0.3	0.0085						-	-	soPb-854943	0.6	0.0090
	12	42.3	soPt-853239	-0.1	0.0089	-	-	soPt-855618	-0.3	0.0090						14	11.4	mbPt-847671	0.5	0.0095
	11	44.1	mbPt-870753	-0.1	0.0094	16	76.9	mbPt-846426	-0.3	0.0095						-	-	soPb-855815	-0.3	0.0101
	11	47.7	soPb-831975	-0.1	0.0099	16	76.9	mbPt-848620	-0.2	0.0100										
						7	40.0	mbPt-846324/ mbPt-868542	26.0											
						11	37.0	soPb-824700/ mbPb-877269	27.7											
	3	75.1	mbPt-848574	-2.5	0.0000	-	-	mbPb-847898	3.0	0.0000	9	22.1	soPb-824730	-1.6	0.0000	-	-	mbPt-870337	-2.5	0.0000
No. of nodes on stem	8	36.7	mbPb-847809	-2.3	0.0005	11	36.7	mbPt-871632	-1.4	0.0005	9	23.2	soPb-825660	-1.4	0.0009	-	-	soPb-854070	-2.4	0.0005
	-	-	mbPt-869240	2.0	0.0010	-	-	mbPb-870338	1.3	0.0010	9	27.5	mbPt-868828	1.3	0.0019	15	25.4	mbPb-877325	1.9	0.0011
	-	-	mbPb-847271	-2.0	0.0015	11	36.3	mbPt-846260	-1.2	0.0015	16	12.4	mbPt-847782	1.2	0.0028	-	-	mbPt-868047	1.4	0.0016
	11	44.2	mbPt-871632	-1.7	0.0020	11	36.3	mbPt-867926	-1.1	0.0020	-	-	soPb-855834	-0.8	0.0038	-	-	mbPt-871830	1.3	0.0021
	8	32.8	mbPb-870338	1.6	0.0025	7	39.8	mbPt-846324	-0.9	0.0025	7	0.0	mbPb-848050	-0.7	0.0047	-	-	mbPt-868993	1.1	0.0026
	-	-	mbPt-877196	1.6	0.0030	11	35.2	soPb-825660	-0.8	0.0030	-	-	mbPt-848756	-0.7	0.0056	-	-	mbPb-868580	-0.9	0.0032
	3	75.1	mbPt-867787	-1.4	0.0035	-	-	mbPt-848688	-0.8	0.0035	16	36.7	mbPb-868581	0.7	0.0066	-	-	mbPt-871774	0.5	0.0037
	5	3.8	mbPt-867879	-1.2	0.0040	-	-	mbPb-867630	-0.8	0.0040	-	-	mbPt-846445	-0.7	0.0075	13	35.3	mbPt-867926	-0.5	0.0042
	1	35.5	mbPt-876485	-1.2	0.0045	11	36.5	mbPt-846471	-0.8	0.0045	9	29.6	mbPb-868828	0.6	0.0085	3	34.9	mbPt-847829	0.4	0.0048
	-	-	mbPb-847471	-1.0	0.0050	-	-	mbPt-877138	-0.7	0.0050	9	21.2	mbPt-867926	-0.6	0.0094	-	-	soPb-824755	-0.3	0.0053
	-	-	soPb-832041	0.9	0.0055	11	36.5	mbPt-870753	-0.7	0.0055	-	-	mbPt-849062	-0.6	0.0104	-	-	soPt-824315	-0.3	0.0058
	9	8.5	mbPt-876465	-0.9	0.0060	-	-	mbPb-846236	0.6	0.0060						-	-	mbPb-871763	-0.3	0.0064
	-	-	mbPt-869447	0.9	0.0065	-	-	mbPt-870460	-0.6	0.0065						-	-	mbPt-876498	0.2	0.0069
	-	-	soPb-824730	-0.8	0.0070	11	46.8	mbPt-877264	-0.5	0.0070						-	-	mbPt-846602	-0.2	0.0074
	12	48.0	soPt-855733	0.8	0.0075	7	39.8	mbPb-849130	-0.5	0.0075						8	7.3	mbPt-869539	0.1	0.0079
			mbPb-870810	-0.7	0.0079	-	-	mbPb-849073	0.5	0.0080						3	34.9	mbPb-847829	0.1	0.0085
	11	40.4	soPb-824843	0.6	0.0084	11	35.6	soPb-831975	-0.5	0.0085						-	-	mbPt-867977	0.1	0.0090
	-	-	mbPb-867780	-0.5	0.0089	-	-	soPt-857502	-0.4	0.0090						-	-	soPt-853461	0.1	0.0095
	-	-	mbPb-869418	-0.4	0.0094	-	-	mbPb-868147	0.4	0.0095						-	-	mbPt-871563	-0.1	0.0101
	9	17.2	mbPb-846543	0.4	0.0099	11	36.0	soPb-824755	-0.4	0.0100										
	8	32.8	mbPb-870338	7.3	0.0000	7	19.6	mbPt-877288	2.6	0.0000	16	36.7	mbPb-868581	3.8	0.0000	-	-	mbPt-870337	-2.0	0.0000
No. of nodes on branches	-	-	mbPb-848278	3.0	0.0005	-	-	mbPb-870338	0.6	0.0005	11	1.7	mbPt-868026	-2.2	0.0009	15	9.5	mbPt-867674	-1.6	0.0005
	8	39.9	mbPt-877288	1.9	0.0010	11	36.4	mbPb-868763	0.6	0.0010	-	-	mbPt-848756	-1.7	0.0019	3	34.9	mbPt-847829	1.5	0.0011
	-	-	mbPt-871774	0.8	0.0015	11	36.3	mbPt-846260	-0.2	0.0015	16	12.4	mbPt-847782	1.6	0.0028	-	-	mbPb-847947	-1.1	0.0016
	8	39.7	mbPb-877288	0.6	0.0020	-	-	soPt-832047	-0.2	0.0020	-	-	mbPb-876726	-1.5	0.0038	-	-	mbPt-849037	-0.8	0.0021

	1xB					1xK					87xB					87xK				
No.of nodes on branches (cont…)	7	42.2	mbPt-871572	-0.4	0.0025	11	46.8	mbPb-868828	0.2	0.0025	-	-	mbPt-847829	1.5	0.0047	-	-	soPt-853812	0.7	0.0026
	11	50.9	mbPb-871281	-0.4	0.0030	-	-	mbPt-871479	-0.2	0.0030	-	-	mbPb-867894	1.2	0.0056	3	34.9	mbPb-847829	0.7	0.0032
	-	-	mbPt-848934	-0.3	0.0035	11	46.8	mbPt-868828	0.1	0.0035	-	-	soPb-855834	-1.2	0.0066	-	-	soPb-854293	0.7	0.0037
	-	-	mbPt-876582	-0.3	0.0040	-	-	mbPb-868534	0.1	0.0040	16	52.4	mbPt-849188	1.0	0.0075	4	25.9	mbPt-847571	-0.4	0.0042
	-	-	soPb-824730	-0.3	0.0045	7	39.8	mbPb-849130	-0.1	0.0045	13	45.1	mbPt-868647	1.0	0.0085	13	0.0	mbPb-847032	-0.3	0.0048
	3	85.1	soPt-855371	-0.2	0.0050	-	-	mbPt-869465	0.1	0.0050	11	2.3	mbPt-846602	-0.9	0.0094	-	-	mbPt-876741	0.3	0.0053
	-	-	mbPb-847271	-0.2	0.0055	7	39.8	mbPt-846324	-0.1	0.0055	13	45.2	mbPt-868067	0.9	0.0104	-	-	mbPt-876498	0.3	0.0058
	-	-	mbPt-869240	0.2	0.0060	-	-	mbPt-847775	-0.1	0.0060						-	-	mbPb-846813	-0.3	0.0064
	12	40.0	mbPt-848161	0.2	0.0065	-	-	soPt-857502	-0.1	0.0065						-	-	mbPt-849188	0.2	0.0069
	11	46.7	soPb-825660	-0.2	0.0070	11	34.4	soPb-824730	-0.1	0.0070						-	-	soPt-856809	-0.2	0.0074
	-	-	soPt-853236	-0.2	0.0075	-	-	mbPt-847947	-0.1	0.0075						-	-	mbPt-846602	-0.2	0.0079
	8	39.9	mbPt-847829	0.1	0.0079	7	19.5	mbPb-877288	0.1	0.0080						-	-	soPb-856883	-0.2	0.0085
	5	16.8	mbPt-848547	0.1	0.0084	-	-	soPb-824057	0.0	0.0085						-	-	mbPt-871774	0.2	0.0090
	-	-	mbPb-848601	-0.1	0.0089	1	23.0	mbPt-871575	0.0	0.0090						12	67.7	mbPt-847239	-0.2	0.0095
	-	-	mbPb-877400	0.1	0.0094	11	35.6	soPb-831975	0.0	0.0095						-	-	mbPt-870444	-0.2	0.0101
	3	90.5	mbPb-846554	0.1	0.0099	-	-	mbPb-847947	0.0	0.0100										
Node of 1st pod	8	32.8	mbPb-870338	4.4	0.0000	-	-	mbPb-870338	11.0	0.0000	9	22.1	soPb-824730	6.6	0.0000	8	7.3	mbPt-869539	1.4	0.0000
	-	-	mbPt-849080	-1.5	0.0005	-	-	mbPb-847947	-3.4	0.0005	-	-	soPb-855834	-3.0	0.0009	-	-	mbPt-871563	-1.3	0.0005
	8	39.9	mbPt-877288	1.3	0.0010	-	-	mbPt-847947	-3.2	0.0010	-	-	mbPb-871145	2.1	0.0019	-	-	mbPt-876498	0.8	0.0011
	-	-	mbPt-868938	0.9	0.0015	11	36.3	mbPt-867926	-1.9	0.0015	16	36.7	mbPb-868581	1.9	0.0028	-	-	mbPb-847045	-0.7	0.0016
	-	-	soPt-855304	0.5	0.0020	-	-	mbPb-876778	-1.8	0.0020	-	-	mbPt-848579	1.9	0.0038	-	-	mbPt-870337	-0.5	0.0021
	-	-	mbPt-869240	0.4	0.0025	11	35.2	soPb-825660	-1.7	0.0025	11	1.7	mbPt-868026	-1.7	0.0047	13	35.3	mbPt-867926	-0.5	0.0026
	11	42.1	mbPt-868763	0.3	0.0030	11	36.8	soPb-824700	-1.6	0.0030	-	-	mbPb-870577	-1.6	0.0056	16	19.3	mbPb-868747	0.4	0.0032
	-	-	mbPt-848934	-0.2	0.0035	11	36.3	mbPt-846260	-1.6	0.0035	16	12.4	mbPt-847782	1.5	0.0066	13	38.7	mbPt-871632	-0.3	0.0037
	11	41.6	mbPb-877269	0.2	0.0040	11	41.7	soPb-832041	1.5	0.0040	-	-	mbPb-876726	-1.5	0.0075	-	-	mbPb-869244	-0.3	0.0042
	-	-	mbPt-868490	0.1	0.0045	18	28.0	mbPb-867765	-1.4	0.0045	-	-	mbPb-868160	1.4	0.0085	3	34.9	mbPb-847829	0.3	0.0048
	-	-	mbPb-847271	-0.1	0.0050	11	46.8	mbPb-868828	1.3	0.0050	-	-	mbPt-877288	1.3	0.0094	3	36.2	mbPb-877288	0.3	0.0053
	11	33.2	mbPb-868828	0.1	0.0055	11	35.6	soPb-831975	-1.1	0.0055	16	28.8	mbPt-846634	-1.3	0.0104	13	12.8	mbPt-848579	-0.2	0.0058
	3	85.1	soPt-855371	-0.1	0.0060	19	34.4	mbPb-871193	-1.1	0.0060	9	23.0	soPb-824730/ soPb-825660	28.0		2	11.6	mbPt-869016	0.2	0.0064
	-	-	mbPt-868133	-0.1	0.0065	11	36.0	soPb-824755	-0.9	0.0065						-	-	soPt-856804	-0.2	0.0069
	11	28.8	soPt-824914	0.1	0.0070	11	36.5	mbPt-846471	-0.7	0.0070						-	-	mbPt-868047	0.2	0.0074
	8	39.7	mbPb-877288	0.1	0.0075	-	-	mbPb-847898	0.7	0.0075						-	-	mbPt-867977	0.2	0.0079
	-	-	mbPt-848183	-0.1	0.0079	11	36.5	mbPt-870753	-0.6	0.0080						3	34.9	mbPt-847829	0.1	0.0085
	13	0.0	mbPb-848300	0.1	0.0084	-	-	soPb-855926	-0.6	0.0085						-	-	mbPb-846813	-0.1	0.0090
	11	46.7	soPb-824755	-0.1	0.0089	-	-	mbPt-869465	0.4	0.0090						-	-	soPt-856741	0.1	0.0095
	11	47.7	soPb-831975	-0.1	0.0094	-	-	soPb-825426	0.4	0.0095						13	24.1	mbPt-846155	-0.1	0.0101
	-	-	mbPt-876582	-0.1	0.0099	4	29.2	mbPt-848599	-0.4	0.0100						3	35.0	mbPt-847829/ mbPt-877288	22.5	
	1	26.0	mbPt-847260/ mbPt-870407 †	38.2		7	40.0	mbPt-846324/ mbPt-868542	20.9							13	36.0	mbPt-867926/ mbPt-871632	35.3	
	1	31.0	mbPt-848299/ mbPt-876899 †	-17.2		11	36.0	soPb-824755/ mbPt-867926	43.2							14	13.0	mbPb-847671/ mbPb-867966	-8.5	
No. of pods per peduncle	6	24.1	mbPt-846949	-1.4	0.0000	4	11.7	mbPt-846797	2.0	0.0000	-	-	mbPt-847829	3.2	0.0000	13	19.2	mbPb-877264	-2.1	0.0000
	-	-	soPt-856219	-1.0	0.0005	4	11.7	mbPb-868485	1.8	0.0005	8	28.6	mbPb-868592	1.9	0.0009	-	-	mbPb-868161	1.8	0.0005
	-	-	mbPt-870553	0.9	0.0010	4	11.7	mbPb-876847	1.2	0.0010	17	15.7	mbPt-870410	1.5	0.0019	13	16.7	mbPb-868763	1.7	0.0011
	8	32.8	mbPb-870338	-0.7	0.0015	-	-	mbPb-870338	-1.1	0.0015	17	25.5	mbPt-871350	-1.2	0.0028	-	-	soPb-855006	-1.5	0.0016
	6	24.0	mbPt-848172	-0.6	0.0020	17	24.3	mbPt-847338	-1.1	0.0020	16	27.8	mbPb-846634	-1.2	0.0038	-	-	soPb-857273	-1.4	0.0021
	-	-	soPt-853745	-0.6	0.0025	-	-	mbPt-868079	1.1	0.0025	15	4.5	mbPt-848876	-1.1	0.0047	10	0.0	mbPt-848229	-1.4	0.0026
	6	50.3	mbPb-847703	0.5	0.0030	18	19.8	mbPt-847817	-1.0	0.0030	17	21.9	mbPt-846869	1.0	0.0056	-	-	mbPb-847045	-1.4	0.0032
	6	25.1	mbPt-868384	0.5	0.0035	17	23.3	mbPb-847272	-0.9	0.0035			soPt-856236	1.0	0.0066	3	34.9	mbPb-847829	1.3	0.0037
	9	3.7	mbPt-848838	-0.4	0.0040	4	11.7	mbPb-848862	0.9	0.0040	13	21.9	mbPb-877071	-0.9	0.0075	5	12.7	mbPt-877042	1.3	0.0042

	1xB							1xK							87xB							87xK				
	6	42.0	mbPt-868047	-0.4	0.0045	-	-	soPt-855179	-0.8	0.0045	13	20.5	mbPb-849187	0.8	0.0085	5	13.5	mbPt-848809	-1.2	0.0048
No. of pods per peduncle (cont…)	6	42.0	mbPt-867977	-0.4	0.0050	18	9.6	mbPt-876498	0.6	0.0050	-	-	mbPb-847272	-0.6	0.0094	-	-	soPt-853803	-1.0	0.0053
	6	23.9	mbPb-848613	-0.4	0.0055	8	20.0	mbPb-847535	-0.6	0.0055	-	-	mbPt-846959	-0.6	0.0104	-	-	soPb-825582	-0.8	0.0058
	9	0.0	mbPb-847372	-0.4	0.0060	-	-	soPt-824253	-0.5	0.0060						5	11.6	mbPb-877063	-0.7	0.0064
	9	3.9	mbPb-847026	-0.4	0.0065	4	12.4	mbPb-877112	0.4	0.0065						13	19.6	mbPb-848513	-0.6	0.0069
	9	3.7	mbPt-848696	-0.3	0.0070	18	9.4	mbPt-848780	0.4	0.0070						14	54.1	mbPb-876709	0.5	0.0074
	4	6.5	mbPt-849092	-0.3	0.0075	16	51.5	mbPt-848665	0.4	0.0075						12	0.0	mbPt-849095	-0.4	0.0079
	4	6.8	mbPt-867995	-0.3	0.0079	4	11.7	mbPb-868193	0.3	0.0080						-	-	soPb-857025	-0.4	0.0085
	3	41.3	mbPb-849024	-0.2	0.0084	18	19.8	mbPb-847817	-0.3	0.0085						-	-	mbPb-871763	-0.4	0.0090
	-	-	mbPb-847314	-0.2	0.0089	16	51.5	mbPb-848665	0.3	0.0090						-	-	soPt-856962	-0.4	0.0095
	-	-	mbPt-871546	-0.2	0.0094	4	11.8	mbPt-848862	0.2	0.0095						3	32.9	mbPt-848400	0.4	0.0101
	6	26.1	mbPb-848613	-0.2	0.0099	4	11.7	mbPb-846797	0.2	0.0100										
Total pod clusters	8	32.8	mbPb-870338	5.5	0.0000	-	-	mbPb-868405	3.0	0.0000	-	-	soPt-831748	0.8	0.0000	-	-	mbPb-847947	-4.6	0.0000
	11	40.4	soPb-824843	2.7	0.0005	-	-	mbPt-848704	-1.5	0.0005	-	-	mbPb-868506	0.7	0.0009	-	-	mbPt-871774	1.9	0.0005
	-	-	mbPb-848278	2.1	0.0010	-	-	mbPt-848820	1.0	0.0010	-	-	mbPt-869482	-0.6	0.0019	-	-	mbPt-868774	-1.8	0.0011
	-	-	mbPt-846811	-1.5	0.0015	-	-	mbPt-877530	0.8	0.0015	5	7.9	mbPt-876620	-0.6	0.0028	3	34.9	mbPt-847829	1.7	0.0016
	4	10.0	mbPb-847445	-0.7	0.0020	11	69.9	mbPt-848192	-0.7	0.0020	-	-	mbPb-847272	-0.5	0.0038	-	-	mbPt-870337	-1.5	0.0021
	11	50.9	mbPb-871281	-0.7	0.0025	11	35.2	soPb-825660	-0.6	0.0025	-	-	mbPt-849056	0.5	0.0047	-	-	mbPb-848622	1.3	0.0026
	11	28.1	soPt-824456	0.6	0.0030	7	39.8	mbPb-849130	-0.6	0.0030	-	-	mbPt-877288	0.5	0.0056	4	25.9	mbPt-847571	-1.1	0.0032
	8	39.7	mbPb-877288	0.6	0.0035	-	-	mbPb-847947	-0.5	0.0035	16	36.7	mbPb-868581	0.5	0.0066	-	-	mbPb-846946	0.8	0.0037
	8	39.9	mbPt-877288	0.5	0.0040	-	-	mbPt-847947	-0.5	0.0040	-	-	mbPb-846983	-0.4	0.0075	3	34.9	mbPb-847829	0.8	0.0042
	11	44.2	mbPt-871632	-0.4	0.0045	7	19.5	mbPb-877288	0.4	0.0045	13	45.1	mbPt-868647	0.4	0.0085	-	-	soPb-854293	0.8	0.0048
	8	47.2	soPt-824253	0.4	0.0050	11	36.0	soPb-824755	-0.4	0.0050	-	-	mbPt-867700	-0.4	0.0094	5	19.8	mbPt-848620	-0.7	0.0053
	9	10.5	mbPb-871067	0.4	0.0055	-	-	mbPt-876467	-0.4	0.0055	3	125.7	mbPt-848859	0.3	0.0104	-	-	soPb-856883	-0.4	0.0058
	6	52.9	mbPb-877486	0.3	0.0060	-	-	mbPb-871479	-0.4	0.0060	16	27.0	mbPt-868172/	-26.4		12	66.0	soPb-824061	-0.4	0.0064
	-	-	mbPt-869537	0.3	0.0065	11	36.8	soPb-824700	-0.3	0.0065			mbPt-868723 †			14	0.0	mbPt-848226	0.4	0.0069
	-	-	soPt-823875	-0.3	0.0070			mbPb-870338	0.3	0.0070	16	38.0	mbPb-868581/	24.5		12	67.6	mbPt-876938	-0.4	0.0074
	8	39.9	mbPt-847829	0.2	0.0075	11	67.5	mbPt-868487	-0.2	0.0075			mbPt-847739 †			3	35.8	mbPt-877288	0.3	0.0079
	-	-	mbPt-846581	-0.2	0.0079	11	36.3	mbPt-846260	-0.2	0.0080						-	-	mbPt-871563	-0.3	0.0085
	11	28.8	soPt-824914	0.2	0.0084	12	26.0	mbPt-868606	0.2	0.0085						4	26.4	mbPb-847571	-0.3	0.0090
	-	-	mbPt-848296	-0.2	0.0089	11	41.7	soPb-832041	0.1	0.0090						-	-	mbPt-876741	0.2	0.0095
	-	-	mbPt-871038	0.2	0.0094	-	-	mbPb-868442	0.1	0.0095						-	-	mbPt-876498	0.2	0.0101
	5	9.2	mbPb-846147	-0.2	0.0099	-	-	mbPt-877414	0.1	0.0100										
	7	118.0	soPt-853076/ soPt-853099	62.6		11	37.0	soPb-824700/ mbPb-877269	15.8							3	34.0	mbPb-848400/ mbPt-847829	24.7	
No. of seeds per pod	8	47.2	soPt-824253	2.6	0.0000	-	-	mbPt-846144	-1.0	0.0000	-	-	mbPb-848630	-4.1	0.0000	3	32.9	mbPt-848400	2.0	0.0000
	-	-	mbPt-867729	2.5	0.0005	-	-	mbPt-848252	-0.9	0.0005	14	31.7	mbPt-848184	3.4	0.0009	3	33.0	mbPb-848400	1.6	0.0005
	-	-	mbPb-869418	1.4	0.0010	-	-	mbPt-848387	-0.8	0.0010	15	13.7	mbPb-848346	2.1	0.0019	-	-	mbPt-847947	-1.2	0.0011
	4	35.7	mbPb-847386	-1.4	0.0015	-	-	mbPb-868556	0.3	0.0015	-	-	mbPt-847829	1.5	0.0028	3	0.0	mbPb-870846	1.2	0.0016
	8	46.1	soPt-855179	1.1	0.0020	14	16.7	mbPb-870582	0.2	0.0020	8	30.3	mbPb-870385	1.5	0.0038	-	-	mbPt-868185	1.1	0.0021
	-	-	soPb-857252	-1.1	0.0025	-	-	mbPt-871227	-0.2	0.0025	11	22.1	mbPt-848838	1.3	0.0047	3	20.0	mbPb-876963	1.0	0.0026
	11	59.0	mbPb-868500	-1.0	0.0030	15	18.9	mbPb-868697	0.1	0.0030	-	-	mbPt-847541	-1.3	0.0056	3	34.9	mbPt-847829	0.8	0.0032
	-	-	mbPb-847947	-1.0	0.0035	-	-	soPb-824650	-0.1	0.0035	17	46.5	mbPt-847488	1.2	0.0066	-	-	mbPb-869080	0.7	0.0037
	8	32.8	mbPb-870338	0.9	0.0040	-	-	soPt-855571	0.1	0.0040	16	39.7	mbPt-847739	1.1	0.0075	-	-	mbPt-876498	0.7	0.0042
	-	-	mbPt-868697	0.8	0.0045	-	-	mbPt-877455	-0.1	0.0045	14	30.3	mbPt-869464	1.0	0.0085	15	21.5	mbPb-876726	-0.7	0.0048
	-	-	mbPb-868608	-0.7	0.0050	12	28.0	mbPb-848643	-0.1	0.0050	-	-	mbPb-849028	-1.0	0.0094	-	-	mbPb-868185	0.6	0.0053
	1	45.7	mbPt-868577	0.7	0.0055	-	-	mbPb-848954	0.1	0.0055	-	-	mbPt-877288	1.0	0.0104	3	36.2	mbPb-877288	0.5	0.0058
	-	-	mbPt-870843	0.7	0.0060	11	37.3	mbPb-877269	0.1	0.0060						-	-	mbPb-867891	0.5	0.0064
	3	28.6	mbPb-846287	-0.6	0.0065	-	-	mbPt-868278	-0.1	0.0065						-	-	mbPb-849005	-0.5	0.0069
	11	63.1	soPb-855762	0.5	0.0070	-	-	mbPb-869237	-0.1	0.0070						-	-	soPt-853719	0.5	0.0074
	-	-	mbPt-876544	-0.5	0.0075	-	-	soPb-825361	0.0	0.0075						3	4.8	mbPb-847372	0.4	0.0079

	1xB					1xK					87xB					87xK				
	11	59.0	mbPb-877485	-0.5	0.0079	-	-	mbPt-868230	0.0	0.0080						3	6.1	soPt-856082	-0.4	0.0085
No. of seeds per	-	-	soPt-854325	-0.3	0.0084	16	84.3	mbPb-876749	0.0	0.0085						11	0.0	mbPb-849156	0.3	0.0090
pod	11	63.1	soPb-854394	0.3	0.0089	-	-	mbPt-846574	0.0	0.0090						10	36.3	mbPb-876479	0.3	0.0095
(cont…)	-	-	mbPb-871654	-0.3	0.0094	12	27.2	mbPb-876821	0.0	0.0095						-	-	mbPt-847794	0.3	0.0101
	-	-	mbPb-870577	-0.3	0.0099	-	-	mbPb-871346	0.0	0.0100										
Pod length	-	-	mbPb-868608	-5.6	0.0000	18	20.2	mbPt-869376	7.5	0.0000	-	-	mbPb-877550	5.4	0.0000	-	-	mbPb-877328	-3.0	0.0000
	6	69.7	soPt-853267	-4.1	0.0005	18	56.3	soPt-853267	-4.4	0.0005	-	-	mbPt-848177	4.5	0.0009	-	-	mbPt-870337	-2.0	0.0005
	5	16.8	mbPt-848177	3.2	0.0010	4	26.2	mbPt-848227	-3.7	0.0010	-	-	mbPt-868023	4.0	0.0019	-	-	soPt-857270	1.4	0.0011
	6	43.5	mbPt-869376	2.3	0.0015	18	19.8	mbPt-847817	-2.4	0.0015	9	23.2	soPb-825660	-3.7	0.0028	-	-	mbPt-867776	-1.2	0.0016
	6	43.8	mbPt-846295	1.9	0.0020	18	19.4	mbPt-847350	2.2	0.0020	-	-	mbPb-877600	2.3	0.0038	3	34.9	mbPt-847829	0.7	0.0021
	-	-	mbPt-869085	1.8	0.0025	18	19.8	mbPb-847817	-2.2	0.0025	-	-	mbPb-847817	-2.2	0.0047	5	12.7	mbPt-877042	0.7	0.0026
	6	43.9	mbPt-847817	-1.7	0.0030	4	12.3	mbPt-848956	1.7	0.0030	15	13.7	mbPb-848346	2.0	0.0056	11	0.0	mbPb-849156	0.7	0.0032
	1	43.3	mbPt-846746	-1.4	0.0035	18	7.3	soPb-853232	1.6	0.0035	-	-	mbPt-847817	-1.9	0.0066	3	32.9	mbPt-848400	0.5	0.0037
	6	52.8	mbPt-877486	-1.2	0.0040	4	8.3	mbPb-847068	-1.6	0.0040	1	21.9	mbPt-868447	1.9	0.0075	10	36.3	mbPb-876479	0.5	0.0042
	-	-	soPt-853543	-1.1	0.0045	4	0.4	mbPt-848455	-1.4	0.0045	-	-	mbPb-849028	-1.6	0.0085	11	14.5	mbPt-848786	-0.3	0.0048
	6	52.9	mbPt-867765	-0.9	0.0050	18	8.0	soPb-855913	1.4	0.0050	12	9.5	mbPb-877486	-1.4	0.0094	4	36.6	mbPt-871418	0.3	0.0053
	6	42.9	mbPt-847350	0.9	0.0055	18	21.5	mbPt-848907	1.1	0.0055	-	-	soPb-855834	-1.3	0.0104	13	38.7	mbPt-871632	-0.3	0.0058
	6	44.4	mbPt-848907	0.7	0.0060	-	-	soPt-824890	1.0	0.0060	9	21.0	soPb-853944/	24.1		14	26.8	mbPt-877276	0.3	0.0064
	3	28.8	mbPt-877159	-0.6	0.0065	18	29.6	mbPt-877453	-0.9	0.0065			mbPt-867926			-	-	mbPt-868047	0.2	0.0069
	6	40.6	soPb-853232	0.5	0.0070	2	9.1	mbPt-848786	-0.9	0.0070						-	-	mbPt-876460	-0.2	0.0074
	6	52.8	mbPb-877453	-0.4	0.0075	18	29.7	mbPt-847443	-0.7	0.0075						14	26.3	mbPt-848285	0.1	0.0079
	5	16.9	mbPt-848405	0.4	0.0079	-	-	soPt-857444	0.7	0.0080						-	-	soPt-832048	-0.1	0.0085
	3	49.9	mbPb-868582	-0.4	0.0084	2	11.6	mbPt-849061	0.6	0.0085						15	25.4	mbPb-877325	0.1	0.0090
	3	28.3	mbPt-877115	-0.3	0.0089	-	-	mbPb-871090	-0.6	0.0090						-	-	mbPt-871774	0.1	0.0095
	6	52.9	mbPt-877453	-0.3	0.0094	18	29.6	mbPt-877486	-0.6	0.0095						-	-	soPb-856273	-0.1	0.0101
	3	52.2	mbPt-849000	-0.3	0.0099	4	22.9	mbPt-868741	0.5	0.0100						5	13.0	mbPt-877042/	-15.4	
	11	3.0	mbPt-847132/	11.5		4	27.0	mbPt-848227/	-29.4									mbPb-847658 †		
			mbPt-867688 †					mbPt-848599								11	0.0	mbPb-849156/	-16.2	
						18	20.0	mbPb-847817/	-23.8									mbPb-848151 †		
								mbPt-869376												
Pod width	8	39.9	mbPt-877288	-3.2	0.0000	-	-	mbPt-846868	2.0	0.0000	-	-	mbPb-848400	-4.8	0.0000	-	-	mbPt-868147	-1.6	0.0000
	-	-	mbPt-876570	1.2	0.0005	-	-	mbPt-868006	0.7	0.0005	6	8.5	mbPt-847626	-1.8	0.0009	14	11.4	mbPt-847671	1.4	0.0005
	6	40.6	soPb-853232	0.4	0.0010	12	26.0	mbPt-868606	-0.6	0.0010	-	-	mbPt-847829	-1.7	0.0019	7	9.1	soPt-855253	0.9	0.0011
	1	16.0	soPt-831593	0.3	0.0015	-	-	mbPt-876574	0.5	0.0015	8	30.4	mbPb-868035	-1.4	0.0028	-	-	mbPt-876465	-0.9	0.0016
	5	32.4	mbPt-848066	0.3	0.0020	4	21.2	mbPt-871533	0.5	0.0020	13	30.6	mbPb-846791	-1.2	0.0038	14	26.6	mbPt-848066	0.9	0.0021
	6	43.9	mbPt-847817	-0.2	0.0025	1	41.3	mbPt-868263	-0.4	0.0025	-	-	mbPt-847794	-1.2	0.0047	9	10.8	mbPb-846503	-0.7	0.0026
	-	-	mbPt-847507	0.2	0.0030	8	40.1	mbPt-848184	-0.4	0.0030	6	19.2	mbPt-867865	-1.1	0.0056	13	0.0	mbPb-847032	0.7	0.0032
	6	52.9	mbPt-867765	-0.2	0.0035	-	-	mbPt-849034	-0.3	0.0035	-	-	mbPt-847947	1.0	0.0066	14	26.4	mbPt-846613	0.6	0.0037
	8	39.7	mbPb-877288	-0.1	0.0040	18	20.2	mbPt-869376	0.2	0.0040	-	-	mbPt-876644	1.0	0.0075	-	-	soPb-825582	-0.5	0.0042
	-	-	mbPt-846502	0.1	0.0045	-	-	mbPb-846748	0.2	0.0045	-	-	mbPt-868763	0.7	0.0085	-	-	mbPt-876460	-0.4	0.0048
	-	-	mbPt-849104	0.1	0.0050	-	-	mbPb-848227	-0.2	0.0050	-	-	mbPb-847055	0.6	0.0094	14	26.3	mbPt-848285	0.4	0.0053
	6	43.5	mbPt-869376	0.1	0.0055	1	35.3	mbPt-876503	-0.2	0.0055	9	32.0	mbPt-846709	0.4	0.0104	-	-	soPt-824315	-0.3	0.0058
	5	35.3	mbPb-868679	0.1	0.0060	13	4.9	mbPb-847923	-0.2	0.0060						-	-	mbPt-868032	-0.3	0.0064
	1	12.0	soPt-855126	0.1	0.0065	7	18.2	mbPb-847372	-0.1	0.0065						-	-	mbPt-870331	-0.3	0.0069
	-	-	mbPt-877071	-0.1	0.0070	1	38.5	mbPt-868756	-0.1	0.0070						13	35.3	mbPt-867926	-0.2	0.0074
	-	-	mbPb-867918	0.1	0.0075	2	13.7	mbPb-868786	-0.1	0.0075						9	9.7	mbPb-847269	-0.2	0.0079
	5	30.2	mbPt-846941	0.1	0.0079	8	32.6	mbPt-846946	-0.1	0.0080						-	-	soPt-854803	-0.2	0.0085
	-	-	mbPt-869373	0.1	0.0084	-	-	soPb-825426	0.1	0.0085						-	-	mbPt-870539	0.2	0.0090
	3	57.9	mbPt-868143	0.1	0.0089	8	33.7	mbPt-847771	-0.1	0.0090						7	11.6	soPt-853667	0.2	0.0095
	-	-	soPb-857252	0.1	0.0094	-	-	mbPt-877204	0.1	0.0095						-	-	soPb-854638	-0.2	0.0101
	5	7.3	mbPt-846147	0.1	0.0099	-	-	soPt-856125	-0.1	0.0100										

	1xB					1xK					87xB					87xK				
Pod width (cont…)						4	21.0	mbPb-871546/ mbPb-871533	-25.3											
						7	19.0	mbPb-847372/ mbPb-877288	-14.2											
						8	34.0	mbPt-847771/ mbPt-848008	-19.7											
Seed size	3	62.6	mbPb-846816	-5.2	0.0000	-	-	soPt-855434	0.6	0.0000	-	-	mbPb-847295	6.5	0.0000	-	-	mbPb-849005	3.3	0.0000
	-	-	mbPb-848652	2.3	0.0005	8	33.7	mbPt-847771	-0.6	0.0005	-	-	mbPt-847817	-3.8	0.0009	-	-	mbPt-876465	-1.7	0.0005
	-	-	mbPt-868152	-2.2	0.0010	13	4.9	mbPb-847923	-0.5	0.0010	-	-	mbPb-848400	-3.5	0.0019	-	-	soPt-832048	-1.7	0.0011
	6	52.9	mbPt-877453	-1.7	0.0015	1	35.3	mbPt-876503	-0.3	0.0015	14	25.4	mbPb-848959	1.6	0.0028	3	0.0	mbPb-870846	-1.6	0.0016
	6	43.9	mbPt-847817	-1.6	0.0020	-	-	mbPb-870730	-0.3	0.0020	-	-	mbPt-848405	1.4	0.0038	13	26.3	soPt-832041	-1.3	0.0021
	6	52.8	mbPt-877486	-1.3	0.0025	13	7.1	mbPb-867898	-0.3	0.0025	-	-	mbPt-868763	1.3	0.0047	-	-	soPb-855231	-1.3	0.0026
	6	40.6	soPb-853232	1.1	0.0030	4	12.3	mbPt-848956	0.2	0.0030	-	-	mbPt-877288	-1.2	0.0056	3	9.1	mbPt-848696	-0.9	0.0032
	-	-	mbPt-846970	0.9	0.0035	8	32.6	mbPt-846946	-0.2	0.0035	15	3.7	mbPt-869171	1.1	0.0066	4	36.6	mbPt-871418	0.9	0.0037
	8	39.9	mbPt-877288	-0.8	0.0040	18	20.2	mbPt-869376	0.2	0.0040	2	3.4	mbPt-869503	0.7	0.0075	-	-	soPt-824315	-0.9	0.0042
	3	57.2	mbPt-868369	-0.8	0.0045	-	-	mbPt-849156	0.2	0.0045	-	-	mbPt-848177	0.7	0.0085	-	-	soPt-854803	-0.7	0.0048
	6	25.4	mbPt-847718	-0.8	0.0050	-	-	soPt-856125	-0.2	0.0050	11	20.1	mbPb-847026	-0.7	0.0094	7	17.2	mbPt-867849	0.6	0.0053
	5	35.3	mbPb-868679	0.7	0.0055	17	16.0	mbPb-867864	-0.2	0.0055	17	42.2	soPt-824113	-0.6	0.0104	-	-	mbPt-846602	0.4	0.0058
	-	-	mbPt-871696	0.6	0.0060	6	0.0	mbPt-847507	0.1	0.0060						3	19.3	mbPt-847520	-0.3	0.0064
	6	43.5	mbPt-869376	0.6	0.0065	10	9.0	mbPb-870614	-0.1	0.0065						-	-	mbPb-847158	-0.3	0.0069
	6	52.9	mbPt-867765	-0.6	0.0070	2	15.9	mbPt-849029	0.1	0.0070						8	7.3	mbPt-869539	0.3	0.0074
	3	57.3	mbPt-848482	-0.5	0.0075	2	17.5	mbPt-876710	0.1	0.0075						7	18.1	mbPt-848800	0.2	0.0079
	3	57.2	mbPt-868642	-0.5	0.0079	2	9.0	mbPb-849156	0.1	0.0080						-	-	soPt-853639	-0.2	0.0085
	6	20.7	mbPt-877442	-0.5	0.0084	-	-	soPb-824664	0.1	0.0085						14	26.4	mbPt-846613	0.2	0.0090
	13	14.0	mbPt-848723	-0.5	0.0089	-	-	soPt-853484	0.1	0.0090						-	-	mbPt-846324	0.2	0.0095
	9	10.1	mbPt-870681	0.4	0.0094	2	13.7	mbPb-868786	-0.1	0.0095						-	-	mbPt-871637	-0.2	0.0101
	6	55.1	mbPb-847443	-0.4	0.0099	2	28.0	mbPt-848179	0.1	0.0100						3	13.0	mbPt-849021/ mbPt-867887	-20.1	
						2	27.0	mbPb-848616/ mbPt-848179	-14.3							4	27.0	mbPb-846398/ mbPt-876807	-19.8	
						4	23.0	mbPt-868741/ mbPt-868438	-8.5							7	19.0	mbPt-848800/ mbPt-848616	-15.4	
						7	38.0	soPb-856649/ mbPt-868516	-7.4											
						8	34.0	mbPt-847771/ mbPt-848008	-26.7							9	15.0	mbPt-868489/ mbPb-877071	-8.3	
						9	4.0	mbPb-868634/ mbPb-846522	-14.1							16	19.0	mbPb-868071/ mbPb-868747	-8.8	
Hardseededness	-	-	mbPt-849188	1.8	0.0000	-	-	soPb-825426	-1.6	0.0000	17	23.4	mbPt-848216	9.4	0.0000	-	-	mbPt-876644	-1.1	0.0000
	-	-	mbPb-849188	1.5	0.0005	-	-	soPb-824603	-1.1	0.0005	5	10.1	mbPb-846147	4.2	0.0009	-	-	mbPt-848087	0.7	0.0005
	13	27.4	mbPb-877550	1.2	0.0010	16	48.3	mbPt-870410	-1.0	0.0010	-	-	mbPb-867948	-3.8	0.0019	-	-	mbPb-847506	-0.7	0.0011
	13	31.4	soPb-824241	1.2	0.0015	-	-	soPb-825015	-0.9	0.0015	16	28.8	mbPt-846634	3.5	0.0028	-	-	mbPb-849005	-0.5	0.0016
	-	-	mbPt-868050	0.9	0.0020	11	14.3	mbPb-868263	0.8	0.0020	-	-	mbPb-868009	-2.1	0.0038	12	56.3	mbPb-868863	-0.4	0.0021
	-	-	mbPt-877243	-0.9	0.0025	8	33.7	mbPt-847771	0.7	0.0025	1	9.1	mbPt-869121	-1.8	0.0047	16	19.3	mbPb-868747	0.4	0.0026
	6	24.1	mbPt-846949	0.9	0.0030	8	34.4	mbPt-848008	0.6	0.0030	-	-	mbPt-868185	1.8	0.0056	16	36.7	mbPt-847817	0.3	0.0032
	1	27.4	mbPt-847027	-0.9	0.0035	-	-	mbPt-877269	0.5	0.0035	-	-	mbPb-869175	-1.5	0.0066	16	19.7	mbPb-877155	0.3	0.0037
	13	28.6	mbPb-877600	0.7	0.0040	-	-	mbPb-868147	0.5	0.0040	9	51.0	mbPt-877498	1.4	0.0075	-	-	soPt-857525	0.2	0.0042
	6	24.0	mbPt-848172	0.5	0.0045	-	-	mbPt-876535	-0.3	0.0045	17	42.2	mbPt-870544	1.4	0.0085	-	-	mbPb-846543	-0.2	0.0048
	-	-	soPt-855247	0.5	0.0050	-	-	soPb-856362	-0.2	0.0050	-	-	mbPt-870982	-1.2	0.0094	-	-	soPt-854615	0.2	0.0053
	6	53.2	mbPb-868534	-0.4	0.0055	8	36.3	mbPb-876816	0.1	0.0055	11	22.1	mbPt-848838	1.1	0.0104	-	-	mbPt-848057	0.2	0.0058
	6	25.1	mbPt-868384	-0.3	0.0060	8	36.3	mbPb-876533	0.1	0.0060	8	27.0	mbPb-848482/ mbPt-868592 †	11.5		-	-	mbPt-870942	0.2	0.0064
	6	17.4	mbPb-846398	0.2	0.0065	-	-	mbPt-869081	0.1	0.0065						5	4.6	mbPt-848650	0.1	0.0069

	1xB					1xK					87xB					87xK				
Hardseededness (cont…)	6	22.3	mbPb-849009	-0.2	0.0070	16	52.7	mbPb-870410	-0.1	0.0070						-	-	mbPb-868035	0.1	0.0074
	13	27.4	mbPb-868602	0.2	0.0075	-	-	mbPt-847509	-0.1	0.0075						-	-	mbPt-876465	0.1	0.0079
	1	26.9	mbPt-847437	-0.2	0.0079	-	-	soPb-824518	-0.1	0.0080						-	-	mbPb-868185	0.1	0.0085
	13	31.1	soPb-824633	0.2	0.0084	-	-	mbPt-869245	0.1	0.0085						12	74.2	mbPb-867920	0.1	0.0090
	6	23.3	mbPt-849009	-0.2	0.0089	-	-	soPb-825430	-0.1	0.0090						15	25.4	mbPb-877325	0.1	0.0095
	13	14.0	mbPt-848723	0.2	0.0094	-	-	mbPb-871385	0.1	0.0095						8	11.8	mbPt-868778	-0.1	0.0101
	11	50.9	mbPb-871281	-0.1	0.0099	16	41.0	mbPt-848057	0.1	0.0100						3	10.0	mbPt-848696/ mbPt-848710 †	16.3	
	7	22.0	mbPt-848539/ mbPt-848588	-25.3		16	41.0	mbPt-848087	0.1											
Dry pod mass	8	20.7	mbPt-846832	4.0	0.0000	7	19.6	mbPt-877288	5.6	0.0000	16	36.7	mbPt-868581	2.2	0.0000	3	34.9	mbPb-847829	1.7	0.0000
	-	-	soPb-824730	-3.8	0.0005	7	19.6	mbPb-847829	3.2	0.0005	17	46.5	mbPt-847488	1.3	0.0009	-	-	soPb-854293	1.4	0.0005
	1	52.3	mbPb-870825	-3.4	0.0010	11	40.6	mbPt-847459	-2.5	0.0010	-	-	mbPt-871474	-1.0	0.0019	15	25.4	mbPb-877325	1.2	0.0011
	-	-	soPb-857598	2.1	0.0015	7	19.5	mbPb-877288	1.9	0.0015	11	2.3	mbPt-846602	-0.9	0.0028	-	-	mbPb-848797	-0.8	0.0016
	8	20.7	mbPb-846687	1.8	0.0020	7	19.6	mbPt-847829	1.9	0.0020	13	45.1	mbPt-868647	0.7	0.0038	3	34.9	mbPt-847829	0.7	0.0021
	-	-	soPb-832238	1.7	0.0025	-	-	mbPb-848954	1.7	0.0025	17	28.9	mbPb-870410	0.7	0.0047	-	-	soPt-853381	0.6	0.0026
	-	-	mbPt-869240	1.5	0.0030	11	36.3	mbPt-846260	-1.6	0.0030	16	52.4	mbPt-849188	0.6	0.0056	13	19.6	mbPb-848513	-0.4	0.0032
	-	-	soPb-857559	1.2	0.0035	11	35.2	soPb-825660	-1.5	0.0035	16	27.5	mbPb-868723	-0.6	0.0066	-	-	soPb-853497	-0.3	0.0037
	11	44.2	mbPt-871632	-1.1	0.0040	-	-	mbPt-871009	-1.4	0.0040	9	14.8	soPb-853944	0.5	0.0075	8	15.0	mbPb-868706	0.3	0.0042
	-	-	mbPt-877396	-1.1	0.0045	-	-	mbPt-869485	1.1	0.0045	-	-	mbPb-868506	0.5	0.0085	8	12.2	mbPt-877316	0.3	0.0048
	8	20.0	mbPt-848266	1.1	0.0050	11	46.8	mbPb-868828	1.0	0.0050	16	27.5	mbPt-868723	-0.5	0.0094	-	-	mbPt-871774	0.3	0.0053
	1	52.6	mbPb-870911	-0.9	0.0055	1	23.0	mbPt-871575	-0.9	0.0055	-	-	soPt-831748	0.5	0.0104	14	54.1	mbPb-876709	0.3	0.0058
	-	-	mbPb-847410	0.8	0.0060	7	39.8	mbPt-846324	-0.9	0.0060	9	29.0	soPt-832041/ mbPb-868828	30.3		4	26.4	mbPb-876807	-0.3	0.0064
	11	40.4	soPb-824843	0.7	0.0065	11	36.4	mbPb-868763	0.8	0.0065						-	-	mbPt-868993	0.3	0.0069
	2	7.1	soPt-857598	0.7	0.0070	-	-	mbPt-876947	0.8	0.0070						-	-	mbPb-869080	0.3	0.0074
	11	42.1	mbPb-868763	0.7	0.0075	11	36.3	mbPt-867926	-0.7	0.0075						-	-	mbPb-846816	-0.2	0.0079
	-	-	mbPb-847393	-0.6	0.0079	2	17.1	mbPb-876710	0.7	0.0080						-	-	soPt-825040	0.2	0.0085
	11	44.1	mbPt-870753	-0.5	0.0084	-	-	mbPt-847775	-0.7	0.0085						14	35.7	mbPb-867780	0.2	0.0090
	8	50.3	soPb-855179	0.5	0.0089	11	40.6	mbPt-847428	-0.7	0.0090						-	-	soPt-831544	-0.2	0.0095
	8	0.0	mbPt-848267	-0.4	0.0094	11	35.6	soPb-831975	-0.6	0.0095						-	-	mbPb-847655	0.2	0.0101
	1	28.0	mbPb-847271	-0.4	0.0099	6	0.0	mbPt-847507	0.6	0.0100						3	34.0	mbPb-848400/ mbPt-847829	26.5	
			mbPt-877268/ mbPt-869029 †	71.8		7	19.0	mbPb-847372/ mbPb-877288	30.7											
	3	57.0	mbPt-847850/ mbPb-868369 †	-29.1		11	35.0	soPb-824730/ soPb-825660	21.9							8	16.0	mbPb-868706/ mbPb-871035	16.1	
	7	96.0	soPt-825622/ soPt-825646 †	-31.7												13	36.0	mbPt-867926/ mbPt-871632	12.9	
Seed yield	-	-	soPb-824730	-2.2	0.0000	7	19.6	mbPt-877288	7.5	0.0000	17	46.5-	mbPt-847488	1.7	0.0000	-	-	soPb-853497	-1.6	0.0000
	1	52.3	mbPb-870825	-2.1	0.0005	-	-	mbPb-848954	6.7	0.0005	11	2.3	mbPt-846602	-1.3	0.0009	-	-	mbPb-848797	-1.3	0.0005
	8	20.7	mbPt-846832	2.0	0.0010	-	-	mbPt-871009	-2.9	0.0010	-	-	mbPb-868506	1.2	0.0019	15	25.4	mbPb-877325	1.1	0.0011
	-	-	soPb-857598	1.4	0.0015	7	19.6	mbPb-847829	1.4	0.0015	-	-	mbPt-871474	-1.1	0.0028	-	-	soPt-853381	0.9	0.0016
	8	20.7	mbPb-846687	1.2	0.0020	-	-	mbPt-869485	1.4	0.0020	16	36.7	mbPb-868581	1.0	0.0038	-	-	soPb-854293	0.8	0.0021
	-	-	mbPt-876544	-1.1	0.0025	2	17.1	mbPb-876710	1.3	0.0025	-	-	mbPt-848472	-1.0	0.0047	3	34.9	mbPb-847829	0.5	0.0026
	-	-	mbPt-869240	0.9	0.0030	11	40.6	mbPt-847459	-1.2	0.0030	17	28.9	mbPb-870410	0.6	0.0056	3	34.9	mbPt-847829	0.4	0.0032
	11	44.2	mbPt-871632	-0.9	0.0035	7	19.5	mbPb-877288	1.1	0.0035	3	28.1	mbPt-849068	0.6	0.0066	3	33.0	mbPb-848400	0.4	0.0037
	1	52.6	mbPb-870911	-0.9	0.0040	-	-	soPb-826131	1.0	0.0040	16	27.5	mbPt-868723	-0.4	0.0075	-	-	mbPt-877415	0.3	0.0042
	11	42.1	mbPt-868763	0.8	0.0045	6	0.0	mbPt-847507	1.0	0.0045	-	-	soPt-831748	0.4	0.0085	-	-	mbPb-869080	0.3	0.0048
	-	-	soPb-857559	0.8	0.0050	-	-	mbPt-876947	0.9	0.0050	-	-	mbPt-848756	-0.4	0.0094	-	-	mbPb-846230	-0.2	0.0053
	11	44.1	mbPt-870753	-0.8	0.0055	1	23.0	mbPt-871575	-0.8	0.0055	15	6.4	mbPt-867989	0.4	0.0104	-	-	mbPt-871774	0.2	0.0058
	8	20.0	mbPt-848266	0.7	0.0060	7	19.6	mbPt-847829	0.7	0.0060	9	29.0	soPt-832041/ mbPb-868828	28.8		-	-	soPt-831544	-0.2	0.0064
	-	-	soPb-832238	0.7	0.0065	-	-	mbPt-847775	-0.6	0.0065						-	-	soPt-824848	-0.2	0.0069
	2	7.1	soPt-857598	0.6	0.0070	11	36.3	mbPt-846260	-0.5	0.0070						8	12.1	mbPb-877305	0.2	0.0074

	1xB					1xK				87xB						87xK		
	-	-	mbPb-871142	-0.6	0.0075	11	36.4	mbPb-868763	0.5	0.0075						4	30.4	soPb-824525	-0.2	0.0079
	11	43.9	mbPt-846471	-0.6	0.0079	2	13.7	mbPb-868786	-0.4	0.0080						-	-	soPt-832155	0.2	0.0085
Seed yield	11	40.4	soPb-824843	0.6	0.0084	11	36.3	mbPt-867926	-0.3	0.0085						-	-	mbPb-847655	0.2	0.0090
(cont…)	-	-	mbPb-847393	-0.6	0.0089	-	-	mbPt-868460	0.3	0.0090						12	66.0	soPb-824061	-0.1	0.0095
	11	42.1	mbPb-868763	0.6	0.0094	7	39.8	mbPt-846324	-0.2	0.0095						-	-	mbPt-868993	0.1	0.0101
			mbPb-868825	-0.5	0.0099	11	36.4	mbPt-868763	0.2	0.0100						3	34.0	mbPb-848400/ mbPt-847829	25.4	
	1	28.0	mbPt-877268/ mbPt-869029 †	63.0		7	19.0	mbPb-847372/ mbPb-877288	24.1							8	15.0	mbPt-848110/ mbPb-868706	12.7	
	3	58.0	mbPt-868143/ mbPt-848973 †	-16.1		11	35.0	soPb-824730/ soPb-825660	23.5							13	36.0	mbPt-867926/ mbPt-871632	13.3	
	7	96.0	soPt-825622/ soPt-825646 †	-19.6																
	-	-	mbPb-848278	3.0	0.0000	11	35.2	soPb-825660	-5.3	0.0000	9	22.1	soPb-824730	-1.2	0.0000			mbPt-871774	2.0	0.0000
Standing dry biomass	8	32.8	mbPb-870338	3.0	0.0005	7	39.8	mbPb-849130	-3.9	0.0005			mbPb-870577	-1.0	0.0009	15	25.4	mbPb-877325	1.4	0.0005
	8	39.9	mbPt-877288	1.9	0.0010	7	39.8	soPt-856272	-3.0	0.0010			mbPb-877485	-0.9	0.0019			soPt-831544	-1.1	0.0011
	-	-	soPb-832041	1.9	0.0015	-	-	mbPt-847947	-2.2	0.0015			mbPb-870825	-0.9	0.0028			mbPb-846230	-1.0	0.0016
	-	-	soPt-853236	-1.7	0.0020	11	36.8	soPb-824700	-2.0	0.0020			mbPb-868500	-0.7	0.0038			mbPb-846816	-0.8	0.0021
	-	-	mbPt-877314	1.0	0.0025	-	-	soPt-853797	-1.9	0.0025	7	6.9	mbPt-871051	0.7	0.0047	-	-	soPt-824253	0.6	0.0026
	8	39.7	mbPb-877288	0.7	0.0030	11	41.7	soPb-832041	1.8	0.0030	13	45.1	mbPt-868647	0.6	0.0056	-	-	soPb-853497	-0.4	0.0032
	-	-	soPb-856075	0.4	0.0035	-	-	mbPb-870338	1.7	0.0035	-	-	mbPt-848756	-0.6	0.0066	-	-	mbPt-849144	-0.3	0.0037
	7	53.0	mbPt-848839	-0.4	0.0040	-	-	mbPb-847947	-1.4	0.0040	-	-	mbPt-848229	-0.6	0.0075	-	-	soPt-855726	-0.2	0.0042
	-	-	mbPt-877233	0.3	0.0045	11	36.0	soPb-824755	-1.4	0.0045	13	45.2	mbPt-868067	0.6	0.0085	-	-	soPb-853425	-0.2	0.0048
	15	34.2	mbPt-868234	0.3	0.0050	-	-	mbPt-848688	-1.3	0.0050	-	-	mbPb-846429	-0.6	0.0094	-	-	soPt-824460	0.2	0.0053
	11	25.4	mbPt-868408	-0.3	0.0055	-	-	soPt-824914	1.3	0.0055	-	-	mbPb-876726	-0.6	0.0104	3	34.9	mbPt-847829	0.2	0.0058
	10	12.2	mbPt-871421	-0.2	0.0060	-	-	soPt-855618	-1.1	0.0060						-	-	mbPt-868993	0.2	0.0064
	3	85.1	mbPt-877609	-0.2	0.0065	11	35.6	soPb-831975	-1.1	0.0065						4	30.4	soPb-824525	-0.2	0.0069
	-	-	soPb-854886	0.2	0.0070	-	-	soPb-855926	-1.0	0.0070						3	34.9	mbPb-847829	0.2	0.0074
	-	-	mbPt-876930	0.2	0.0075	-	-	soPt-825848	1.0	0.0075						3	36.2	mbPb-877288	0.2	0.0079
	-	-	mbPt-869240	0.2	0.0079	11	69.9	mbPt-848192	-1.0	0.0080						3	33.0	mbPb-848400	0.2	0.0085
	1	40.3	mbPt-869184	-0.1	0.0084	-	-	mbPb-876778	-1.0	0.0085						-	-	soPb-854293	0.2	0.0090
	3	85.1	soPt-824889	-0.1	0.0089	11	67.5	mbPt-868487	-0.9	0.0090						-	-	soPt-824315	-0.2	0.0095
	-	-	mbPb-877400	0.1	0.0094	-	-	mbPt-869245	-0.9	0.0095						-	-	mbPt-870337	-0.1	0.0101
	-	-	mbPt-877501	0.1	0.0099	-	-	mbPt-877530	0.8	0.0100						3	32.0	mbPb-847621/ mbPt-848400	18.1	
	7	118.0	soPt-853076/ soPt-853099	62.0		7	40.0	mbPt-846324/ mbPt-868542	32.7							15	25.0	mbPb-871281/ mbPb-877325	13.7	
						11	37.0	soPb-824700/ mbPb-877269	19.6											
						18	56.0	mbPb-847443/ soPt-853267	-7.2											
						19	25.0	soPb-825612/ mbPb-846990	-10.9											
Harvest index	8	39.7	mbPb-877288	-4.4	0.0000	-	-	mbPb-876778	3.3	0.0000	-	-	mbPt-876830	-2.3	0.0000	-	-	soPb-857025	-4.7	0.0000
	8	32.8	mbPb-870338	-3.7	0.0005	-	-	soPb-855926	3.1	0.0005	-	-	mbPb-847671	-1.2	0.0009	-	-	soPb-855100	2.5	0.0005
	8	39.9	mbPt-877288	-3.0	0.0010	8	24.1	mbPt-846792	2.8	0.0010	-	-	mbPb-877550	0.7	0.0019	13	28.1	soPt-825848	-1.5	0.0011
	-	-	soPt-855878	2.3	0.0015	8	20.0	mbPb-847535	-2.3	0.0015	17	23.4	mbPb-848216	0.6	0.0028	-	-	soPt-855671	-0.8	0.0016
	-	-	mbPt-846880	2.0	0.0020	2	20.3	mbPt-847660	1.7	0.0020	-	-	mbPb-868602	0.6	0.0038	-	-	mbPt-869019	0.8	0.0021
	7	53.0	mbPt-848839	1.9	0.0025	7	42.6	mbPt-848267	1.6	0.0025	-	-	mbPb-870825	0.5	0.0047	-	-	soPb-856133	0.7	0.0026
	8	39.9	mbPt-847829	-1.9	0.0030	-	-	mbPt-848368	1.6	0.0030	8	14.3	mbPb-867904	0.4	0.0056	-	-	soPt-854454	-0.7	0.0032
	6	20.7	mbPt-877442	-1.2	0.0035	-	-	soPb-826147	-1.5	0.0035	-	-	mbPt-870641	0.4	0.0066	-	-	mbPb-848797	-0.5	0.0037
	3	85.1	soPt-855371	1.1	0.0040	-	-	soPt-855710	1.3	0.0040	8	14.3	mbPb-849168	0.4	0.0075	12	68.4	soPb-824363	0.4	0.0042
	-	-	mbPt-868663	-1.1	0.0045	-	-	soPt-857002	-1.1	0.0045	-	-	soPt-825848	-0.3	0.0085	16	25.6	mbPt-867765	-0.4	0.0048

Harvest index (cont…)

1xB					1xK					87xB					87xK				
-	-	mbPt-846340	-0.9	0.0050	-	-	mbPt-868063	-1.1	0.0050	-	-	mbPb-877600	0.3	0.0094	-	-	soPb-856301	-0.4	0.0053
15	34.2	mbPt-868234	-0.7	0.0055	7	39.8	mbPb-849130	1.0	0.0055	8	49.4	mbPt-867668	0.2	0.0104	-	-	mbPt-871421	-0.3	0.0058
-	-	soPb-832041	-0.6	0.0060	8	24.1	mbPt-849009	1.0	0.0060	9	22.0	mbPt-867926/ soPb-824730	17.8		16	24.7	mbPt-877453	-0.3	0.0064
6	24.4	mbPt-849032	-0.5	0.0065	-	-	mbPb-870338	-1.0	0.0065						6	7.4	mbPt-847437	-0.2	0.0069
6	20.7	mbPt-849131	-0.4	0.0070	-	-	mbPt-847947	0.8	0.0070						15	9.8	mbPt-848586	0.2	0.0074
11	22.6	mbPt-868656	0.3	0.0075	2	17.1	mbPb-876710	0.8	0.0075						-	-	mbPb-848622	0.2	0.0079
12	31.4	mbPt-846824	-0.3	0.0079	-	-	mbPt-871054	-0.7	0.0080						1	5.2	soPt-856768	0.2	0.0085
-	-	mbPt-848996	0.3	0.0084	1	38.5	mbPt-868756	-0.6	0.0085						-	-	mbPb-871594	0.1	0.0090
-	-	mbPt-869467	0.2	0.0089	-	-	soPb-857600	0.6	0.0090						-	-	mbPb-848054	0.1	0.0095
12	31.4	mbPt-877400	-0.2	0.0094	-	-	soPb-855618	0.5	0.0095						-	-	mbPt-849175	0.1	0.0101
11	63.1	soPb-824256	-0.2	0.0099	7	39.8	soPt-856272	0.5	0.0100						14	42.0	mbPt-848177/ mbPb-849151	10.9	
					2	20.0	mbPt-876710/ mbPt-847660	10.9							15	10.0	soPt-832053/ soPt-854393	15.6	
					7	43.0	mbPt-848267/ mbPt-876614	20.2							16	24.0	mbPb-877453/ mbPt-877453	13.1	
					8	26.0	mbPt-868381/ mbPt-847654	15.8											

Appendix 5.3. Congruent QTLs resolved in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on LG (in cM); PVE = Phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect);

♪: Overlapped QTLs for a trait are highlighted in the same colour;

‡: Underlined LG and Pos are positions of SML QTLs which are assigned based on the genotype correlation ($r \geq 0.84$) between markers linked to SML QTLs and markers mapped on the individual linkage map;

✓: Indicate markers/QTLs present

		1B					1K					87B					87K				
Traits	**Common marker♪**	**LG‡**	**Pos (cM)**		**PVE %**	***P* - value**	**LG**	**Pos (cM)**		**PVE %**	***P* - value**	**LG**	**Pos (cM)**		**PVE %**	***P* - value**	**LG**	**Pos (cM)**		**PVE %**	***P* - value**
Hypocotyl pigment	mbPb-868147	-	-	✓	-1.1	0.0005	-	-	✓	-5.8	0.0000										
Leaf rachis colour	mbPt-846869	4	33.9	✓	0.3	0.0099						17	21.9	✓	1.0	0.0009					
	mbPt-847829	8	39.9	✓	1.3	0.0020						-	-	✓	0.9	0.0019					
	mbPt-877288	8	39.9	✓	1.6	0.0005						-	-	✓	0.8	0.0047					
	soPb-824730	-	-	✓	-1.6	0.0015	11	34.4	✓	-1.3	0.0000										
Plant hair density	mbPb-877453	6	52.8	✓	-0.4	0.0045						12	8.3	✓	-0.7	0.0028					
	mbPb-877486	6	52.9	✓	-0.4	0.0040						12	9.5	✓	-1.2	0.0000					
	mbPt-867765						18	29.6	✓	-0.7	0.0065						16	25.6	✓	30.1	-
	mbPt-869376						18	20.2	✓	1.7	0.0020						16	49.5	✓	2.9	0.0000
Plant hair colour	mbPb-847829						7	19.6	✓	-0.2	0.0100						3	34.9	✓	-0.5	0.0053
	mbPb-877288						7	19.5	✓	-2.1	0.0015						3	36.2	✓	-0.3	0.0079
	mbPt-877288						7	19.6	✓	-0.4	0.0080			✓	-0.6	0.0038	3	35.8	✓	-0.5	0.0048
Growth habit	mbPb-877288	8	39.7	✓	13.4	-	7	19.5	✓	3.7	0.0015										
	mbPt-847829						7	19.6	✓	0.3	0.0080						3	34.9	✓	1.4	0.0042
	mbPt-877288	8	39.9	✓	0.5	0.0065	7	19.6	✓	1.0	0.0050										
Twining	mbPb-848500						8	24.5	✓	0.2	0.0075						4	15.2	✓	20.1	-
	mbPb-848781	6	24.1	✓	0.1	0.0075	8	23.9	✓	0.8	0.0050										
	mbPt-868381						8	25.7	✓	-1.0	0.0040						4	16.4	✓	-0.2	0.0079
	mbPb-849009											14	18.2	✓	21.1	-	4	12.7	✓	-0.4	0.0042
	soPt-824253						-	-	✓	4.6	0.0000						-	-	✓	0.1	0.0090
Leaf lobing	mbPb-848586	11	67.4	✓	-0.1	0.0084	11	10.6	✓	-5.1	0.0005	-	-	✓	-8.7	0.0009					
	mbPb-848641	11	68.4	✓	-0.4	0.0040						-	-	✓	15.5	0.0000	15	9.7	✓	-4.5	0.0011
	mbPb-867674						11	10.7	✓	-3.2	0.0015	-	-	✓	-3.7	0.0019	15	9.7	✓	4.9	0.0005
	mbPb-868263						11	14.3	✓	-3.3	0.0010	-	-	✓	-2.9	0.0028	15	16.9	✓	-0.6	0.0101
	mbPt-848586	11	68.3	✓	-1.1	0.0010	11	10.8	✓	-6.6	0.0000						15	9.8	✓	-3.5	0.0016
	mbPt-848641	11	68.3	✓	-1.4	0.0000	11	10.7	✓	2.3	0.0020						15	9.7	✓	-5.6	0.0000
	mbPt-876514	11	68.3	✓	23.4	-	11	10.7	✓	-2.0	0.0030						15	6.6	✓	-2.3	0.0026
	soPb-825492	11	63.1	✓	0.5	0.0035	-	-	✓	0.4	0.0070						15	11.2	✓	2.0	0.0032
	soPb-832053						-	-	✓	0.4	0.0075						15	7.4	✓	1.5	0.0048
	soPb-853645	11	63.1	✓	0.3	0.0055											15	13.7	✓	1.6	0.0042
	soPb-853806											9	10.3	✓	33.6	-	15	9.9	✓	1.4	0.0058
	soPb-854393	11	62.9	✓	1.0	0.0015											15	9.3	✓	0.7	0.0079
	soPb-855071						-	-	✓	0.6	0.0065						15	11.8	✓	0.6	0.0095
	soPb-855762	11	63.1	✓	0.8	0.0020	11	4.6	✓	0.3	0.0095										
	mbPb-877325						11	22.9	✓	1.7	0.0035	9	43.6	✓	2.8	0.0038	15	25.4	✓	1.3	0.0064

Traits	Common markers	1B					1K					87B					87K				
		LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value
Flower colour	mbPb-870577	-	-	✓	-0.3	0.0084						-	-	✓	-0.7	0.0066					
	mbPb-876726						11	0.0	✓	-0.1	0.0095	-	-	✓	-0.6	0.0075					
	mbPb-877485	11	59.0	✓	-0.5	0.0065	11	0.6	✓	17.2	-										
	mbPt-848586	11	68.3	✓	-0.4	0.0079	11	10.8	✓	-0.1	0.0100										
	mbPt-849047			✓	-0.3	0.0089											9	7.7	✓	-0.2	0.0101
Inflorescence structure	mbPb-846949	6	24.0	✓	-40.0	-						14	12.2	✓	-0.7	0.0094					
	mbPb-848781	6	24.1	✓	-0.1	0.0040						14	12.4	✓	-1.4	0.0019					
	mbPt-848781											14	10.8	✓	-1.1	0.0028	4	16.0	✓	-0.6	0.0037
	mbPb-848500						8	24.5	✓	-0.1	0.0095	14	12.7	✓	-0.9	0.0056					
	mbPb-868160	3	73.1	✓	-0.1	0.0084						-	-	✓	-0.6	0.0104					
	mbPt-868384	6	25.1	✓	1.3	0.0000	8	11.0	✓	0.1	0.0045						4	5.1	✓	0.3	0.0101
Dry pod colour	mbPb-868706											15	19.0	✓	3.7	0.0000	8	15.0	✓	2.8	0.0021
	mbPt-848184						8	40.1	✓	0.4	0.0075	14	31.7	✓	2.6	0.0019					
	mbPt-848800	3	72.3	✓	22.8	-						3	119.2	✓	-1.6	0.0047					
	mbPt-876498						18	9.6	✓	0.8	0.0030						-	-	✓	12.8	0.0000
Pod dehiscence	mbPt-846949	6	24.1	✓	0.3	0.0050						14	10.0	✓	1.1	0.0009					
Powdery mildew	mbPb-849188			✓	-1.3	0.0055													✓	-0.6	0.0079
Thrips	soPt-855180			✓	-0.1	0.0089						9	6.4	✓	-1.2	0.0085					
Perenniality	mbPb-868706											15	19.0	✓	5.8	0.0000	8	15.0	✓	1.8	0.0011
Testa colour	mbPb-847032						11	42.3	✓	-4.1	0.0005						13	0.0	✓	24.2	-
	mbPb-868032											-	-	✓	3.6	0.0009	-	-	✓	1.9	0.0011
	mbPb-868147	-	-	✓	40.8	0.0000	-	-	✓	59.6	0.0000	-	-	✓	79.6	0.0000					
	mbPt-868032											-	-	✓	0.7	0.0047	-	-	✓	70.6	0.0000
	mbPt-868147	-	-	✓	14.5	0.0005											-	-	✓	1.6	0.0021
Seed mottling	mbPb-868032											-	-	✓	4.3	0.0009	-	-	✓	5.9	0.0011
	mbPb-868147	-	-	✓	55.9	0.0000	-	-	✓	64.7	0.0000	-	-	✓	76.8	0.0000					
	mbPt-868032											-	-	✓	0.1	0.0085	-	-	✓	52.1	0.0000
	mbPt-868147	-	-	✓	9.2	0.0005											-	-	✓	7.1	0.0005
	mbPb-847032						11	42.3	✓	-2.7	0.0005						13	0.0	✓	-0.4	0.0053
Seed coat ridging	mbPb-868763											-	-	✓	18.0	0.0000	13	16.7	✓	0.1	0.0053
	mbPt-868260											-	-	✓	0.6	0.0094	13	32.6	✓	17.4	-
	soPb-824730											9	22.1	✓	26.2	-	13	47.9	✓	-0.1	0.0064
Lustre	mbPt-876498													✓	-1.0	0.0085			✓	1.6	0.0021
Texture layer depth	mbPb-868763											-	-	✓	13.5	0.0000	13	16.7	✓	0.8	0.0026
	mbPb-877269	11	41.6	✓	0.2	0.0045	11	37.3	✓	0.4	0.0085						13	16.7	✓	3.5	0.0000
	mbPt-868763	11	42.1	✓	0.4	0.0015	11	36.4	✓	1.7	0.0015	-	-	✓	3.3	0.0009					
	mbPb-849156						2	9.0	✓	-0.6	0.0060						11	0.0	✓	0.3	0.0095
	mbPb-877325	11	51.5	✓	0.9	0.0005	11	22.9	✓	22.5	-	9	43.6	✓	1.3	0.0047	15	25.4	✓	1.0	0.0011
	soPb-824730						11	34.4	✓	-2.7	0.0000						13	47.9	✓	-0.4	0.0069

Traits	Common markers	1B					1K					87B					87K				
		LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value
Hilum colour	mbPb-868763	11	42.1	✓	3.9	0.0010	11	36.4	✓	53.7	0.0000	-	-	✓	36.6	0.0000	13	16.7	✓	6.7	0.0005
	mbPb-877269	11	41.6	✓	50.7	0.0000	11	37.3	✓	2.7	0.0010						13	16.7	✓	55.5	0.0000
	mbPt-868763	11	42.1	✓	14.5	0.0005	11	36.4	✓	13.0	0.0005	-	-	✓	7.1	0.0009					
	mbPt-846471	11	43.9	✓	-1.7	0.0015	11	36.5	✓	-0.1	0.0090										
	mbPt-870753	11	44.1	✓	-0.4	0.0055											13	40.4	✓	-0.2	0.0069
	mbPt-871632	11	44.2	✓	-0.2	0.0084	11	36.7	✓	-0.1	0.0085	9	13.5	✓	-1.0	0.0075					
	soPb-824730						11	34.4	✓	-0.1	0.0080	9	22.1	✓	-0.6	0.0104	13	47.9	✓	-0.6	0.0048
	soPb-824755	11	46.7	✓	-0.7	0.0035											-	-	✓	-0.1	0.0074
	soPb-825660	11	46.7	✓	-0.7	0.0030						9	23.2	✓	-0.8	0.0094					
	soPb-831975	11	47.7	✓	-0.1	0.0094	11	35.6	✓	-0.6	0.0040										
	mbPb-868828	11	33.2	✓	0.6	0.0045											13	30.1	✓	0.9	0.0042
	mbPt-868828	11	33.0	✓	0.5	0.0050											13	30.7	✓	0.9	0.0032
	mbPt-868260											-	-	✓	2.0	0.0028	13	32.6	✓	3.2	0.0011
	mbPt-877498											9	51.0	✓	3.5	0.0019	13	33.1	✓	2.9	0.0016
	mbPb-877325	11	51.5	✓	0.2	0.0079	11	22.9	✓	0.3	0.0050						15	25.4	✓	1.3	0.0026
Texture layer colour	mbPb-868763	11	42.1	✓	3.8	0.0010	11	36.4	✓	99.0	0.0000	-	-	✓	50.6	0.0000	13	16.7	✓	0.8	0.0026
	mbPb-877269	11	41.6	✓	54.9	0.0000											13	16.7	✓	67.9	0.0000
	mbPt-868763	11	42.1	✓	14.9	0.0005						-	-	✓	10.8	0.0009					
	mbPb-868828	11	33.2	✓	0.5	0.0040											13	30.1	✓	0.2	0.0074
	mbPt-868828	11	33.0	✓	1.4	0.0015	11	46.8	✓	0.1	0.0005	9	27.5	✓	37.3	-	13	30.7	✓	37.3	-
	mbPt-870753	11	44.1	✓	-0.2	0.0084											13	40.4	✓	-0.3	0.0058
	soPb-825660	11	46.7	✓	-1.3	0.0025						9	23.2	✓	-0.8	0.0075					
	mbPb-876592											9	38.8	✓	21.9	-	13	25.2	✓	0.3	0.0064
	mbPb-877325	11	51.5	✓	0.3	0.0055						9	43.6	✓	1.5	0.0028	15	25.4	✓	3.7	0.0005
	mbPt-868260													✓	1.6	0.0019	13	32.6	✓	0.7	0.0037
	soPb-824730	-	-	✓	-0.3	0.0060						9	22.1	✓	-0.5	0.0094	13	47.9	✓	-1.9	0.0011
Overall visual seed traits	mbPb-868763	11	42.1	✓	1.0	0.0045	11	36.4	✓	18.3	0.0005	-	-	✓	20.5	0.0009					
	mbPb-877269	11	41.6	✓	1.3	0.0030	11	37.3	✓	12.1	-						-	-	✓	3.3	0.0016
	mbPt-868763	11	42.1	✓	5.9	0.0015	11	36.4	✓	0.7	0.0025	-	-	✓	1.4	0.0047					
	mbPb-868032											-	-	✓	7.8	0.0019	-	-	✓	4.0	0.0005
	mbPb-868147	-	-	✓	30.8	0.0000	-	-	✓	55.3	0.0000	-	-	✓	39.2	0.0000					
	mbPb-868828											9	29.6	✓	0.6	0.0075	13	30.1	✓	0.9	0.0042
	mbPt-868828											9	27.5	✓	30.8	-	13	30.7	✓	0.2	0.0101
	mbPt-868147	-	-	✓	10.0	0.0010											-	-	✓	3.5	0.0011
	soPb-825660	11	46.7	✓	-0.1	0.0099						9	23.2	✓	-2.5	0.0038					
Time to flowering	mbPb-877288	8	39.7	✓	2.5	0.0005											3	36.2	✓	0.9	0.0005
	mbPt-847829	8	39.9	✓	0.4	0.0089						-	-	✓	1.3	0.0009					
	mbPt-867926						11	36.3	✓	-0.7	0.0045						13	35.3	✓	-0.2	0.0058
	mbPt-870753	11	44.1	✓	-0.5	0.0079	11	36.5	✓	-0.2	0.0100										
	soPb-825660	11	46.7	✓	-0.5	0.0084	11	35.2	✓	-3.0	0.0010	9	23.2	✓	21.0	-					
	mbPb-868828						11	46.8	✓	0.4	0.0065						13	30.1	✓	0.2	0.0042
	mbPb-870338	8	32.8	✓	15.7	0.0000	-	-	✓	18.5	0.0000										
	mbPt-867904											8	14.0	✓	-0.5	0.0094	-	-	✓	-0.2	0.0069
	soPb-824730	-	-	✓	-0.6	0.0065						9	22.1	✓	21.0	-	13	47.9	✓	-0.2	0.0074
Duration of flowering	mbPb-870338	8	32.8	✓	1.7	0.0000	-	-	✓	6.6	0.0000										
	soPb-824730	-	-	✓	-0.6	0.0055						9	22.1	✓	-0.5	0.0085					
	soPt-824253						-	-	✓	0.6	0.0090						-	-	✓	2.3	0.0021

Traits	Common markers	1B					1K					87B					87K				
		LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value
Pod growth duration	soPb-824755	11	46.7	✓	-1.1	0.0045	11	36.0	✓	-1.0	0.0055										
	soPb-825660	11	46.7	✓	-0.8	0.0060	11	35.2	✓	-2.1	0.0000										
	mbPb-870338	8	32.8	✓	2.6	0.0015	-	-	✓	1.9	0.0005										
Growth duration	mbPb-868706											15	19.0	✓	6.6	0.0009	8	15.0	✓	3.3	0.0000
Leaflet length	mbPb-877288	8	39.7	✓	-0.9	0.0065	7	19.5	✓	-16.7	-										
	mbPb-867674	11	68.5	✓	-3.1	0.0010	11	10.7	✓	-1.0	0.0025										
	mbPt-876514	11	68.3	✓	-1.9	0.0030	11	10.7	✓	-0.4	0.0075										
	mbPt-848179	3	75.7	✓	0.6	0.0084	2	28.0	✓	0.7	0.0030										
	mbPt-848616	3	75.1	✓	0.6	0.0094	2	29.1	✓	0.3	0.0090										
Leaflet width	mbPt-847350	6	42.9	✓	0.5	0.0084	18	19.4	✓	0.4	0.0070										
	mbPt-869376	6	43.5	✓	1.2	0.0015	18	20.2	✓	0.7	0.0050										
Leaflet ratio	mbPb-848586	11	67.4	✓	-0.1	0.0030						-	-	✓	-0.9	0.0085					
	mbPt-847350	6	42.9	✓	-0.1	0.0050	18	19.4	✓	-1.4	0.0015										
	mbPt-877316											15	12.8	✓	-1.1	0.0056	8	12.2	✓	0.5	0.0058
Stem diameter	mbPt-867926											9	21.2	✓	22.3	-	13	35.3	✓	-1.1	0.0053
	soPb-825660						11	35.2	✓	-0.6	0.0055	9	23.2	✓	-1.3	0.0047					
Internode length	mbPb-870338	8	32.8	✓	-0.8	0.0030	-	-	✓	-2.5	0.0005										
	mbPt-877288						7	19.6	✓	-2.7	0.0000	-	-	✓	-1.0	0.0094					
Floral standard width	mbPt-848267	8	0.0	✓	0.5	0.0045	7	42.6	✓	-10.7	-										
	mbPb-849130	8	0.0	✓	0.2	0.0084	7	39.8	✓	0.2	0.0065										
	mbPb-867966											-	-	✓	1.7	0.0056	14	13.8	✓	1.3	0.0005
	mbPb-870338	8	32.8	✓	-0.6	0.0035	-	-	✓	-0.3	0.0050										
Stem length	mbPb-848172	6	24.0	✓	2.0	0.0010						14	12.7	✓	1.0	0.0075					
	mbPb-848500	6	24.4	✓	0.7	0.0079	8	24.5	✓	2.0	0.0010	14	12.7	✓	1.6	0.0028					
	mbPb-848781	6	24.1	✓	1.8	0.0015	8	23.9	✓	2.1	0.0005	14	12.4	✓	2.0	0.0000					
	mbPt-847535	6	24.0	✓	0.6	0.0089	8	24.4	✓	0.9	0.0040										
	mbPt-848781	6	24.0	✓	1.2	0.0040	8	24.6	✓	0.6	0.0075										
	mbPb-848641											-	-	✓	-1.9	0.0019	15	9.7	✓	-1.8	0.0005
	mbPb-867674											-	-	✓	-1.4	0.0047	15	9.7	✓	-1.6	0.0011
	mbPb-846792	6	25.1	✓	-0.6	0.0094	8	23.9	✓	-0.7	0.0070										
	mbPt-868384	6	25.1	✓	-1.5	0.0020	8	11.0	✓	-1.4	0.0025										
	mbPb-847400	-	-	✓	0.8	0.0070	8	0.0	✓	2.1	0.0000										
	mbPt-876952	-	-	✓	2.7	0.0005	-	-	✓	1.8	0.0015										
Branch length	mbPb-848781	6	24.1	✓	1.6	0.0010	8	23.9	✓	1.7	0.0035										
	mbPt-848172	6	24.0	✓	2.7	0.0000											4	15.7	✓	0.4	0.0079
	mbPt-848781	6	24.0	✓	1.2	0.0025											4	16.0	✓	16.6	-
	mbPb-847400	-	-	✓	0.9	0.0055	8	0.0	✓	2.0	0.0020										
	mbPb-849009	6	22.3	✓	-0.9	0.0050						14	18.2	✓	-1.1	0.0019					
	mbPb-877325	11	51.5	✓	0.6	0.0099											15	25.4	✓	1.2	0.0037
	mbPt-868384	-	-	✓	-1.6	0.0015	8	11.0	✓	-0.7	0.0080										
	mbPt-877288											-	-	✓	0.7	0.0056	3	35.8	✓	0.4	0.0090

Traits	Common markers	1B					1K					87B					87K				
		LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value
Peduncle length	soPb-824755	11	46.7	✓	1.9	0.0010	11	36.0	✓	0.3	0.0080										
	soPb-825660	11	46.7	✓	2.3	0.0000						9	23.2	✓	-0.5	0.0085					
	mbPb-877269	11	41.6	✓	-0.5	0.0070	11	37.3	✓	-1.0	0.0050										
	mbPt-846260	11	43.5	✓	0.6	0.0060	11	36.3	✓	3.0	0.0005										
	mbPt-846471	11	43.9	✓	0.9	0.0035	11	36.5	✓	1.5	0.0030										
	mbPt-867926	11	44.1	✓	2.1	0.0005	11	36.3	✓	1.7	0.0015										
	mbPt-870753	11	44.1	✓	0.9	0.0030	11	36.5	✓	6.4	0.0000										
	mbPt-871632	11	44.2	✓	0.7	0.0050	11	36.7	✓	1.5	0.0025										
	soPb-824730	-	-	✓	0.8	0.0040	11	34.4	✓	1.3	0.0035										
No. of branches per plant	mbPb-877288						7	19.5	✓	0.2	0.0045						3	36.2	✓	1.4	0.0064
	mbPt-847829											-	-	✓	2.1	0.0038	3	34.9	✓	2.4	0.0016
	mbPt-877288	8	39.9	✓	0.1	0.0079	7	19.6	✓	4.4	0.0000	-	-	✓	1.5	0.0056	3	35.8	✓	0.9	0.0090
	mbPb-847947						-	-	✓	-0.1	0.0060						-	-	✓	-2.8	0.0011
	mbPb-870338	8	32.8	✓	0.6	0.0020	-	-	✓	0.1	0.0070										
No. of leaves on stem	soPb-825660						11	35.2	✓	-1.5	0.0020	9	23.2	✓	-0.9	0.0009					
	soPb-831975	11	47.7	✓	-0.1	0.0099	11	35.6	✓	-0.5	0.0070										
	mbPb-870338	8	32.8	✓	2.0	0.0005	-	-	✓	1.8	0.0005										
	mbPt-877288	8	39.9	✓	0.5	0.0025											3	35.8	✓	0.8	0.0069
	soPb-824730	-	-	✓	-0.5	0.0020						9	22.1	✓	-0.9	0.0000					
No. of nodes on stem	mbPt-867926						11	36.3	✓	-1.1	0.0020	9	21.2	✓	-0.6	0.0094	13	35.3	✓	-0.5	0.0042
	mbPt-871632	11	44.2	✓	-1.7	0.0020	11	36.7	✓	-1.4	0.0005										
	soPb-824755						11	36.0	✓	-0.4	0.0100						-	-	✓	-0.3	0.0053
	soPb-825660						11	35.2	✓	-0.8	0.0030	9	23.2	✓	-1.4	0.0009					
	mbPb-870338	8	32.8	✓	1.6	0.0025	-	-	✓	1.3	0.0010										
	soPb-824730	-	-	✓	-0.8	0.0070						9	22.1	✓	-1.6	0.0000					
No. of nodes on branches	mbPb-877288	8	39.7	✓	0.6	0.0020	7	19.5	✓	0.1	0.0080										
	mbPt-847829	8	39.9	✓	0.1	0.0079						-	-	✓	1.5	0.0047	3	34.9	✓	1.5	0.0011
	mbPt-877288	8	39.9	✓	1.9	0.0010	7	19.6	✓	2.6	0.0000										
	mbPb-847947						-	-	✓	0.0	0.0100						-	-	✓	-1.1	0.0016
	mbPb-870338	8	32.8	✓	7.3	0.0000	-	-	✓	0.6	0.0005										
	mbPt-846602											11	2.3	✓	-0.9	0.0094	-	-	✓	-0.2	0.0079
	mbPt-849188											16	52.4	✓	1.0	0.0075	-	-	✓	0.2	0.0069
	mbPt-871774	-	-	✓	0.8	0.0015											-	-	✓	0.2	0.0090
	soPb-824730	-	-	✓	-0.3	0.0045	11	34.4	✓	-0.1	0.0070										
Node of 1st pod	mbPb-877288	8	39.7	✓	0.1	0.0075											3	36.2	✓	0.3	0.0053
	mbPt-877288	8	39.9	✓	1.3	0.0010						-	-	✓	1.3	0.0094	3	35.8	✓	22.5	-
	mbPt-867926						11	36.3	✓	-1.9	0.0015						13	35.3	✓	-0.5	0.0026
	soPb-824755	11	46.7	✓	-0.1	0.0089	11	36.0	✓	-0.9	0.0065										
	soPb-825660						11	35.2	✓	-1.7	0.0025	9	23.2	✓	28.0	-					
	soPb-831975	11	47.7	✓	-0.1	0.0094	11	35.6	✓	-1.1	0.0055										
	mbPb-868828	11	33.2	✓	0.1	0.0055	11	46.8	✓	1.3	0.0050										
	mbPb-870338	8	32.8	✓	4.4	0.0000	-	-	✓	11.0	0.0000										
	mbPt-848579											-	-	✓	1.9	0.0038	13	12.8	✓	-0.2	0.0058
No. of pods per peduncle	mbPb-847272						17	23.3	✓	-0.9	0.0035	-	-	✓	-0.6	0.0094					
	mbPb-870338	8	32.8	✓	-0.7	0.0015	-	-	✓	-1.1	0.0015										

Traits	Common markers	1B					1K					87B					87K				
		LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value	LG	Pos (cM)		PVE %	*P* - value
Total pod clusters	mbPb-877288	8	39.7	✓	0.6	0.0035	7	19.5	✓	0.4	0.0045										
	mbPt-847829	8	39.9	✓	0.2	0.0075											3	34.9	✓	1.7	0.0016
	mbPt-877288	8	39.9	✓	0.5	0.0040						-	-	✓	0.5	0.0056	3	35.8	✓	0.3	0.0079
	mbPb-847947						-	-	✓	-0.5	0.0035						-	-	✓	-4.6	0.0000
	mbPb-870338	8	32.8	✓	5.5	0.0000	-	-	✓	0.3	0.0070										
No. of seeds per pod	mbPb-847947	-	-	✓	-1.0	0.0035											-	-	✓	-1.2	0.0011
	mbPt-847829											-	-	✓	1.5	0.0028	3	34.9	✓	0.8	0.0032
Pod length	mbPb-847817						18	19.8	✓	-2.2	0.0025			✓	-2.2	0.0047					
	mbPt-847350	6	42.9	✓	0.9	0.0055	18	19.4	✓	2.2	0.0020										
	mbPt-848907	6	44.4	✓	0.7	0.0060	18	21.5	✓	1.1	0.0055										
	mbPt-869376	6	43.5	✓	2.3	0.0015	18	20.2	✓	7.5	0.0000										
	mbPt-877453	6	52.9	✓	-0.3	0.0094	18	29.6	✓	-0.9	0.0065										
	mbPt-877486	6	52.8	✓	-1.2	0.0040	18	29.6	✓	-0.6	0.0095										
	mbPt-847817	6	43.9	✓	-1.7	0.0030	18	19.8	✓	-2.4	0.0015	-	-	✓	-1.9	0.0066					
	mbPt-848177	5	16.8	✓	3.2	0.0010						-	-	✓	4.5	0.0009					
	mbPt-848786						2	9.1	✓	-0.9	0.0070						11	14.5	✓	-0.3	0.0048
	soPb-853232	6	40.6	✓	0.5	0.0070	18	7.3	✓	1.6	0.0035										
	soPt-853267	6	69.7	✓	-4.1	0.0005	18	56.3	✓	-4.4	0.0005										
Pod width	mbPb-877288	8	39.7	✓	-0.1	0.0040	7	19.5	✓	-14.2	-										
	mbPt-848066	5	32.4	✓	0.3	0.0020											14	26.6	✓	0.9	0.0021
	mbPt-869376	6	43.5	✓	0.1	0.0055	18	20.2	✓	0.2	0.0040										
Seed size	mbPt-847817	6	43.9	✓	-1.6	0.0020						-	-	✓	-3.8	0.0009					
	mbPt-869376	6	43.5	✓	0.6	0.0065	18	20.2	✓	0.2	0.0040										
	mbPt-877288	8	39.9	✓	-0.8	0.0040						-	-	✓	-1.2	0.0056					
Hardseededness	mbPt-848057						16	41.0	✓	0.1	0.0100						-	-	✓	0.2	0.0058
	mbPt-848087						16	41.0	✓	0.1	0.0100						-	-	✓	0.7	0.0005
Dry pod mass	mbPb-847829						7	19.6	✓	3.2	0.0005						3	34.9	✓	1.7	0.0000
	mbPt-847829						7	19.6	✓	1.9	0.0020						3	34.9	✓	0.7	0.0021
	mbPt-867926						11	36.3	✓	-0.7	0.0075						13	35.3	✓	12.9	-
	soPb-824730	-	-	✓	-3.8	0.0005	11	34.4	✓	21.9	-										
	mbPb-868828						11	46.8	✓	1.0	0.0050	9	29.6	✓	30.3	-					
	mbPt-871632	11	44.2	✓	-1.1	0.0040											13	38.7	✓	12.9	-
Seed yield	mbPb-847829						7	19.6	✓	1.4	0.0015						-	-	✓	0.5	0.0026
	mbPt-847829						7	19.6	✓	0.7	0.0060						3	34.9	✓	0.4	0.0032
	mbPb-868763	11	42.1	✓	0.6	0.0094	11	36.4	✓	0.5	0.0075										
	mbPt-868763	11	42.1	✓	0.8	0.0045	11	36.4	✓	0.2	0.0100										
	mbPt-867926						11	36.3	✓	-0.3	0.0085						13	35.3	✓	13.3	-
	mbPt-871632	11	44.2	✓	-0.9	0.0035											13	38.7	✓	13.3	-
	soPb-824730	-	-	✓	-2.2	0.0000	11	34.4	✓	23.5	-										
Standing dry biomass	mbPb-870338	8	32.8	✓	3.0	0.0005	-	-	✓	1.7	0.0035										
	mbPb-877288	8	39.7	✓	0.7	0.0030											3	36.2	✓	0.2	0.0079
	soPb-832041	-	-	✓	1.9	0.0015	11	41.7	✓	1.8	0.0030										
Harvest index	mbPb-870338	8	32.8	✓	-3.7	0.0005	-	-	✓	-1.0	0.0065										
	soPt-825848											-	-	✓	-0.3	0.0085	13	28.1	✓	-1.5	0.0011

Appendix 5.4. Locations and effects of QTLs associated with qualitatively inherited morphological traits detected by the SML (*P*-value ≤ 0.001) and ICIM - ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on LG (in cM); LOD = Logarithm of odds score; PVE = Phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect)

♪: Underlined LG and Pos are positions of SML QTLs which are assigned based on the genotype correlation ($r \geq 0.84$) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Highlighted markers indicate overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold PVE numbers indicate major QTLs with PVE % ≥ 20% for SML and ≥ 30% for ICIM methods respectively

a: QTLs detected by SML method at significant *P*-value of 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for an ICIM QTL region

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. Hypocotyl pigment	11	14.5	mbPt-846225 a		-1.7	3	10.0	mbPt-870444/ mbPt-848692	3.7	-15.8										
	-	-	mbPb-868147 a		-1.1															
	-	-	mbPt-868147 a		-1.0	11	42.3	mbPb-847032 a		1.7										
						11	66.0	mbPt-877021/ mbPt-868487	4.3	-18.6										
						19	40.0	soPb-832205/ soPb-854765	3.4	-15.5										
						-	-	mbPb-868147 a		5.8										
						-	-	soPb-825015 a		1.7										
b. Stem colour	1	24.6	soPt-856642 a		-3.3	7	32.4	mbPt-867887 a		-1.1	17	21.3	mbPt-877415 a		0.9	14	59.6	mbPt-848061 a		3.0
	1	87.0	mbPt-847742/ mbPb-849011	4.5	-12.3	7	33.0	mbPt-867887/ soPb-825779 †	6.3	21.0	-	-	mbPt-847817 a		1.0	-	-	mbPb-870682 a		-2.5
																-	-	mbPt-868732 a		1.7
	1	87.1	mbPb-849011 a		4.2	7	32.4	mbPb-849021 a		-1.3										
	3	71.0	mbPb-846221/ mbPt-847660	4.0	12.8	19	5.0	mbPb-870677/ mbPb-846988 †	5.4	16.6										
	5	7.5	mbPt-848651 a		-2.8	-	-	mbPt-868086 a		-1.0										
	11	40.0	mbPt-847459/ soPb-824843	5.1	14.9															

Appendix 5.4. Continued 1...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
c. Leaf rachis colour	4	16.0	soPb-831980/ mbPt-848196	3.5	-22.7	1	28.0	mbPt-867902/ mbPt-869470 †	3.5	10.7	17	21.3	mbPt-877415 a		1.4	13	0.0	mbPb-847032 a		1.0
											17	21.9	mbPt-846869 a		1.0	3	39.1	mbPb-870338 a		0.8
	8	39.0	mbPb-847809/ mbPb-877288	3.5	18.0	8	18.0	mbPt-868384/ mbPb-847535†	5.7	19.5						-	-	mbPb-846720 a		0.6
	8	39.7	mbPb-877288 a		1.6	8	26.0	mbPt-868381/ mbPt-847654 †	9.6	**-30.3**										
	8	39.9	mbPt-877288 a		1.6															
	-	-	mbPt-869240 a		2.4	11	34.4	soPb-824730 a		-1.3										
						11	36.5	mbPt-870753 a		-1.1										
						11	48.0	mbPb-848513/ mbPt-849182 †	6.7	-20.7										
						17	40.0	mbPt-849118/ mbPt-848472 †	4.4	-12.0										
						-	-	soPt-855536 a		1.2										
d. Leaf petiole colour	1	63.0	mbPt-869213/ mbPt-868339 †	3.9	-10.2	3	32.0	mbPt-870568/ mbPt-868868 †	11.9	27.0	11	12.4	mbPt-849021 a		-5.6	-	-	soPb-832155 a		3.8
											-	-	mbPb-847817 a		3.5	-	-	mbPb-847286 a		2.2
	3	50.0	mbPb-868582/ mbPb-849156 †	4.9	10.3	3	46.0	soPb-831873/ mbPb-871848 †	6.2	-12.6						-	-	soPt-832155 a		1.6
	4	15.0	soPb-825932/ soPb-831980 †	7.2	-19.6	7	18.0	soPt-855854/ mbPb-847372 †	5.6	11.4										
	8	39.7	mbPb-877288 a		1.4	9	7.0	mbPt-847916/ mbPt-847896 †	6.0	-13.6										
	-	-	soPb-824730 a		-2.1															
	-	-	mbPt-869240 a		1.8	-	-	mbPb-868147 a		-2.8										
						-	-	soPb-832055 a		-2.2										
						-	-	soPt-853484 a		2.2										

Appendix 5.4. Continued 2...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval‡	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
e. Plant hair density	3	37.0	mbPt-867694/ mbPb-868464 †	12.6	-12.7	16	48.0	mbPt-846915/ mbPt-870410	3.8	19.7	12	9.5	mbPb-877486 a		-1.2	16	32.0	mbPt-867765/ mbPb-848907	4.7	**30.1**
	4	6.8	mbPb-846685 a		-0.9	16	48.3	mbPt-870410 a		3.4	-	-	soPt-825889 a		1.1	16	49.5	mbPt-869376 a		2.9
	4	9.0	mbPb-847658/ soPt-855555 †	14.8	20.4	-	-	mbPt-871155 a		-2.2						-	-	mbPt-846445 a		-2.5
						-	-	soPb-824602 a		1.9						-	-	mbPb-847947 a		-2.3
	6	52.9	mbPt-877453 a		-0.6															
	7	32.8	mbPt-846835 a		0.6															
	7	33.0	mbPt-846835/ soPt-855837 †	15.1	17.1															
	7	42.0	soPt-854555/ mbPt-871572 †	12.4	-14.0															
	12	65.0	mbPb-876762/ mbPb-846334 †	10.8	-10.6															
f. Plant hair colour	3	0.0	mbPt-849095/ mbPt-867792 †	13.8	**32.8**	16	70.0	mbPt-877344/ mbPt-847627	3.5	-18.3	-	-	mbPb-848400 a		-1.1	3	30.0	mbPt-847626/ soPb-857559 †	3.0	11.7
											-	-	mbPt-876534 a		0.7					
	3	8.0	mbPb-846396/ mbPt-876637 †	9.6	-21.4	-	-	mbPt-869245 a		3.3						3	34.9	mbPt-847829 a		-1.5
						-	-	mbPt-867794 a		-2.6						3	35.0	mbPt-847829/ mbPt-877288 †	3.8	-15.1
	8	14.5	soPb-855550 a		0.9	-	-	soPb-856981 a		-2.3										
	8	19.5	mbPb-876991 a		-1.0											-	-	soPb-854119 a		-0.7
	11	51.0	mbPb-871281/ mbPb-877325 †	6.3	-13.0											-	-	mbPb-869222 a		-0.7
	11	51.5	mbPb-877325 a		-0.8															
	12	59.0	mbPt-848460/ mbPt-846334 †	5.4	11.5															
g. Growth habit	3	62.0	mbPt-846669/ mbPb-868471	4.3	13.5	11	46.8	mbPt-868828 a		3.8	3	40.6	mbPt-870630 a		2.9	3	32.0	mbPb-847621/ mbPt-848400	3.2	20.3
	8	39.0	mbPb-847809/ mbPb-877288	3.6	13.4	1	21.0	mbPt-848946/ mbPt-848646	3.6	12.0	11	3.0	mbPt-846602/ mbPt-867887	4.3	19.6	3	32.9	mbPt-848400 a		5.9
						-	-	mbPt-871661 a		6.6	-	-	soPb-855834 a		-3.7	3	33.0	mbPb-848400 a		2.7
	11	42.1	mbPt-868763 a		2.4	-	-	mbPt-871497 a		4.1						-	-	mbPt-848943 a		6.6
	11	51.0	mbPb-871281/ mbPb-877325	4.7	16.0															
	11	51.5	mbPb-877325 a		2.0															
	8	47.2	soPt-824253 a		1.8															

Appendix 5.4. Continued 3...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
h. Twining	6	24.0	mbPt-847535/ mbPb-846949	16.6	**48.3**	1	21.0	mbPt-848946/ mbPt-848646	6.2	17.3	14	19.0	mbPb-849009/ mbPt-849166	4.0	21.1	3	13.0	mbPt-849021/ mbPt-867887	4.1	15.6
	6	25.1-	mbPt-868384 a		-1.2	-	-	soPt-824253 a		4.6	17	0.0	soPb-856544 a		4.6	4	15.0	mbPt-848613/ mbPb-848500	5.0	20.1
						-	-	soPt-857318 a		-2.3	-	-	mbPb-877550 a		5.2					
	9	10.0	mbPt-876465/ mbPt-870681	4.9	10.2	-	-	soPb-825355 a		2.0						-	-	mbPt-870337 a		-3.0
			mbPb-868263 a													-	-	mbPb-848706 a		-2.4
	-	-	mbPb-847393 a		-0.5											-	-	mbPt-848885 a		-1.6
i. Leaflet lobing	11	63.0	soPb-854393/ soPb-855762	6.1	14.8	11	9.0	soPb-853216/ mbPb-848586	17.0	**66.2**	9	11.0	soPb-853806/ soPt-853944	8.3	**33.6**	11	13.0	mbPb-849000/ mbPb-868592 †	7.4	14.3
	11	68.0	mbPb-848586/ mbPt-876514	8.9	23.4	11	10.6	mbPb-848586 a		-5.1	12	0.0	soPb-831551/ soPb-857372	3.1	11.0	15	9.7	mbPb-867674 a		4.9
						11	10.8	mbPt-848586 a		-6.6						15	9.7	mbPb-848641 a		-4.5
	11	68.3	mbPt-848641 a		-1.4	11	14.3	mbPb-868263 a		-3.3	-	-	mbPb-848641 a		15.5	15	9.7	mbPt-848641 a		-5.6
	11	68.3	mbPt-848586 a		-1.1						-	-	mbPb-848586 a		-8.7	15	14.0	soPb-853645/ mbPb-870853 †	7.4	15.4
	-	-	mbPt-849022 a		1.1															
j. Flower colour	11	67.4	mbPb-848586 a	4.8	-1.3	11	0.0	mbPb-876726/ mbPb-877485	3.3	17.2	6	14.7	mbPt-847184 a	8.9	4.0	9	48.3	mbPt-868815 a		0.9
	11	68.0	mbPb-848586/ mbPt-876514		23.4						9	2.0	mbPb-846264/ soPt-832240		**41.8**	-	-	soPt-854506 a		0.9
						-	-	soPt-854823 a		1.0						-	-	soPt-825659 a		0.8
	11	68.4	mbPb-848641 a		-1.4	-	-	mbPt-849064 a		0.7	9	10.3	soPb-853575 a		6.3					
	11	68.5	mbPb-867674 a		-1.4	-	-	soPt-854770 a		0.4										
k. Inflorescence structure	6	24.0	mbPt-847535/ mbPb-846949	11.1	**-40.0**	7	55.0	mbPt-847642/ mbPt-848023	6.3	-16.8	14	11.2	mbPt-848172 a		-1.6	4	0.0	mbPb-847400 a		-1.3
											-	-	mbPb-868285 a		-1.6	4	2.0	mbPb-847400/ mbPt-868384	3.6	-21.8
	6	25.1	mbPt-868384 a		1.3	-	-	mbPb-848919 a		1.3										
	11	59.0	mbPb-870853 a		0.6	-	-	mbPt-868631 a		-1.0						4	20.3	mbPb-868023 a		1.1
	-	-	mbPt-876725 a		0.5	-	-	mbPt-876766 a		-0.9						10	15.9	mbPb-876675 a		-1.6

Appendix 5.4. Continued 4...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
l. Dry pod colour	3	57.2	mbPt-868642 a		1.6	16	18.3	mbPt-848870 a		1.1	15	13.7	mbPb-848346 a		3.2	2	12.0	mbPt-869016/ soPt-857021 †	18.0	**45.2**
	3	67.7	mbPb-846221 a		-1.4	16	18.4	mbPt-849070 a		1.3	15	19.0	mbPb-868706 a		3.7					
	3	73.0	mbPt-848800/ mbPb-868160	5.3	22.8	16	19.0	mbPt-848221/ mbPt-846885	4.8	20.2	15	21.0	mbPb-868706/ mbPt-869539	5.4	**31.6**	13	38.0	mbPt-867926/ mbPt-871632 †	9.8	18.1
	3	73.1	mbPb-868160 a		2.4	16	19.1	mbPt-846885 a		1.7						14	42.0	mbPt-848177/ mbPb-849151 †	6.9	12.5
																16	14.0	soPt-853267/ soPb-857372 †	5.6	10.7
																-	-	mbPt-876498 a		12.8
																-	-	mbPb-847045 a		-5.0
																-	-	soPb-856883 a		-4.4
m. Pod dehiscence	1	10.0	mbPt-846139/ mbPt-876878 †	4.4	-19.2	7	39.0	mbPt-868516/ mbPt-846324 †	4.0	18.7	14	10.0	mbPt-846949/ mbPt-848613	4.1	15.6	8	18.0	mbPb-868706/ mbPb-871035	3.6	20.7
	6	41.0	soPb-853232/ mbPt-868047 †	3.4	10.4	11	36.8	soPb-824700 a		1.0	14	10.0	mbPt-846949 a		1.1	8	18.7	mbPb-871035 a		-1.3
						18	19.8	mbPt-847817 a		1.6	-	-	mbPt-848285 a		-1.2	16	16.4	soPb-857372 a		-1.3
	11	59.0	mbPt-848587/ mbPb-870853 †	5.8	14.6	18	19.8	mbPb-847817 a		1.5						-	-	mbPt-870744 a		1.4
	-	-	mbPt-846215 a		-2.4															
	-	-	soPt-853635 a		1.9															
	-	-	mbPt-877231 a		-1.6															
n. Powdery mildew	5	46.0	mbPb-867966/ mbPb-847671	4.3	10.7	4	29.2	mbPt-848599 a		0.9	3	106.0	mbPt-848435/ mbPt-848441	3.8	15.7	13	19.2	mbPb-877264 a		4.3
	5	46.8	mbPb-847671 a		-2.4	7	40.0	mbPt-846324/ mbPt-868542	4.3	-19.0	7	10.9	mbPt-871507 a		-4.0	13	22.0	mbPb-848100/ mbPb-846991	3.8	23.2
	6	13.0	mbPt-869281/ mbPt-876816	6.3	-16.6	-	-	mbPt-877076 a		-1.0	7	12.0	mbPt-871507/ soPt-825810	6.3	-24.0	-	-	soPt-824890 a		4.8
						-	-	mbPt-871365 a		0.9						-	-	mbPb-869240 a		-4.6
	8	32.0	mbPt-876991/ mbPb-870338	5.9	-17.4						17	23.0	mbPt-846869/ mbPt-848216	3.7	14.5					
	11	0.0	mbPt-847209/ mbPt-847132	4.1	-9.5						17	23.4	mbPt-848216 a		3.5					
	11	42.0	mbPb-877269/ mbPt-868763	3.5	-8.9															
	-	-	mbPt-848368 a		2.5															
	-	-	mbPt-876596 a		2.2															

Appendix 5.4. Continued 5...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
o. Thrips	11	22.6	mbPt-846155 a		2.5	4	5.0	mbPb-867668/ mbPb-877185 †	5.1	16.9	12	1.0	soPb-857372/ mbPb-868823	3.8	-16.4	3	34.0	mbPb-848400/ mbPt-847829	3.4	-18.0
	11	22.6	mbPt-868656 a		3.1															
	11	22.6	mbPt-876612 a		1.5	4	11.7	mbPt-876847 a		1.4	-	-	mbPb-848630 a		3.1	13	24.7	mbPb-877602 a		2.4
	11	23.0	mbPt-877602/ mbPt-868118	4.9	-22.3	15	19.9	mbPb-867920 a		1.1	-	-	mbPt-868763 a		-2.4	13	24.8	mbPb-876477 a		1.1
						15	20.0	mbPb-867920/ soPb-855725 †	17.6	**74.4**						13	24.8	mbPb-876817 a		1.1
						-	-	mbPt-868952 a		1.5										
p. Perenniality											8	28.6	mbPb-868592 a		1.5	3	32.9	mbPt-848400 a		1.9
											15	19.0	mbPb-868706 a		5.8	3	33.0	mbPb-848400 a		1.9
											17	18.0	mbPt-870410/ mbPt-877415 †	5.3	-22.6	8	18.0	mbPb-868706/ mbPb-871035	5.3	17.5
																8	19.0	mbPb-871035/ mbPb-847338	4.0	17.4
																8	15.0	mbPb-868706 a		1.8

Appendix 5.5. Locations and effects of QTLs associated with qualitative seed traits detected by the SML (*P* - value ≤ 0.001) and ICIM-ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on linkage group (in cM); LOD = Logarithm of odds score; PVE = Phenotypic variation explanation (%) (+ve or -ive depending on whether the QTL increased or decreased the trait effect

♪: Underlined LG and Pos are positions of SML QTLs which are assigned based on the genotype correlation ($r \geq 0.84$) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Highlighted markers indicate overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold PVE numbers indicate major QTLs with PVE % ≥ 20% for SML and ≥ 30% for ICIM methods respectively

a: QTLs detected by SML method at significant *P* – value of 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for an ICIM QTL region

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. Testa colour	11	14.0	mbPt-867688/ mbPt-846225	8.0	**45.6**	11	42.3	mbPb-847032 a	6.1	-4.1	-	-	mbPb-868147 a		**79.6**	13	6.0	mbPb-847032/ mbPt-848579	4.7	24.2
	11	14.5	mbPt-846225 a		5.5	11	43.0	mbPb-847032/ mbPb-847470		29.1	-	-	mbPb-868032 a		3.6	-	-	mbPt-868032 a		**70.6**
	-	-	mbPb-868147 a		**40.8**	-	-	mbPb-868147 a		**59.6**						-	-	soPb-854233 a		-1.9
	-	-	mbPt-868147 a		14.5	-	-	mbPt-868810 a		-1.7						-	-	mbPb-868032 a		1.9
b. Seed mottling	11	14.5	mbPt-846225 a		3.9	11	42.0	soPb-832041/ mbPb-847032	6.5	**33.6**	-	-	mbPb-868147 a		**76.8**	13	7.0	mbPb-847032/ mbPt-848579	4.1	22.4
	11	13.0	mbPt-867688/ mbPt-846225	9.5	**54.7**						-	-	mbPb-868032 a		4.3					
						11	42.3	mbPb-847032 a		-2.7						-	-	mbPt-868032 a		**52.1**
	11	33.0	mbPt-877422/ mbPt-868828	4.2	-18.6	-	-	mbPb-868147 a		**64.7**						-	-	mbPt-868147 a		7.1
						-	-	mbPb-870721 a		-1.1						-	-	mbPb-868032 a		5.9
	-	-	mbPb-868147 a		**55.9**															
	-	-	mbPt-868147 a		9.2															
c. Seed coat ridging											9	23.0	soPb-824730/ soPb-825660 †	10.0	26.2	13	16.7	mbPb-877269 a		3.3
																13	33.0	mbPt-868260/ mbPt-877498	4.6	17.4
											-	-	mbPb-868763 a		18.0					
											-	-	mbPt-868763 a		6.7	15	25.4	mbPb-877325 a		1.1
																-	-	mbPt-847646 a		-4.0
d. Lustre																4	7.5	mbPb-848613 a		-5.3
																-	-	soPb-825303 a		-3.7
																-	-	mbPb-847655 a		-2.4

Appendix 5.5. Continued…

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
e. Texture layer depth	3	103.7	soPt-854190 a		0.7	11	34.0	mbPb-877325/ soPb-824730	4.4	22.5	9	25.0	soPb-825660/ mbPt-868828	8.8	**46.3**	13	16.7	mbPb-877269 a		3.5
	11	51.5	mbPb-877325 a		0.9											13	25.2	mbPb-876592 a		1.3
	-	-	mbPt-877004 a		-1.5	11	34.4	soPb-824730 a		-2.7	-	-	mbPb-868763 a		13.5	15	25.4	mbPb-877325 a		1.0
						11	35.2	mbPb-847088 a		-1.7	-	-	mbPt-868763 a		3.3	13	33.0	mbPt-868260/ mbPt-877498	4.1	20.4
						11	36.4	mbPb-868763 a		2.1										
f. Hilum colour	11	41.6	mbPb-877269 a		**50.7**	11	36.4	mbPb-868763 a		**53.7**	9	23.0	soPb-824730/ soPb-825660	4.6	16.1	13	16.0	mbPt-848579/ mbPb-868763	13.5	29.7
	11	42.1	mbPt-868763 a		14.5	11	36.4	mbPt-868763 a		13.0										
	11	42.1	mbPb-868763 a		3.9	11	37.3	mbPb-877269 a		2.7	-	-	mbPb-868763 a		**36.6**	13	16.7	mbPb-868763 a		6.7
											-	-	mbPt-868763 a		7.1	13	16.7	mbPb-877269 a		**55.5**
																13	32.0	mbPt-868828/ mbPt-868260	16.6	**37.7**
															3.5	13	32.6	mbPt-868260 a		3.2
g. Texture layer colour	11	41.6	mbPb-877269 a		**54.9**	11	36.4	mbPb-868763 a		**99.0**	9	24.0	soPb-825660/ mbPt-868828	9.2	27.0	13	16.7	mbPb-877269 a		**67.9**
	11	42.0	mbPb-877269/ mbPt-868763	38.9	**92.3**	-	-	mbPt-868828 a		0.1										
											9	42.0	mbPb-876592/ mbPb-877325	6.6	21.9	13	32.0	mbPt-868828/ mbPt-868260	13.0	**37.3**
	11	42.1	mbPt-868763 a		14.9											13	47.9	soPb-824730 a		-1.9
	11	42.1	mbPb-868763 a		3.8						-	-	mbPb-868763 a		**50.6**	15	25.0	mbPb-871281/ mbPb-877325	5.6	13.2
											-	-	mbPt-868763 a		10.8	15	25.4	mbPb-877325 a		3.7
h. Overall visual seed traits	11	12.0	mbPt-867688/ mbPt-846225	23.1	**67.7**	11	36.4	mbPb-868763 a	3.8	18.3	9	28.0	mbPt-868828/ soPt-824812	8.5	**38.0**	13	25.0	mbPb-876897/ mbPb-876592	9.0	**41.7**
	11	14.5	mbPt-846225 a		10.2	11	38.0	mbPb-877269/ mbPt-847428		12.1	-	-	mbPb-868147 a		**39.2**	-	-	mbPt-868032 a		**33.7**
	11	42.0	mbPb-877269/ mbPt-868763	4.5	13.8	11	42.3	mbPb-847032 a		-2.0	-	-	mbPb-868763 a		**20.5**	-	-	mbPb-868032 a		4.0
						11	43.0	mbPb-847032/ mbPb-847470	6.8	23.2						-	-	mbPt-868147 a		3.5
	-	-	mbPb-868147 a		**30.8**															
	-	-	mbPt-868147 a		10.0	-	-	mbPb-868147 a		**55.3**										

Appendix 5.6. Location and effects of QTLs associated with phenological traits detected by the SML (*P*-value ≤ 0.001) and ICIM-ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on linkage group (cM); LOD = Logarithm of odds score; PVE = phenotypic variation explanation (%) (+ve or -ive depending on whether the QTL increased or decreased the trait effect

♪: Underlined LG and Pos are positions of SML QTLs which are assigned based on the genotype correlation ($r \geq 0.84$) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Highlighted markers indicate overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold PVE numbers indicate major QTLs with PVE % ≥ 10% for SML and ≥ 20% for ICIM methods respectively

a: QTLs detected by SML method at significant *P* – value of 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for an ICIM QTL region

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. Time to flowering	3	85.1	soPt-855371 a		-2.0	7	40.0	mbPt-846324/ mbPt-868542	10.6	**27.2**	9	23.0	soPb-824730/ soPb-825660	5.1	**21.0**	3	36.2	mbPb-877288 a		0.9
	8	32.8	mbPb-870338 a		**15.7**											4	1.0	mbPb-847400/ mbPt-868384	3.7	11.6
	8	39.7	mbPb-877288 a		2.5	11	35.2	soPb-825660 a		-3.0	16	15.0	mbPt-847782/ mbPt-847390	3.0	11.2	13	30.7	mbPt-868828 a		1.0
						11	36.0	soPb-824755/ mbPt-867926	11.6	**28.6**	-	-	mbPb-871145 a		2.8	13	31.0	mbPt-868828/ mbPt-868260	8.6	**29.9**
						19	27.0	mbPb-846990/ soPb-832109	3.7	-8.1	-	-	mbPt-847829 a		1.3	-	-	soPb-853363		0.4
						-	-	mbPb-870338 a		**18.5**										
						-	-	mbPb-876778 a		-3.3										
b. Duration of flowering	8	12.7	mbPb-848296 a		-1.1	7	39.0	mbPt-868516/ mbPt-846324	29.4	**29.4**	1	14.0	mbPt-868911/ mbPt-869413	6.8	**32.4**	8	11.8	mbPt-868778 a		-2.6
	8	32.8	mbPb-870338 a		1.7											8	12.1	mbPb-877316 a		4.7
	-	-	mbPb-846581 a		-1.1	-	-	mbPb-870338 a		6.6	1	6.0	mbPt-869143/ mbPt-869315	11.6	- **66.1**	14	17.0	mbPb-867966/ mbPt-847297	3.4	12.5
						-	-	mbPb-848079 a		2.5										
						-	-	soPb-854003 a		1.5	16	36.7	mbPb-868581 a		5.2	14	17.1	mbPt-847297 a		-3.0
											-	-	mbPt-848376 a		2.8					
c. Pod growth duration	1	35.5	mbPt-876485 a		-2.7	7	40.0	mbPt-846324/ mbPt-868542	4.7	16.7	4	1.0	mbPt-846518/ mbPt-871643	3.9	- 19.8	4	0.0	mbPb-847400 a		0.8
	-	-	mbPt-877231 a		9.6											-	-	soPb-855048 a		-0.9
	-	-	mbPt-868938 a		2.7	11	35.2	soPb-825660 a		-2.1	-	-	mbPt-848177 a		3.8	-	-	soPb-855733 a		0.8
						11	37.0	soPb-824700/ mbPb-877269	4.0	14.9	-	-	mbPb-867891 a		-2.1					
						19	25.0	soPb-825612/ mbPb-846990	3.7	-14.0										
						-	-	mbPb-870338 a		1.9										
						-	-	mbPt-847947 a		-1.8										

Appendix 5.6. Continued…

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
d. Growth duration	3	55.0	mbPb-868786/ mbPt-868786 †	4.6	**-21.0**	1	27.0	mbPt-877351/ mbPt-867902 †	3.1	10.5	15	19.0	mbPb-868706/ mbPt-869539	4.3	18.2	3	35.0	mbPt-847829/ mbPt-877288	4.0	11.4
	11	46.7	soPb-825660 a		-2.7	2	3.0	mbPt-868143/ mbPb-846816 †	3.9	-12.9	15	19.0	mbPb-868706 a		6.6	8	12.1	mbPb-877305 a		2.7
	11	59.0	mbPb-868500 a		-4.1						16	36.7	mbPb-868581 a		7.8	8	12.2	mbPt-877316 a		1.6
	11	59.0	mbPb-877485 a		-3.4	7	35.0	mbPt-867887/ soPb-825779 †	3.9	-11.4	16	37.0	mbPb-868581/ mbPt-847739	3.5	15.8	8	15.0	mbPb-868706 a		3.3
																8	16.0	mbPb-868706/ mbPb-871035	7.2	**25.4**
						7	40.0	mbPt-846324/ mbPt-868542 †	9.5	**33.9**										
						11	34.4	soPb-824730 a		-1.1						16	9.0	soPt-853267/ soPb-857372	5.0	**21.5**
						-	-	soPt-853630 a		2.0										
						-	-	soPb-824080 a		-1.3										

Appendix 5.7. Location and effects of QTLs associated with quantitative morphological traits detected by the SML (*P*-value ≤ 0.001) and ICIM-ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on LG (cM); LOD = Logarithm of odds score; PVE = Phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect)

♪: Underlined LG and Pos are positions of SML QTLs which are assigned based on the genotype correlation (r ≥ 0.84) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Highlighted markers indicate overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold PVE numbers indicate major QTLs with PVE % ≥ 10% for SML and ≥ 20% for ICIM methods respectively

a: QTLs detected by SML method at significant *P*- value of 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for an ICIM QTL region

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. Leaflet size	1	32.0	mbPt-870422/ mbPt-847689 †	3.4	**21.4**	2	28.9	mbPb-848179 a		1.2	17	15.7	mbPt-870410 a		3.2	14	11.4	mbPt-847671 a		2.8
Leaflet length						6	0.0	mbPt-847507/ mbPt-846276 †	5.2	-12.3	17	16.0	mbPt-870410/ mbPt-877415	3.6	-18.6	-	-	mbPb-846816 a		-2.0
	3	71.3	mbPt-847660 a		3.5											-	-	soPt-855342 a		1.2
	8	39.9	mbPt-877288 a		-3.7	7	19.0	mbPb-847372/ mbPb-877288 †	4.4	-16.7	-	-	mbPb-868763 a		2.7					
	11	68.5	mbPb-867674 a		-3.1															
						11	14.3	mbPb-868263 a		-1.3										
						11	58.0	mbPb-877020/ mbPb-848590 †	7.9	**-26.2**										
						12	40.0	soPt-825518/ soPt-853239 †	4.1	-10.2										
						-	-	soPt-853360 a		1.1										
Leaflet width	5	35.3	mbPb-868679 a		2.4	7	13.0	mbPt-848629/ mbPb-871067 †	4.3	-10.5	15	12.2	mbPb-877305 a		1.7	3	34.9	mbPb-847829 a		0.9
	6	43.8	mbPt-846295 a		1.2						15	19.0	mbPb-868706/ mbPt-869539	5.0	**24.4**					
	6	43.9	mbPt-847817 a		-1.3	7	42.0	mbPt-868161/ mbPt-848267 †	4.3	-10.5	15	19.0	mbPb-868706 a		2.1	14	11.0	mbPt-848226/ mbPt-847671	3.3	-15.1
						12	24.0	mbPt-877400/ soPt-857221 †	4.0	-10.3						14	11.4	mbPt-847671 a		1.6
																-	-	mbPt-870331 a		-1.1
						-	-	mbPt-846299 a		2.9										
						-	-	mbPt-871360 a		-2.5										
						-	-	soPt-855586 a		2.0										
b. Leaflet ratio	-	-	mbPt-868675 a		1.4	11	36.4	mbPb-868763 a		1.6	4	5.0	mbPb-876653/ mbPt-847087 †	4.0	19.9	4	0.0	mbPb-847400/ mbPt-868384	3.2	-11.4
	-	-	mbPt-876728 a		-0.8	-	-	mbPt-846229 a		-2.3										
	-	-	soPt-857628 a		0.7	-	-	mbPt-869395 a		-1.6	16	18.4	mbPt-868581 a		1.9	8	12.1	mbPb-877305 a		0.9
											-	-	mbPb-848641 a		-1.8	10	15.0	mbPt-847457/ mbPb-876675	8.4	**38.9**
																-	-	mbPt-848943 a		1.9
																-	-	mbPt-868185 a		0.8

Appendix 5.7. Continued 1...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
c. Petiole length	6	52.9	mbPt-877453 a		-3.8	15	29.0	soPt-831671/ mbPb-868039 †	4.9	-15.8	9	25.0	soPb-825660/ mbPt-868828 †	3.0	16.3	10	45.7	mbPt-848723 a		1.7
	7	51.1	soPt-853126 a		1.4											14	11.4	mbPt-847671 a		1.6
	-	-	soPb-857598 a		2.0	-	-	mbPt-869465 a		4.9	17	21.9	mbPt-846869 a		2.8	-	-	mbPb-847045 a		-3.3
						-	-	soPt-855537 a		-3.4	-	-	mbPt-868763 a		3.7					
						-	-	soPb-857306 a		1.5										
d. Stem diameter	1	27.4	mbPt-847027 a		-1.2	6	10.0	mbPt-876555/ mbPt-847297 †	5.4	-13.9	9	22.0	mbPt-867926/ soPb-824730	5.3	**22.3**	13	16.7	mbPb-868763 a		4.7
	5	50.8	soPt-853688 a		1.6											13	17.0	mbPb-868763/ mbPt-848513	4.3	**22.8**
	-	-	soPb-854886 a		0.8	6	10.0	mbPt-876555 a		1.7	17	21.9	mbPt-846869 a		6.9	-	-	soPt-824253 a		2.5
						7	39.0	mbPt-868516/ mbPt-846324 †	9.7	**38.0**	17	22.0	mbPt-846869/ mbPt-848216	3.6	- 14.3	-	-	soPt-854310 a		-2.3
						16	86.0	mbPt-849105/ mbPt-846677 †	4.6	-12.1	-	-	mbPb-847272 a		-2.0					
						-	-	mbPb-847947 a		-1.4										
						-	-	mbPt-848688 a		-1.2										
e. Internode length	7	47.3	soPt-855771 a		1.6	7	19.0	mbPb-847372/ mbPb-877288	3.3	**-20.7**	-	-	soPt-853358 a		3.8	-	-	mbPb-847703 a		-1.3
	11	33.2	mbPb-868828 a		-2.1						-	-	mbPt-870886 a		-3.6	-	-	mbPb-846789 a		-0.8
	-	-	mbPt-869240 a		-1.5	7	19.6	mbPt-877288 a		-2.7						-	-	soPb-857163 a		0.8
						-	-	mbPb-870338 a		-2.5										
						-	-	mbPb-847947 a		2.2										
f. Floral standard width	8	9.3	soPb-856272 a		5.3	4	18.0	mbPt-867694/ mbPb-849024	6.6	**-23.1**	8	12.8	mbPb-877140 a		-5.9	14	13.8	mbPb-867966 a		1.3
	-	-	mbPb-870769 a		-1.6						8	35.6	mbPb-868412 a		4.0	14	14.0	mbPb-867966/ mbPt-847297	3.3	-17.2
	-	-	mbPt-868682 a		-1.2	4	21.2	mbPt-871533 a		0.9										
						7	43.0	mbPt-848267/ mbPt-876614	3.6	-10.7						-	-	mbPt-870769 a		1.3
																-	-	mbPt-848216 a		0.9
						9	3.8	mbPb-868634 a		2.8										
						11	37.0	soPb-824700/ mbPb-877269	3.5	-11.4										
						-	-	mbPb-876778 a		1.7										

Appendix 5.7. Continued 2...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
g. Stem length	6	24.0	mbPt-848172 a		3.8	8	0.0	mbPb-847400/ mbPb-868626	5.6	16.6	14	12.4	mbPb-848781 a		2.0	3	35.0	mbPt-847829/ mbPt-877288	3.0	9.7
	6	24.0	mbPb-848172 a		2.0						14	18.2	mbPb-849009 a		-1.9					
	-	-	mbPt-876952 a		2.7	8	0.0	mbPb-847400 a		2.1	14	19.0	mbPb-849009/ mbPt-849166	5.6	**30.5**	4	24.0	mbPb-876816/ mbPb-846828	4.5	15.3
						8	23.9	mbPb-848781 a		2.1										
						8	24.5	mbPb-848500 a		2.0						15	9.7	mbPb-848641 a		-1.8
						11	23.0	mbPb-877325/ soPb-824730	4.0	11.4						15	9.7	mbPb-867674 a		-1.6
																15	22.7	mbPb-871281 a		-1.9
h. Branch length	5	0.0	mbPt-846370/ soPt-824786 †	6.1	**27.1**	1	30.0	mbPt-848952/ mbPt-848611	4.6	18.7	-	-	mbPt-848196 a		-1.9	3	32.0	mbPb-847621/ mbPt-848400	5.9	**32.4**
											-	-	mbPb-867966 a		1.3					
	5	10.0	mbPb-846370/ mbPt-876620 †	9.3	**-49.2**	8	7.1	mbPt-847673 a		3.5						3	34.9	mbPt-847829 a		3.1
						11	46.8	mbPt-868828 a		3.3						4	16.0	mbPb-846949/ mbPt-848781	4.8	16.6
	6	24.0	mbPt-848172 a		2.7	-	-	mbPt-868063 a		**11.2**										
	6	24.0	mbPb-848172 a		2.0											-	-	soPb-854416 a		-3.4
	6	24.1	mbPb-848781 a		1.6											-	-	soPb-854205 a		2.3
i. Peduncle length	11	44.1	mbPt-867926 a		2.1	11	36.3	mbPt-846260 a		3.0	13	40.5	mbPb-846131 a		1.2	3	20.8	mbPb-868715 a		1.2
	11	46.7	soPb-825660 a		2.3	11	36.5	mbPt-870753 a		6.4	-	-	mbPb-876762 a		-1.2	5	13.5	mbPt-848809 a		-1.0
	11	46.7	soPb-824755 a		1.9	-	-	mbPt-871187 a		2.0						14	11.4	mbPt-847671 a		0.7
	12	40.0	mbPt-868108/ mbPt-848161	3.1	-15.7															
j. No. of branches per plant	-	-	mbPb-847271 a		-2.3	7	19.6	mbPt-877288 a		4.4	16	36.7	mbPb-868581 a		3.8	3	33.0	mbPb-848400 a		4.6
	-	-	mbPb-848278 a		1.3	-	-	soPb-857384 a		0.7	-	-	soPb-855834 a		-4.0	-	-	mbPt-876498 a		3.7
	-	-	mbPt-869359 a		1.2	-	-	soPt-855225 a		0.6						-	-	mbPb-847947 a		-2.8
k. No. of leaves on stem	8	32.8	mbPb-870338 a		2.0	7	40.0	mbPt-846324/ mbPt-868542	8.9	**26.0**	9	22.1	soPb-824730 a		-0.9	3	32.9	mbPt-848400 a		2.1
	-	-	mbPt-871774 a		2.8						9	23.2	soPb-825660 a		-0.9	3	33.0	mbPb-848400 a		3.0
	-	-	mbPt-867812 a		-0.7	11	36.3	mbPt-846260 a		-2.7	9	24.0	soPb-825660/ mbPt-868828 †	4.2	18.3	-	-	mbPb-871843 a		2.7
						11	37.0	soPb-824700/ mbPb-877269	9.1	**27.7**										
						-	-	mbPb-870338 a		1.8	13	50.0	mbPt-868067/ mbPt-867692 †	4.0	16.6					
						-	-	mbPt-871187 a		-1.8										

Appendix 5.7. Continued 3...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
l. Nodes on stem	3	75.1	mbPt-848574 a		-2.5	11	36.7	mbPb-847898 a		3.0	9	22.1	soPb-824730 a		-1.6	15	25.4	mbPb-877325 a		1.9
	8	36.7	mbPb-847809 a		-2.3	-	-	mbPt-871632 a		-1.4	9	23.2	soPb-825660 a		-1.4	-	-	mbPt-870337 a		-2.5
	-	-	mbPt-869240 a		2.0	-	-	mbPb-870338 a		1.3						-	-	soPb-854070 a		-2.4
m. No. nodes on branches	8	32.8	mbPb-870338 a		7.3	7	19.6	mbPt-877288 a		2.6	16	36.7	mbPb-868581 a		3.8	3	34.9	mbPt-847829 a		1.5
	8	39.9	mbPt-877288 a		1.9	11	36.4	mbPb-868763 a		0.6	11	1.7	mbPt-868026 a		-2.2	15	9.5	mbPt-867674 a		-1.6
	-	-	mbPb-848278 a		3.0	-	-	mbPb-870338 a		0.6						-	-	mbPt-870337 a		-2.0
n. Node of 1st pod	1	26.0	mbPt-847260/ mbPt-870407 †	8.4	**38.2**	7	40.0	mbPt-846324/ mbPt-868542	7.5	**20.9**	9	22.1	soPb-824730 a		6.5	3	35.0	mbPt-847829/ mbPt-877288	7.5	**22.5**
	1	31.0	mbPt-848299/ mbPt-876899 †	5.1	-17.2	11	36.0	soPb-824755/ mbPt-867926	13.7	**43.2**	9	23.0	soPb-824730/ soPb-825660	5.9	**28.0**	8	7.3	mbPt-869539 a		1.4
	8	32.8	mbPb-870338 a		4.4	-	-	mbPb-870338 a		**11.0**	-	-	soPb-855834 a		-3.0	13	36.0	mbPt-867926/ mbPt-871632	10.5	**35.3**
	8	39.9	mbPt-877288 a		1.3	-	-	mbPb-847947 a		-3.4						14	13.0	mbPb-847671/ mbPb-867966	3.2	-8.5
	-	-	mbPt-849080 a		-1.5	-	-	mbPt-847947 a		-3.2						-	-	mbPt-871563 a		-1.3
																-	-	mbPt-876498 a		0.8

Appendix 5.8. Locations and effects of QTLs associated with pod and seed traits detected by the SML (*P* - value ≤ 0.001) and ICIM-ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on LG (in cM); LOD = Logarithm of odds score; PVE = Phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect)

♪: Underlined LG and Pos are positions of SML QTLs which are assigned based on the genotype correlation (r ≥ 0.84) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Highlighted markers indicate overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold PVE numbers indicate major QTLs with PVE % ≥ 10% for SML and ≥ 20% for ICIM methods respectively

a: QTLs detected by SML method at significant P – value of 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for an ICIM QTL region

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. No. pods per peduncle	6	24.1	mbPt-846949 a		-1.4	4	11.7	mbPt-846797 a		2.0	8	28.6	mbPb-868592 a		1.9	13	16.7	mbPb-868763 a		1.7
	-	-	soPt-856219 a		-1.0	4	11.7	mbPb-868485 a		1.8	-	-	mbPt-847829 a		3.2	13	19.2	mbPb-877264 a		-2.1
	-	-	mbPt-870553 a		0.9	4	11.7	mbPb-876847 a		1.2						-	-	mbPb-868161 a		1.8
b. Total pod clusters	7	118.0	soPt-853076/ soPt-853099	4.7	**62.6**	11	37.0	soPb-824700/ mbPb-877269	5.1	15.8	16	27.0	mbPt-868172/ mbPt-868723 †	6.3	**-26.4**	3	34.0	mbPb-848400/ mbPt-847829	5.4	**24.7**
	8	32.8	mbPb-870338 a		5.5	-	-	mbPb-868405 a		3.0	16	38.0	mbPb-868581/ mbPt-847739 †	5.6	**24.5**	-	-	mbPb-847947 a		-4.6
	11	40.4	soPb-824843 a		2.7	-	-	mbPt-848704 a		-1.5						-	-	mbPt-871774 a		1.9
	-	-	mbPb-848278 a		2.1	-	-	mbPt-848820 a		1.0	-	-	soPt-831748 a		0.8	-	-	mbPt-868774 a		-1.8
											-	-	mbPb-868506 a		0.7					
c. No. seeds per pod	8	47.2	soPt-824253 a		2.6	-	-	mbPt-846144 a		-1.0	14	31.7	mbPt-848184 a		3.4	3	32.9	mbPt-848400 a		2.0
	-	-	mbPt-867729 a		2.5	-	-	mbPt-848252 a		-0.9	-	-	mbPb-848630 a		-4.1	3	33.0	mbPb-848400 a		1.6
	-	-	mbPb-869418 a		1.4	-	-	mbPt-848387 a		-0.8						-	-	mbPb-847947 a		-1.2
d. Pod size																				
Pod length	5	16.8	mbPt-848177 a		3.2	4	26.2	mbPt-848227 a		-3.7	9	21.0	soPb-853944/ mbPt-867926	4.9	**24.1**	5	13.0	mbPt-877042/ mbPb-847658 †	3.5	-15.4
	6	69.7	soPt-853267 a		-4.1	4	27.0	mbPt-848227/ mbPt-848599	7.0	**-29.4**										
	11	3.0	mbPt-847132/ mbPt-867688 †	3.2	11.5						-	-	mbPb-877550 a		5.4	11	0.0	mbPb-849156/ mbPb-848151 †	4.5	-16.2
						18	20.0	mbPb-847817/ mbPt-869376	7.0	**-23.8**	-	-	mbPt-848177 a		4.5					
	-	-	mbPb-868608 a		-5.6											-	-	mbPb-877328 a		-3.0
						18	20.2	mbPt-869376 a		7.5						-	-	mbPt-870337 a		-2.0
						18	56.3	soPt-853267 a		-4.4						-	-	soPt-857270 a		1.4

Appendix 5.8. Continued 2...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
Pod width	6	40.6	soPb-853232 a		0.4	4	21.0	mbPb-871546/ mbPb-871533	7.2	**-25.3**	6	8.5	mbPt-847626 a		-1.8	7	9.1	soPt-855253 a		0.9
	8	39.9	mbPt-877288 a		-3.2						-	-	mbPb-848400 a		-4.8	14	11.4	mbPt-847671 a		1.4
	-	-	mbPt-876570 a		1.2	7	19.0	mbPb-847372/ mbPb-877288	3.6	-14.2						-	-	mbPt-868147 a		-1.6
						8	34.0	mbPt-847771/ mbPt-848008	5.1	-19.7										
						12	26.0	mbPt-868606 a		-0.6										
						-	-	mbPt-846868 a		2.0										
						-	-	mbPt-868006 a		0.7										
e. Seed size	3	62.6	mbPb-846816 a		-5.2	2	27.0	mbPb-848616/ mbPt-848179	6.7	-14.3	-	-	mbPb-847295 a		6.5	3	13.0	mbPt-849021/ mbPt-867887	8.4	**-20.1**
	-	-	mbPb-848652 a		2.3						-	-	mbPt-847817 a		-3.8					
	-	-	mbPt-868152 a		-2.2	4	23.0	mbPt-868741/ mbPt-868438	3.9	-8.5						4	27.0	mbPb-846398/ mbPt-876807	6.1	-19.8
						7	38.0	soPb-856649/ mbPt-868516	3.7	-7.4						7	19.0	mbPt-848800/ mbPt-848616	6.3	-15.4
						8	33.7	mbPt-847771 a		-0.6						9	15.0	mbPt-868489/ mbPb-877071	3.9	-8.3
						8	34.0	mbPt-847771/ mbPt-848008	10.1	**-26.7**										
						9	4.0	mbPb-868634/ mbPb-846522	6.3	-14.1						16	19.0	mbPb-868071/ mbPb-868747	4.1	-8.8
																-	-	mbPb-849005 a		3.3
						13	4.9	mbPb-847923 a		-0.5						-	-	mbPt-876465 a		-1.7
						-	-	soPt-855434 a		0.6						-	-	soPt-832048 a		-1.7
f. Hard-seededness	7	22.0	mbPt-848539/ mbPt-848588	4.5	**-25.3**	16	48.3	mbPt-870410 a		-1.0	5	10.1	mbPb-846147 a		4.2	3	10.0	mbPt-848696/ mbPt-848710 †	5.1	16.3
						-	-	soPb-825426 a		-1.6	8	27.0	mbPb-848482/ mbPt-868592 †	3.9	11.5					
	13	27.4	mbPb-877550 a		1.2	-	-	soPb-824603 a		-1.1						-	-	mbPt-876644 a		-1.1
	-	-	mbPt-849188 a		1.8						17	23.4	mbPt-848216 a		9.4	-	-	mbPt-848087 a		0.7
	-	-	mbPb-849188 a		1.5											-	-	mbPb-847506 a		-0.7

Appendix 5.9. Locations and effects of QTLs associated with yield-related traits detected by the SML (*P*-value ≤ 0.001) and ICIM-ADD methods in the four mungbean F_5 RIL populations derived from crosses between two cultivars (Berken and Kiloga) and two wild accessions (ACC 1 and ACC 87)

LG = Linkage group; Pos = QTL position on LG (cM); LOD = Logarithm of odds score; PVE = phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect)

♪: Underlined LG and Pos are position of SML QTLs which are assigned based on the genotype correlation (r ≥ 0.84) between markers linked to SML QTLs and markers mapped on the individual linkage map

‡: Highlighted markers indicate overlapped markers/QTLs for a trait in a population; Underlined markers indicate common markers/ QTLs for a trait across populations.

¶: Bold PVE numbers indicate major QTLs with PVE % ≥ 10% for SML and ≥ 20% ICIM methods respectively

a: QTLs detected by SML method at significant *P*- value of 0.001 while others detected by ICIM – ADD method; †: QTLs detected by ICIM with relaxed PIN of 0.01; /: separates the left and right flanking markers for an ICIM QTL region

Traits	1xB					1xK					87xB					87xK				
	LG♪	Pos (cM)	Marker interval‡	LOD	PVE¶ (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
a. Dry pod mass	1	28.0	mbPt-877268/ mbPt-869029 †	15.8	**71.8**	7	19.0	mbPb-847372/ mbPb-877288	5.7	**30.7**	9	29.0	soPt-832041/ mbPb-868828	5.5	**30.3**	3	34.0	mbPb-848400/ mbPt-847829	6.6	**26.5**
	1	52.3	mbPb-870825 a		-3.4	7	19.6	mbPt-877288 a		5.6	16	36.7	mbPb-868581 a		2.2	3	34.9	mbPb-847829 a		1.7
						7	19.6	mbPb-847829 a		3.2	17	46.5	mbPt-847488 a		1.3					
	3	57.0	mbPt-847850/ mbPb-868369 †	10.8	**-29.1**	11	35.0	soPb-824730/ soPb-825660	6.0	**21.9**						8	16.0	mbPb-868706/ mbPb-871035	4.0	16.1
	7	96.0	soPt-825622/ soPt-825646 †	11.5	**-31.7**	11	40.6	mbPt-847459 a		-2.5						13	36.0	mbPt-867926/ mbPt-871632	3.4	12.9
	8	20.7	mbPt-846832 a		4.0											-	-	soPb-854293 a		1.4
	-	-	soPb-824730 a		-3.8															
b. Seed yield	1	28.0	mbPt-877268/ mbPt-869029 †	12.3	**63.0**	7	19.0	mbPb-847372/ mbPb-877288	4.6	**24.1**	9	29.0	soPt-832041/ mbPb-868828	5.2	**28.8**	3	34.0	mbPb-848400/ mbPt-847829	6.2	**25.4**
	3	58.0	mbPt-868143/ mbPt-848973 †	5.1	-16.1	7	19.6	mbPt-877288 a		7.5	11	2.3	mbPt-846602 a		-1.3	8	15.0	mbPt-848110/ mbPb-868706	3.5	12.7
						11	35.0	soPb-824730/ soPb-825660	6.9	**23.5**	17	46.5	mbPt-847488 a		1.7					
	1	52.3	mbPb-870825 a		-2.1											13	36.0	mbPt-867926/ mbPt-871632	3.3	13.3
	7	96.0	soPt-825622/ soPt-825646 †	6.2	-19.6	-	-	mbPb-848954 a		6.7										
						-	-	mbPt-871009 a		-2.9						15	25.4	mbPb-877325 a		1.1
	8	20.7	mbPt-846832 a		2.0											-	-	soPb-853497 a		-1.6
	-	-	soPb-824730 a		-2.2											-	-	mbPb-848797 a		-1.3

Appendix 5.9. Continued...

Traits	1xB					1xK					87xB					87xK				
	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)	LG	Pos (cM)	Marker interval	LOD	PVE (%)
c. Standing dry biomass	7	118.0	soPt-853076/ soPt-853099	6.4	**62.0**	7	39.8	mbPb-849130 a		-3.9	9	22.1	soPb-824730 a		-1.2	3	32.0	mbPb-847621/ mbPt-848400	3.3	18.1
						7	39.8	soPt-856272 a		-3.0	-	-	mbPt-870577 a		-1.0					
	8	32.8	mbPb-870338 a		3.0	7	40.0	mbPt-846324/ mbPt-868542	11.1	**32.7**						15	25.0	mbPb-871281/ mbPb-877325	3.3	13.7
	8	39.9	mbPt-877288 a		1.9	11	35.2	soPb-825660 a		-5.3										
	-	-	mbPb-848278 a		3.0											15	25.4	mbPb-877325 a		1.4
						11	37.0	soPb-824700/ mbPb-877269	7.0	19.6						-	-	mbPt-871774 a		2.0
																-	-	soPt-831544 a		-1.1
						18	56.0	mbPb-847443/ soPt-853267	3.2	-7.2										
						19	25.0	soPb-825612/ mbPb-846990	4.1	-10.9										
d. Harvest index (HI)	8	32.8	mbPb-870338 a		-3.7	2	20.0	mbPt-876710/ mbPt-847660	3.7	10.9	9	22.0	mbPt-867926/ soPb-824730	3.5	17.8	13	28.1	soPt-825848 a		-1.5
	8	39.7	mbPb-877288 a		-4.4											14	42.0	mbPt-848177/ mbPb-849151	3.3	10.9
	8	39.9	mbPt-877288 a		-3.0	7	43.0	mbPt-848267/ mbPt-876614	6.3	**20.2**	-	-	mbPt-876830 a		-2.3					
											-	-	mbPb-847671 a		-1.2	15	10.0	soPt-832053/ soPt-854393	4.9	15.6
						8	24.1	mbPt-846792 a		2.8										
						8	26.0	mbPt-868381/ mbPt-847654	5.1	15.8						16	24.0	mbPb-877453/ mbPt-877453	4.2	13.1
						-	-	mbPb-876778 a		3.3						-	-	soPb-857025 a		-4.7
						-	-	soPb-855926 a		3.1						-	-	soPb-855100 a		2.5

Appendix 5.10. Common and co-localized QTLs detected in the four F_5 RIL mungbean populations derived from crosses between two cultivars and two wild accessions (a) ACC 1 x Berken; (b) ACC 1 x Kiloga; (c) ACC 87 x Berken; (d) ACC 87 x Kiloga

LG = Linkage group; Pos = Position; Segregation distortion: significant at $P < 0.05$ (*) and $P < 0.01$ (**)

♪: Underlined LG and Pos are position of SML QTLs which are assigned based on the genotype correlation ($r \geq 0.84$) between markers linked to SML QTLs and markers mapped on the individual linkage map

a: QTLs detected by SML method at significant P-value ≤ 0.001 while others detected by ICIM – ADD method; "or" indicated overlapped QTLs

(a) ACC 1 x Berken

LG♪	Pos (cM)	Traits	Marker interval
	10.0	Pod dehiscence	mbPt-846139 **/ mbPt-876878 *
	24.6	Stem colour	soPt-856642 a
	26.0	Node of 1st pod	mbPt-847260 **/ mbPt-870407 **
1	27.4	Stem diameter	mbPt-847027 a **
1	28.0	Pod dry mass Seed yield	mbPt-877268 **/ mbPt-869029
	31.0	Node of 1st pod	mbPt-848299 **/ mbPt-876899 **
	32.0	Leaflet length	mbPt-870422/ mbPt-847689
1	35.5	Pod growth duration	mbPt-876485 a
-	52.3	Pod dry mass Seed yield	mbPb-870825 a **
	63.0	Leaf petiole colour	mbPt-869213 **/ mbPt-868339
	87.0	Stem colour	mbPt-847742 **/ mbPb-849011 ** or mbPb-849011 a **
	0.0	Plant hair colour	mbPt-849095/ mbPt-867792
	8.0	Plant hair colour	mbPb-846396/ mbPt-876637
	37.0	Plant hair density	mbPt-867694 **/ mbPb-868464 *
	50.0	Leaf petiole colour	mbPb-868582/ mbPb-849156
	55.0	Growth duration	mbPb-868786/ mbPt-868786
	57.0	Dry pod mass	mbPt-847850/ mbPb-868369
	57.2	Dry pod colour	mbPt-868642 a
	58.0	Seed yield	mbPt-868143/ mbPt-848973
3	62.0	Growth habit	mbPt-846669/ mbPb-868471
	62.6	Seed size	mbPb-846816 a
	67.7	Dry pod colour	mbPb-846221 a
	or 71.0	Stem colour	or mbPb-846221/ mbPt-847660 *
	or 71.3	Leaflet length	or mbPt-847660 a *
	73.0	Dry pod colour	mbPt-848800/ mbPb-868160 or mbPb-868160 a
	75.1	No. of nodes on on stem	mbPt-848574 a
3	85.1	Time to flowering	soPt-855371 a **
	103.7	Texture layer depth	soPt-854190 a *
	6.8	Plant hair density	mbPb-846685 a **
4	9.0	Plant hair density	mbPb-847658 **/ soPt-855555 **
	15.0	Leaf petiole colour	soPb-825932/ soPb-831980 **
	16.0	Leaf rachis colour	soPb-831980 **/ mbPt-848196
	0.0	Branch length	mbPt-846370/ soPt-824786 **

(c) ACC 87 x Berken

LG	Pos (cM)	Traits	Marker interval
1	6.0	Duration of flowering	mbPt-869143/ mbPt-869315
	14.0		mbPt-868911 */ mbPt-869413 **
3	40.6	Growth habit	mbPt-870630 a **
	106.0	Powdery mildew	mbPt-848435/ mbPt-848441
4	1.0	Pod growth duration	mbPt-846518/ mbPt-871643
	5.0	Leaflet ratio	mbPb-876653/ mbPt-847087
5	10.1	Hardseededness	mbPb-846147 a
6	8.5	Pod width	mbPt-847626 a
	14.7	Flower colour	mbPb-847184 a
7	10.9 or 12.0	Powdery mildew	mbPt-871507/ soPt-825810 or mbPt-871507 a
8	12.8	Floral standard width	mbPb-877140 a
	27.0	Hardseededness	mbPb-848482 */ mbPt-868592
	28.6	Perenniality No. pods per peduncle	mbPb-868592 a
	35.6	Floral standard width	mbPb-868412 a
9	2.0	Flower colour	mbPb-846264/ soPt-832240
	10.3	Flower colour	soPb-853575 a
	11.0	Leaflet lobing	soPb-853806/ soPt-853944
	21.0	Pod length	soPb-853944/ mbPt-867926
	22.0	Stem diameter Harvest index	mbPt-867926/ soPb-824730
	22.1	No. of leaves on stem No. of nodes on stem Node of 1st pod Standing dry biomass	soPb-824730 a
	23.0 or 23.2	Seed coat ridging Hilum colour Time to flowering No. of leaves on stem No. of nodes on stem Node of 1st pod	soPb-824730/ soPb-825660 or soPb-825660 a
	24.0 or 25	Texture layer depth Texture layer colour Petiole length No. of leaves on stem	soPb-825660/ mbPt-868828
	28.0	Overall visual seed traits	mbPt-868828/ soPt-824812

	7.5	Stem colour	mbPt-848651 a
	10.0	Branch length	mbPb-846370/ mbPt-876620
5	16.8	Pod length	mbPt-848177 a
	35.3	Leaflet width	mbPb-868679 a
	46.0 or 46.8	Powdery mildew	mbPb-867966/ mbPb-847671 or mbPb-847671 a
	50.8	Stem diameter	soPt-853688 a
	13.0	Powdery mildew	mbPt-869281/ mbPt-876816 **
	24.0	Twining Inflorescence structure	mbPt-847535 */ mbPb-846949 **
	24.0 or 24.1	Stem length Branch length	mbPb-848172 a * or mbPt-848172 a * or mbPb-848781 a *
6	24.1	No. of pods per peduncle	mbPt-846949 a *
6	25.1	Twining Inflorescence structure	mbPt-868384 a *
	40.6 or 41.0	Pod dehiscence Pod width	soPb-853232/ mbPt-868047 or soPb-853232 a
	43.8	Leaflet width	mbPt-846295 a
	43.9	Leaflet width	mbPt-847817 a
	52.9	Plant hair density Petiole length	mbPt-877453 a
	69.7	Pod length	soPt-853267 a *
	22.0	Hardseededness	mbPt-848539 **/ mbPt-848588
	32.8 33.0	Plant hair density	mbPt-846835 a ** or mbPt-846835 **/ soPt-855837 **
7	42.0	Plant hair density	soPt-854555 **/ mbPt-871572
	47.3	Internode length	soPt-855771 a
	51.1	Petiole length	soPt-853126 a **
	96.0	Dry pod mass Seed yield	soPt-825622/ soPt-825646
	118.0	Total pod clusters Standing dry biomass	soPt-853076 **/ soPt-853099 **
8	9.3	Floral standard width	soPb-856272 a
	12.7	Duration of flowering	mbPb-848296 a
	14.5	Plant hair colour	soPb-855550 a
	19.5	Plant hair colour	mbPb-876991 a
8	20.7	Dry pod mass Seed yield	mbPt-846832 a
8	32.0 or 32.8	Powdery mildew Time to flowering Duration of flowering No. of leaves on stem No. of nodes on branches Node of 1st pod Total pod clusters	mbPt-876991 */ mbPb-870338 or mbPb-870338 a
9	29.0	Dry pod mass Seed yield	soPt-832041/ mbPb-868828
	42.0	Texture layer colour	mbPb-876592/ mbPb-877325
	1.7	No. nodes on branches	mbPt-868026 a
	2.3	Growth habit	mbPt-846602 a **
11	or 3.0	Seed yield	or mbPt-846602 **/ mbPt-867887
	12.4	Leaf petiole colour	mbPt-849021 a
	0.0	Leaflet lobing	soPb-831551/ soPb-857372
12	1.0	Thrips	soPb-857372/ mbPb-868823
	9.5	Plant hair density	mbPb-877486 a*
13	40.5	Peduncle length	mbPb-846131 a
	50.0	No. of leaves on stem	mbPt-868067 */ mbPt-867692
	10.0	Pod dehiscence	mbPt-846949 a or mbPt-846949/ mbPt-848613
	11.2	Inflorescence structure	mbPt-848172 a
14	12.4	Stem length	mbPb-848781 a
	18.2	Stem length	mbPb-849009 a **
	19.0	Twining Stem length	mbPb-849009 **/ mbPt-849166 **
	31.7	No. of seeds per pod	mbPt-848184 a
	12.2	Leaflet width	mbPb-877305 a
	13.7	Dry pod colour	mbPb-848346 a
15	19.0 or 21.0	Dry pod colour Perenniality Growth duration Leaflet width	mbPb-868706 a or mbPb-868706/ mbPt-869539
	15.0	Time to flowering	mbPt-847782/ mbPt-847390 *
	18.4	Leaflet ratio	mbPt-868581 a
	27.0	Total pod clusters	mbPt-868172 */ mbPt-868723 *
16	36.7 or 37.0 or 38.0	Duration of flowering Growth duration No. of branches per plant No. of nodes on branches Total pod clusters Dry pod mass	mbPb-868581 a or mbPb-868581/ mbPt-847739
	0.0	Twining	soPb-856544 a
	15.7 or 16.0 or 18	Leaflet length Perenniality	mbPt-870410 a or mbPt-870410/ mbPt-877415
17	21.3 or 21.9 or 22.0 or 23.4	Stem colour Leaf rachis colour Powdery mildew Petiole length Stem diameter Hardseededness	mbPt-877415 a or mbPt-846869 a or mbPt-846869/ mbPt-848216 or mbPt-848216 a
17	46.5	Dry pod mass Seed yield	mbPt-847488 a **

		Standing dry biomass		-	-	Plant hair colour	mbPb-848400 a
		Harvest index				Pod width	
		No. of nodes on stem		-	-	Thrips	mbPb-848630 a
		Leaf rachis colour				No. seeds per pod	
		Leaf petiole colour	mbPb-847809 a	-	-	Leaflet lobing	mbPb-848586 a
	36.7	Growth habit	mbPb-847809/ mbPb-877288			Leaflet ratio	or mbPb-848641 a *
	or 39.0	Time to flowering	or mbPb-877288 a			Testa colour	mbPb-868032 a
	or 39.7	Leaflet length	or mbPt-877288 a	-	-	Seed mottling	or mbPb-868147 a *
	or 39.9	No. of nodes on branches				Overall visual seed traits	
		Node of 1st pod				Thrips	
		Pod width				Seed coat ridging	
		Standing dry biomass				Texture layer depth	mbPb-868763 a
		Harvest index		-	-	Hilum colour	or mbPt-868763 a
	47.2	Growth habit	soPt-824253 a			Texture layer colour	
		No. of seeds per pod				Overall visual seed traits	
9	10.0	Twining	mbPt-876465/ mbPt-870681			Leaflet length	
	0.0	Powdery mildew	mbPt-847209/ mbPt-847132			Petiole length	
	3.0	Pod length	mbPt-847132/ mbPt-867688	-	-	Twining	mbPb-877550 a
	12.0	Hypocotyl pigment				Pod length	
11	or 13	Testa colour	mbPt-846225 a	-	-	Stem colour	mbPt-847817 a
	or 14	Seed mottling	or mbPt-867688/ mbPt-846225			Seed size	
	or 14.5	Overall visual seed traits		-	-	Time to flowering	mbPt-847829 a
	22.6		mbPt-846155 a			No. of pods per peduncle	
11	22.6	Thrips	or mbPt-868656 a	-	-	Pod growth duration	mbPt-848177 a
11	22.6		or mbPt-876612 a			Pod length	
	23.0	Thrips	mbPt-877602 */ mbPt-868118			Growth habit	
	33.0	Seed mottling	mbPt-877422/ mbPt-868828	-	-	No. of branches per plant	soPb-855834 a
	or 33.2	Internode length	or mbPb-868828 a			Node of 1st pod	
	40.0	Stem colour	mbPt-847459 */ soPb-824843 **	-	-	Leaf petiole colour	mbPt-847817 a
11	or 40.4	Total pod clusters		-	-	Plant hair colour	mbPt-876534 a
	41.6	Growth habit	mbPb-877269/ mbPt-868763	-	-	Plant hair density	soPt-825889 a
	or 42	Powdery mildew	or mbPb-868763 a	-	-	Inflorescence structure	mbPb-868285 a
	or 42.1	Hilum colour	or mbPt-868763 a	-	-	Pod dehiscence	mbPt-848285 a **
		Texture layer colour	or mbPb-877269 a	-	-	Time to flowering	mbPb-871145 a
		Overall visual seed traits		-	-	Duration of flowering	mbPt-848376 a **
	44.1	Peduncle length	mbPt-867926 a	-	-	Pod growth duration	mbPb-867891 a *
	46.7	Growth duration	soPb-825660 a	-	-	Stem diameter	mbPb-847272 a
11		Peduncle length	or soPb-824755 a	-	-	Internode length	soPt-853358 a **
		Plant hair colour	mbPb-871281/ mbPb-877325	-	-		mbPt-870886 a *
	51.0	Growth habit	or mbPb-877325 a	-	-	Branch length	mbPb-867966 a
		Texture layer depth		-	-		mbPt-848196 a **
11	59.0	Inflorescence structure	mbPb-870853 a	-	-	Peduncle length	mbPb-876762 a
		Pod dehiscence	or mbPt-848587/ mbPb-870853	-	-	Total pod clusters	soPt-831748 a *
11	59.0	Growth duration	mbPb-868500 a	-	-		mbPb-868506 a
	59.0		or mbPb-877485 a	-	-	Seed size	mbPb-847295 a **

	63.0	Leaflet lobing	soPb-854393/ soPb-855762
	65.9	Twining	mbPb-868263 a
	67.4	Leaflet lobing	mbPb-848586 a
11	or 68.0	Flower colour	or mbPb-848586/ mbPt-876514
	68.3	Leaflet lobing	or mbPt-848641 a or mbPb-848641 a
	or 68.4	Flower colour	or mbPt-848586 a
	or 68.5	Leaflet length	or mbPb-867674 a
	40.0	Peduncle length	mbPt-868108 **/ mbPt-848161 **
12	59.0	Plant hair colour	mbPt-848460 **/ mbPt-846334 *
	65.0	Plant hair density	mbPb-876762/ mbPb-846334
13	27.4	Hardseededness	mbPb-877550 a **
-	-	No. of branches per plant	mbPb-848278 a **
-	-	No. of nodes on branches	
-	-	Total pod clusters	
-	-	Standing dry biomass	
-	-	Hypocotyl pigment	
-	-	Testa colour	mbPb-868147 a *
-	-	Seed mottling	
-	-	Overall visual seed traits	
-	-	Hypocotyl pigment	mbPt-868147 a *
		Testa colour	
		Seed mottling	
		Overall visual seed traits	
-	-	Leaf rachis colour	mbPt-869240 a
-	-	Leaf petiole colour	
-	-	Internode length	
-	-	No. of nodes on stem	
-	-	Pod dehiscence	mbPt-877231 a **
		Pod growth duration	
		Leaf petiole colour	
-	-	Dry pod mass	soPb-824730 a
		Seed yield	
-	-	Hardseededness	mbPb-849188 a **
			or mbPt-849188 a **
-	-	Twining	mbPb-847393 a **
-	-	Leaflet lobing	mbPt-849022 a **
-	-	Inflorescence structure	mbPt-876725 a **
-	-	Pod dehiscence	soPt-853635 a **
-	-		mbPt-846215 a **
-	-	Powdery mildew	mbPt-848368 a
-	-		mbPt-876596 a **
-	-	Texture layer depth	mbPt-877004 a
-	-	Duration of flowering	mbPb-846581 a
-	-	Pod growth duration	mbPt-868938 a **
-	-	Leaflet ratio	mbPt-868675 a
-	-		mbPt-876728 a **
-	-		soPt-857628 a *
-	-	Standing dry biomass	mbPb-870577 a **
-	-	Harvest index	mbPb-847671 a
-	-		mbPt-876830 a **

-	-	Petiole length	soPb-857598 a **
-	-	Stem diameter	soPb-854886 a **
-	-	Floral standard width	mbPb-870769 a
-	-		mbPt-868682 a *
-	-	Stem length	mbPt-876952 a
-	-	No. of branches per plant	mbPt-869359 a **
-	-		mbPb-847271 a **
-	-	No. of leaves on stem	mbPt-871774 a
-	-		mbPt-867812 a **
-	-	Node of 1st pod	mbPt-849080 a **
-	-	No. of pods per peduncle	mbPt-870553 a *
-	-		soPt-856219 a
-	-	No. of seeds per pod	mbPb-869418 a
-	-		mbPt-867729 a
-	-	Pod length	mbPb-868608 a **
-	-	Pod width	mbPt-876570 a
-	-	Seed size	mbPb-848652 a *
-	-		mbPt-868152 a *

Appendix 5.10. Continued…

(b) ACC 1 x Kiloga

LG	Pos (cM)	Traits	Marker interval
	21.0	Growth habit Twining	mbPt-848946/ mbPt-848646 *
1	27.0	Growth duration	mbPt-877351/ mbPt-867902
	28.0	Leaf rachis colour	mbPt-867902/ mbPt-869470
	30.0	Branch length	mbPt-848952 mbPt-848611
	3.0	Growth duration	mbPt-868143/ mbPb-846816 **
2	20.0	Harvest index	mbPt-876710 **/ mbPt-847660 *
	27.0	Seed size	mbPb-848616/ mbPt-848179
	28.9	Leaflet length	or mbPb-848179 a
	10.0	Hypocotyl pigment	mbPt-870444 mbPt-848692
3	32.0	Leaf petiole colour	mbPt-870568/ mbPt-868868
	46.0	Leaf petiole colour	soPb-831873 */ mbPb-871848 **
	5.0	Thrips	mbPb-867668 mbPb-877185
	11.7	No. of pods per peduncle Thrips	mbPt-846797 a ** or mbPb-876847 a * or mbPb-868485 a ** or mbPt-876847 a **
4	18.0	Floral standard width	mbPt-867694 */ mbPb-849024 *
	21.0	Pod width Floral standard width	mbPb-871546 */ mbPb-871533 * or mbPt-871533 a *
	23.0	Seed size	mbPt-868741 */ mbPt-868438
	26.2	Pod length	mbPt-848227 a *
	or 27.0	Powdery mildew	or mbPt-848227 */ mbPt-848599 **
	or 29.2		or mbPt-848599 a **
	0.0	Leaflet length	mbPt-847507/ mbPt-846276
6	10.0	Stem diameter	mbPt-876555/ mbPt-847297 or mbPt-876555 a
	13.0	Leaflet width	mbPt-848629 */ mbPb-871067
	18.0	Leaf petiole colour	soPt-855854 */ mbPb-847372
7	19.0	Leaflet length Internode length Pod width Dry pod mass Seed yield	mbPb-847372/ mbPb-877288
	19.6	Internode length No. of branches per plant No. of nodes on branches Dry pod mass Seed yield	mbPb-847829 a or mbPt-877288 a
<u>7</u>	<u>32.4</u>	Stem colour	mbPb-849021 a
7	32.4	Stem colour	or mbPt-867887 a
	or 33.0	Growth duration	or mbPt-867887/ soPb-825779
	or 35.0		

(d) ACC 87 x Kiloga

LG	Pos (cM)	Traits	Marker interval
2	12.0	Dry pod colour	mbPt-869016 **/ soPt-857021**
	10.0	Hardseededness	mbPt-848696/ mbPt-848710*
	13.0	Twining Seed size	mbPt-849021/ mbPt-867887
	20.8	Peduncle length	mbPb-868715 a
	30.0	Plant hair colour	mbPt-847626/ soPb-857559**
3	32.0 or 32.9 or 33.0	Growth habit Perenniality Branch length No. of branches per plant No.of leaves on stem No. of seeds per pod Standing dry biomass	mbPb-847621/ mbPt-848400 or mbPt-848400 a or mbPb-848400 a
	34.0	Thrips Total pod clusters Dry pod mass Seed yield	mbPb-848400/ mbPt-847829
	34.9	Plant hair colour Growth duration Branch length	mbPt-847829 a
<u>**3**</u>	<u>34.9</u>	Leaflet width	or mbPb-847829 a
<u>**3**</u>	<u>34.9</u>	Dry pod mass	
	or 35.0	Stem length No. nodes on branches Node of 1st pod	or mbPt-847829/ mbPt-877288
	36.2	Time to flowering	mbPb-877288 a
	39.1	Leaf rachis colour	mbPb-870338 a
4	0.0 or 1.0 or 2.0	Infloresence structure Time to flowering Pod growth duration Leaflet ratio	mbPb-847400 a or mbPb-847400/ mbPt-868384
	7.5	Lustre	mbPb-848613 a
	15.0	Twining	mbPt-848613/ mbPb-848500
	16.0	Branch length	mbPb-846949/ mbPt-848781
	20.3	Infloresence structure	mbPb-868023 a
	24.0	Stem length	mbPb-876816/ mbPb-846828
	27.0	Seed size	mbPb-846398/ mbPt-876807
5	13.0	Pod length	mbPt-877042 **/ mbPb-847658**
	13.5	Peduncle length	mbPt-848809 a **
7	9.1	Pod width	soPt-855253 a
	19.0	Seed size	mbPt-848800/ mbPt-848616
8	7.3	Node of 1st pod	mbPt-869539 a
	11.8	Duration of flowering	mbPt-868778 a **

7	38.0	Seed size	soPb-856649/ mbPt-868516
	39.0	Pod dehiscence Duration of flowering	mbPt-868516/ mbPt-846324
	or 39.8	Stem diameter Standing dry biomass	or mbPb-849130 a or soPt-856272 a
7	40.0	Powdery mildew Time to flowering Pod growth duration Growth duration No. of leaves on stem Nodes of 1st pod Standing dry biomass	mbPt-846324/ mbPt-868542
	42.0	Leaflet width	mbPt-868161/ mbPt-848267 *
	43.0	Floral standard width	mbPt-848267 */ mbPt-876614 **
	43.0	Harvest index	
	55.0	Inflorescence structure	mbPt-847642 ** mbPt-848023 **
8	0.0	Stem length	mbPb-847400 a or mbPb-847400/ mbPb-868626
	7.1	Branch length	mbPt-847673 a
	18.0	Leaf rachis colour	mbPt-868384/ mbPb-847535†
	23.9	Stem length	mbPb-848781 a
	24.1	Harvest index	mbPt-846792 a
	24.5	Stem length	mbPb-848500 a
	26.0	Leaf rachis colour Harvest index	mbPt-868381/ mbPt-847654
	33.7	Seed size	mbPt-847771 a
	or 34.0	Pod width	or mbPt-847771/ mbPt-848008
9	4.0	Seed size	mbPb-868634 **/ mbPb-846522 **
	3.8	Floral standard width	mbPb-868634 a **
	7.0	Leaf petiole colour	mbPt-847916 **/ mbPt-847896 **
	0.0	Flower colour	mbPb-876726 mbPb-877485
	9.0		soPb-853216/ mbPb-848586
	or 10.6	Leaflet lobing	or mbPb-848586 a
	or 10.8		or mbPt-848586 a
	14.3	Leaflet lobing Leaflet length	mbPb-868263 a
11	23.0	Leaf rachis colour	
	or 34.0	Texture layer depth	mbPb-877325/ soPb-824730
	or 34.4	Growth duration	or soPb-824730 a
	34.4	Stem length	
11	35.0	Texture layer depth Time to flowering	soPb-824730/ soPb-825660
	or 35.2	Pod growth duration	or soPb-825660 a
		Dry pod mass	or mbPb-847088 a
		Duration of flowering	mbPb-877316 a
	12.1	Growth duration	or mbPb-877316 a
		Leaflet ratio	or mbPb-877305 a
8	15.0	Perenniality Growth duration Seed yield	mbPt-848110/ mbPb-868706 or mbPb-868706 a
	16.0 or 18.0	Pod dehiscence Perenniality Growth duration Dry pod mass	mbPb-868706/ mbPb-871035
	18.7 or 19.0	Pod dehiscence Perenniality	mbPb-871035 a or mbPb-871035/ mbPb-847338 **
9	15.0	Seed size	mbPt-868489 **/ mbPb-877071 **
	48.3	Flower colour	mbPt-868815 a
10	15.0	Leaflet ratio	mbPt-847457/ mbPb-876675
	or 15.9	Inflorescence structure	or mbPb-876675 a
	45.7	Petiole length	mbPt-848723 a
11	0.0	Pod length	mbPb-849156/ mbPb-848151 †
	13.0	Leaflet lobing	mbPb-849000/ mbPb-868592 †
13	0.0 or 6.0 or 7.0	Leaf rachis colour Testa colour Seed mottling	mbPb-847032 a or mbPb-847032/ mbPt-848579
	16.0 or 16.7	Hilum colour Stem diameter	mbPt-848579/ mbPb-868763 * or mbPb-868763 a *
13	16.7 16.7 16.7 16.7	Seed coat ridging Texture layer depth Hilum colour Texture layer colour	or mbPb-877269 a **
	17.0	No. pods per peduncle	or mbPb-868763 */ mbPt-848513
	19.2	Powdery mildew No. pods per peduncle	mbPb-877264 a
	22.0	Powdery mildew	mbPb-848100 */ mbPt-846991
13	24.7 or 24.8 24.8	Thrips	mbPb-877602 a or mbPb-876817 a or mbPb-876477 a
	25.0 or 25.2	Overall visual seed traits Texture layer depth	mbPb-876897/ mbPb-876592 * or mbPb-876592 a *
	28.1	Harvest index	soPt-825848 a **
	31.0 or 32.0 or 32.6	Time to flowering Hilum colour Texture layer colour	mbPt-868828 */ mbPt-868260 ** or mbPt-868260 a **
	33.0	Seed coat ridging Texture layer depth	mbPt-868260 **/ mbPt-877498 **
	36.0	Dry pod colour Node of 1st pod	mbPt-867926 */ mbPt-871632

		Seed yield	
		Standing dry biomass	
	36.0	Time to flowering	soPb-824755/ mbPt-867926
		Nodes of 1st pod	
	36.3	Peduncle length	mbPt-846260 a
		No. of leaves on stem	
	36.4	Texture layer depth	mbPb-868763 a or mbPt-868763 a
		Hilum colour	
		Texture layer colour	
		Overall visual seed traits	
		Leaflet ratio	
		No. of nodes on branches	
	36.5	Leaf rachis colour	mbPt-870753 a
		Peduncle length	
11	36.7	No. of nodes on stem	mbPt-871632 a
	36.8 or 37.0	Pod dehiscence	soPb-824700 a or oPb-824700/ mbPb-877269
		Pod growth duration	
		Floral standard width	
		No. of leaves on stem	
		Total pod clusters	
		Standing dry biomass	
	37.3 or 38.0	Hilum colour	mbPb-877269 a or mbPb-877269/ mbPt-847428 **
		Overall visual seed traits	
11	40.6	Pod dry mass	mbPt-847459 a **
	42.0 or 42.3 or 43.0	Hypocotyl pigment	soPb-832041 ** mbPb-847032 or mbPb-847032 a or mbPt-847032/ mbPb-847470 **
		Testa colour	
		Seed mottling	
11	46.8	Growth habit	mbPt-868828 a
		Branch length	
	48.0	Leaf rachis colour	mbPb-848513/ mbPt-849182
	58.0	Leaflet length	mbPb-877020/ mbPb-848590
	66.0	Hypocotyl pigment	mbPt-877021/ mbPt-868487
12	24.0	Leaflet width	mbPt-877400/ soPt-857221 **
12	26.0	Pod width	mbPt-868606 a
	40.0	Leaflet length	soPt-825518/ soPt-853239
13	4.9	Seed size	mbPb-847923 a **
15	19.9 or 20.0	Thrips	mbPb-867920 a or mbPb-867920 soPb-855725
	29.0	Petiole length	soPt-831671/ mbPb-868039
16	18.3 or 18.4	Dry pod colour	mbPt-848870 a * or mbPt-849070 a *
	19.0 or 19.1	Dry pod colour	mbPt-848221 */ mbPt-846885 ** or mbPt-846885 a **
	48.0	Plant hair density	mbPt-846915/ mbPt-870410
16	or 48.3	Hardseededness	mbPt-870410 a
	70.0	Plant hair colour	mbPt-877344/ mbPt-847627 **
	or 38.0	Dry pod mass	
		Seed yield	
	47.9	Texture layer colour	soPb-824730 a
		Leaflet length	
	11.0 or 11.4	Leaflet width	mbPt-848226 */ mbPt-847671 or mbPt-847671 a
		Petiole length	
		Peduncle length	
		Pod width	
14	13.0	Node of 1st pod	mbPb-847671/ mbPb-867966
	13.8 or 14.0 or 17.0 or 17.1	Floral standard width	mbPb-867966 a or mbPb-867966/ mbPt-847297 ** or mbPt-847297 a **
		Duration of flowering	
	42.0	Dry pod colour	mbPt-848177/ mbPb-849151
		Harvest index	
	59.6	Stem colour	mbPt-848061 a
	9.5 or 9.7	Leaflet lobing	mbPt-867674 a ** or mbPb-848641 a ** or mbPb-867674 a ** or mbPt-848641 a **
		Stem length	
		No. of nodes on branches	
15	or 9.7	Leaflet lobing	
	10.0	Harvest index	soPt-832053/ soPt-854393
	14.0	Leaflet lobing	soPb-853645 **/ mbPb-870853 †
	22.7 or 25.0 or 25.4	Seed coat ridging	mbPb-871281 a ** or mbPb-871281 **/ mbPb-877325 * or mbPb-877325 a *
		Texture layer depth	
		Texture layer colour	
15		Stem length	
		No. of nodes on stem	
		Dry pod mass	
		Seed yield	
		Standing dry biomass	
	9.0 or 14.0 or 16.4	Dry pod colour	soPt-853267 **/ soPb-857372 or soPb-857372 a
		Pod dehiscence	
		Growth duration	
16	19.0	Seed size	mbPb-868071 */ mbPb-868747 *
	24.0	Harvest index	mbPb-877453/ mbPt-877453
	32.0	Plant hair density	mbPt-867765/ mbPb-848907
	49.5	Plant hair density	mbPt-869376 a
-	-	Internode length	mbPb-846789 a
-	-		or mbPb-847703 a
-	-	Dry pod colour	mbPb-847045 a **
-	-	Petiole length	
-	-	Plant hair density	
-	-	No. of branches per plant	mbPb-847947 a
-	-	Total pod clusters	mbPb-847947 a
-	-	No. of seeds per pod	
-	-	Testa colour	

	86.0	Stem diameter	mbPt-849105 **/ mbPt-846677 **
17	40.0	Leaf rachis colour	mbPt-849118/ mbPt-848472
18	19.8	Pod dehiscence	mbPt-847817 a or mbPb-847817 a *
	or 20.0	Pod length	or mbPb-847817 */ mbPt-869376
	or 20.2		or mbPt-869376 a
	56.0	Pod length	mbPb-847443 **/ soPt-853267 **
	or 56.3	Standing dry biomass	or soPt-853267 a **
19	5.0	Stem colour	mbPb-870677/ mbPb-846988
	25.0	Pod growth duration Standing dry biomass	soPb-825612/ mbPb-846990 **
	27.0	Time to flowering	mbPb-846990 **/ soPb-832109
	40.0	Hypocotyl pigment	soPb-832205 **/ soPt-854765 *
-	-	Pod growth duration	
-	-	Stem diameter	mbPb-847947 a
-	-	Internode length	or mbPt-847947 a
-	-	Nodes of 1st pod	
-	-	Hypocotyl pigment	
-	-	Leaf petiole colour	
-	-	Testa colour	mbPb-868147 a
-	-	Seed mottling	
-	-	Overall visual seed traits	
-	-	Time to flowering	
-	-	Duration of flowering	
-	-	Pod growth duration	
-	-	Internode length	mbPb-870338 a
-	-	No. of leaves on stem	
-	-	No.of nodes on stem	
-	-	No. of nodes on branches	
-	-	Nodes of 1st pod	
-	-	Time to flowering	
-	-	Floral standard width	mbPb-876778 a **
-	-	Harvest index	
-	-	Peduncle length	mbPt-871187 a
-	-	No. of leaves on stem	
-	-	Hypocotyl pigment	soPb-825015 a *
-	-	Stem colour	mbPt-868086 a **
-	-	Leaf rachis colour	soPt-855536 a
-	-	Leaf petiole colour	soPb-832055 a **
-	-		soPt-853484 a
-	-	Plant hair colour	mbPt-867794 a **
-	-		mbPt-869245 a **
-	-		soPb-856981 a
-	-	Plant hair density	mbPt-871155 a
-	-		soPb-824602 a **
-	-	Growth habit	mbPt-871497 a **
-	-		mbPt-871661 a **
-	-		soPb-825355 a **
-	-	Seed mottling	mbPb-868032 a *
-	-	Overall visual seed traits	
-	-	Plant hair density	mbPt-846445 a
-	-	Pod dehiscence	or mbPt-870744 a
-	-	Hardseededness	mbPt-848087 a *
-	-	Floral standard width	or mbPt-848216 a *
-	-	Growth habit	mbPt-848943 a
-	-	Leaflet ratio	or mbPt-868185 a
-	-	Testa colour	
-	-	Seed mottling	mbPt-868032 a
-	-	Overall visual seed traits	or mbPt-868147 a
-	-	Pod width	
-	-	Total pod clusters	mbPt-871774 a **
-	-	Standing dry biomass	
-	-	Dry pod colour	
-	-	No. of branches per plant	mbPt-876498 a
-	-	Node of 1st pod	
-	-	Stem colour	mbPb-870682 a **
-	-		mbPt-868732 a **
-	-	Leaf rachis colour	mbPb-846720 a
-	-	Leaf petiole colour	soPb-832155 a **
-	-		soPt-832155 a **
-	-		mbPb-847286 a
-	-	Plant hair colour	soPb-854119 a
-	-		mbPb-869222 a *
-	-	Twining	mbPb-848706 a **
-	-		mbPt-848885 a **
-	-		mbPt-870337 a
-	-	Flower colour	soPt-825659 a **
-	-		soPt-854506 a **
-	-	Dry pod colour	soPb-856883 a *
-	-	Powdery mildew	soPt-824890 a **
-	-		mbPb-869240 a
-	-	Testa colour	soPb-854233 a
-	-		mbPt-847646 a
-	-	Lustre	soPb-825303 a **
-	-		mbPb-847655 a
-	-	Time to flowering	soPb-853363
-	-	Pod growth duration	soPb-855048 a
-	-		soPb-855733 a
-	-	Leaflet length	soPt-855342 a **
-	-		mbPb-846816 a **
-	-	Leaflet width	mbPt-870331 a
-	-	Stem diameter	soPt-824253 a **
-	-		soPt-854310 a *
-	-	Branch length	soPb-854205 a
-	-		soPb-854416 a
-	-	Internode length	soPb-857163 a **

-	-	Twining	soPt-824253 a **
-	-		soPt-857318 a **
-	-	Flower colour	mbPt-849064 a
-	-		soPt-854770 a **
-	-		soPt-854823 a **
-	-	Inflorescence structure	mbPt-868631 a
-	-		mbPt-876766 a *
-	-		mbPb-848919 a **
-	-	Powdery mildew	mbPt-871365 a
-	-		mbPt-877076 a **
-	-	Thrips	mbPt-868952 a
-	-	Testa colour	mbPt-868810 a **
-	-	Seed mottling	mbPb-870721 a
-	-	Duration of flowering	soPb-854003 a
-	-		mbPb-848079 a **
-	-	Growth duration	soPb-824080 a **
-	-		soPt-853630 a **
-	-	Leaflet length	soPt-853360 a
-	-	Leaflet width	mbPt-846299 a *
-	-		mbPt-871360 a
-	-		soPt-855586 a **
-	-	Leaflet ratio	mbPt-846229 a **
-	-		mbPt-869395 a **
-	-	Petiole length	mbPt-869465 a **
-	-		soPb-857306 a
-	-		soPt-855537 a **
-	-	Stem diameter	mbPt-848688 a *
-	-	Branch length	mbPt-868063 a **
-	-	No. of branches per plant	soPb-857384 a
-	-		soPt-855225 a
-	-	No. of nodes on stem	mbPb-847898 a
-	-	Total pod clusters	mbPt-848704 a
-	-		mbPt-848820 a **
-	-		mbPb-868405 a **
-	-	No. of seeds per pod	mbPt-846144 a
-	-		mbPt-848252 a **
-	-		mbPt-848387 a
-	-	Pod width	mbPt-846868 a **
-	-		mbPt-868006 a **
-	-	Seed size	soPt-855434 a **
-	-	Hardseededness	soPb-824603 a
-	-		soPb-825426 a
-	-	Seed yield	mbPt-871009 a
-	-		mbPb-848954 a
-	-	Harvest index	soPb-855926 a *
-	-	Floral standard width	mbPt-870769 a **
-	-	No. of leaves on stem	mbPb-871843 a **
-	-	No. of nodes on stem	soPb-854070 a **
-	-		mbPt-870337 a
-	-	No. of nodes on branches	mbPt-870337 a
-	-	Node of 1st pod	mbPt-871563 a
-	-	No. of pods per peduncle	mbPb-868161 a
-	-	Total pod clusters	mbPt-868774 a *
-	-	Pod length	soPt-857270 a **
-	-		mbPb-877328 a
-	-		mbPt-870337 a
-	-	Seed size	soPt-832048 a **
-	-		mbPb-849005 a **
-	-		mbPt-876465 a **
-	-	Hardseededness	mbPt-876644 a **
-	-		mbPb-847506 a
-	-	Dry pod mass	soPb-854293 a
-	-	Seed yield	soPb-853497 a **
-	-		mbPb-848797 a
-	-	Standing dry biomass	soPt-831544 a **
-	-	Harvest index	soPb-855100 a
-	-		soPb-857025 a

Appendix 6.1. ANOVA for relative water content (RWC, %), epidermal conductance (g_e, mm/s), g_e (with PAW adopted as a covariate) in the parental plants and the soybean RIL populations over four sampling times in two drought stress runs

Traits	Sources of variation	d.f.	S.S.	M.S.	F	*P*
RWC	Run stratum	1	295.9	295.9	0.0	
	Sample stratum	3	387605.0	129201.7	16.8	
	Run.Sample stratum	3	23116.8	7705.6	95.0	
	Run.Sample.*Units*stratum					
	Crossing_pops	3	5853.5	1951.2	24.1	< 0.001
	Crossing_pops.Genotype	187	50519.0	270.2	3.3	< 0.001
	Residual	1266	102643.6	81.1		
	Total	1463	514255.4			
g_e	Run stratum	1	0.05	0.05	0.1	
	Sample stratum	3	62.84	20.95	23.8	
	Run.Sample stratum	3	2.64	0.88	31.5	
	Run.Sample.*Units*stratum					
	Crossing_pops	3	6.78	2.26	81.0	< 0.001
	Crossing_pops.Genotype	187	11.97	0.06	2.3	< 0.001
	Residual	1265	35.30	0.03		
	Total	1462	111.04			
g_e (with PAW as covariate)	Run stratum					
	Covariate	1	0.06	0.06		
	Sample stratum					
	Covariate	1	62.53	62.53	1309.8	< 0.001
	Residual	2	0.10	0.05	0.4	
	Run.Sample stratum					
	Covariate	1	2.34	2.34	18.3	0.05
	Residual	2	0.26	0.13	5.1	
	Run.Sample.*Units*stratum					
	Crossing_pops	3	6.63	2.21	88.3	< 0.001
	Crossing_pops.Genotype	187	7.09	0.04	1.5	< 0.001
	Covariate	1	3.58	3.58	143.1	< 0.001
	Residual	1262	31.61	0.03		
	Total	1460	110.86			

Appendix 6.2. ANOVA for recovery traits in the parental plants and the three soybean RIL populations derived from crosses between two oilseed cultivars (Valder and CPI 26671) and a landrace variety (G2120) over two drought stress runs

NS1/ NS2 = No. of stem nodes before/ after re-watering, respectively; nNS = No. of new stem nodes; rLL = No. of remnant leaflets; LL = No. of leaflets; nLL = No. of new leaflets; rLA = Remnant leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score
ns = Not significant

Traits	Sources of variation	d.f.	S.S.	M.S.	F	*P*
NS1	Run stratum	1	180.0	180.0	163.9	
	Run.*Units*Stratum					
	Populations	3	49.8	16.6	15.1	< 0.001
	Populations.Genotypes	187	333.7	1.8	1.6	< 0.001
	Residual	207	227.4	1.1		
	Total	398	790.0			
rLL	Run	1	1.5	1.5	0.0	
	Run.*Units*Stratum					
	Populations	3	1056.6	352.2	6.3	< 0.001
	Populations.Genotypes	187	15302.2	81.8	1.5	0.004
	Residual	207	11634.7	56.2		
	Total	398	27908.8			
rLA	Run	1	794165	794165	15.0	
	Run.*Units*Stratum					
	Populations	3	1661330	553777	10.4	< 0.001
	Populations.Genotypes	187	12320754	65886	1.2	0.064 *ns*
	Residual	207	10978450	53036		
	Total	398	25732362			
NS2	Run	1	11.9	11.9	4.7	
	Run.*Units*Stratum					
	Populations	3	145.6	48.5	19.2	< 0.001
	Populations.Genotypes	187	1026.3	5.5	2.2	< 0.001
	Residual	141	356.0	2.5		
	Total	332	1238.1			
LL	Run	1	3102.9	3102.9	11.2	
	Run.*Units*Stratum					
	Populations	3	8258.7	2752.9	10.0	< 0.001
	Populations.Genotypes	187	75420.6	403.3	1.5	0.004
	Residual	207	57266.6	276.7		
	Total	398	143208.7			
LA	Run	1	562300	562300	2.3	
	Run.*Units*Stratum					
	Populations	3	9428000	3143000	13.1	< 0.001
	Populations.Genotypes	187	55050000	294400	1.2	0.077 *ns*
	Residual	207	49750000	240300		
	Total	398	114500000			

Appendix 6.2. Continued…

Traits	Sources of variation	d.f.	S.S.	M.S.	F	*P*
nNS	Run	1	154.316	154.316	98.64	
	Run.*Units*Stratum					
	Populations	3	18.398	6.133	3.92	0.01
	Populations.Genotypes	187	372.140	1.99	1.27	0.049
	Residual	192	300.385	1.565		
	Total	383	790.102			
nLL	Run	1	3495.4	3495.4	31.31	
	Run.*Units*Stratum					
	Populations	3	3291.7	1097.2	9.83	< 0.001
	Populations.Genotypes	187	28964.3	154.9	1.39	0.011
	Residual	206	23000.2	111.7		
	Total	397	58290.1			
nLA	Run	1	39852	39852	0.45	
	Run.*Units*Stratum					
	Populations	3	3428836	1142945	12.83	< 0.001
	Populations.Genotypes	187	21132755	113009	1.27	0.048
	Residual	206	18357428	89114		
	Total	397	42788038			
Rc	Run	1	11.341	11.341	9.36	
	Run.*Units*Stratum					
	Populations	3	10.435	3.478	2.87	0.038
	Populations.Genotypes	187	336.121	1.797	1.48	0.003
	Residual	192	232.639	1.212		
	Total	383	555.872			
Rc	Run stratum					
(with PAW	Covariate	1	11.341	11.341		
as covariate)	Run.*Units*Stratum					
	Populations	3	9.301	3.100	2.61	0.053
	Populations.Genotypes	187	330.255	1.766	1.49	0.003
	Covariate	1	6.053	6.053	5.10	0.025
	Residual	191	226.586	1.186		
	Total	383	555.872			

Appendix 6.3. Linkage groups (LGs) on the integrated soybean map corresponding with linkage groups on the three component maps, for the three soybean RIL populations derived from crosses between two oilseed cultivars (Valder and CPI 26671) and a landrace variety (G2120)

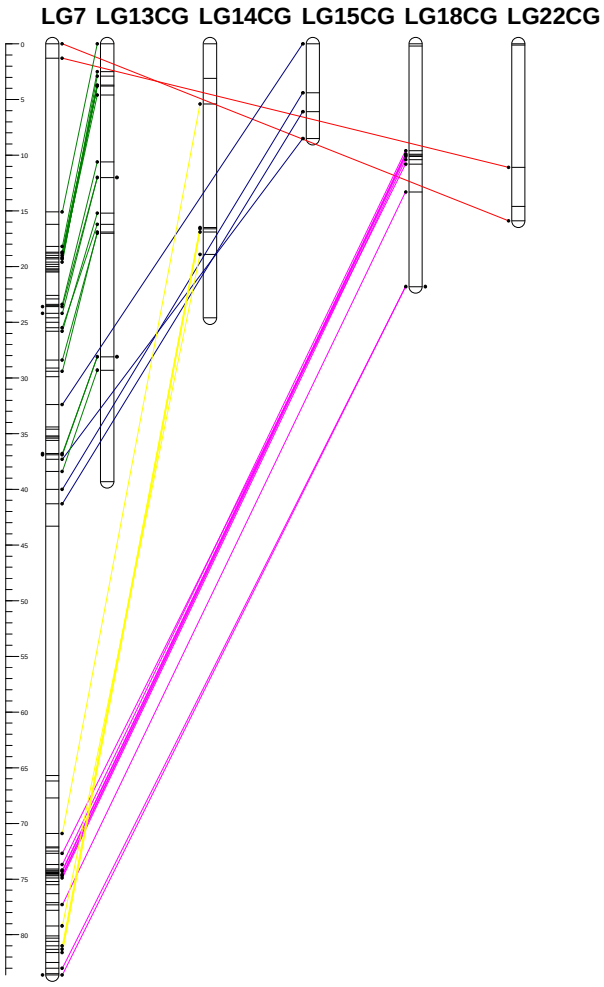
♪: Numbers in ( ) are numbers of shared markers between the LGs of the integrated map and LGs of the component maps; the 8LGs in bold and highlighted consisted of more than two LGs within or from different component maps

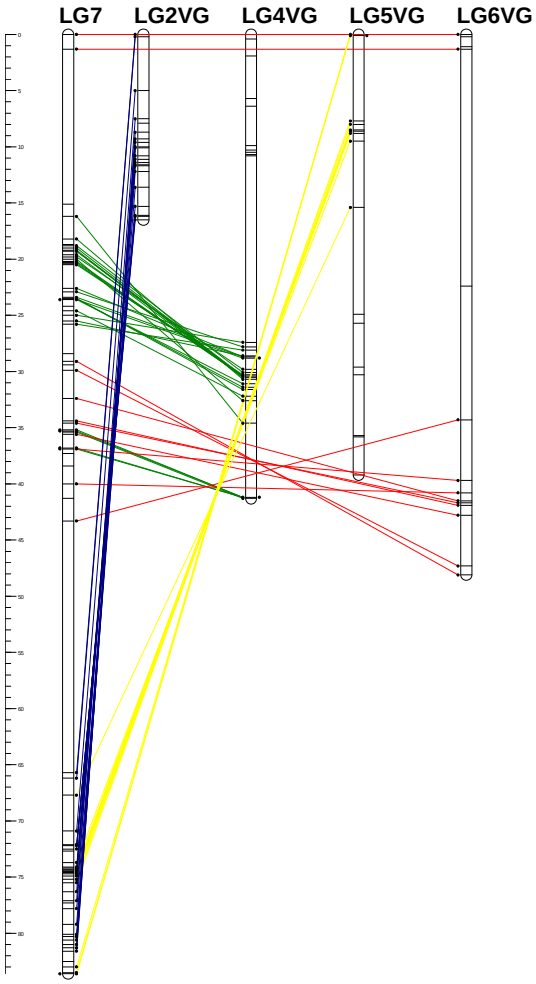
CG = CPI26671 x G2120; CV = CPI26671 x Valder; VG = Valder x G2120

Integrated map			**Component maps**		
			CG	**CV**	**VG**
LG	Length (cM)	No. markers	LG♪ (no. shared markers)	LG (no. shared markers)	LG (no. shared markers)
LG1	75.2	195	LG1 (65), LG2(72), LG3 (16), LG4 (7), LG5 (4), LG6 (10), LG7 (14)	LG6 (11)	
LG2	35.3	29	LG10 (29)		
LG3	35.6	4	LG11 (4)		
LG4	13.9	5	LG12 (5)		
LG5	28	46	LG8 (46)		LG13 (1)
LG6	26	4	LG9 (4)		
LG7	83.6	115	LG13 (31), LG14 (5), LG15 (4), LG18 (16), LG22 (2)	LG11 (1), LG12 (4)	LG2 (22), LG4 (47), LG5 (20), LG6 (11)
LG8	9.2	105			LG1 (105)
LG9	7.8	7		LG12 (5)	LG12 (6)
LG10	18.8	27		LG11 (21)	LG4 (18)
LG11	15.5	8		LG12 (3)	LG5 (7)
LG12	17.3	12			LG3 (12)
LG13	58.4	55		LG1 (49); LG5 (6)	
LG14	50.8	23		LG2 (14); LG4 (9)	
LG15	8.1	22	LG17 (22)		
LG16	15.9	15	LG19 (15)		
LG17	25.1	13			LG8 (13)
LG18	21.1	10			LG9 (10)
LG19	35.8	9		LG8 (9)	
LG20	14	8	LG20 (8)		
LG21	28	7		LG10 (7)	
LG22	8.3	7		LG9 (7)	
LG23	27.5	7		LG15 (5)	LG15 (5)
LG24	24.9	6		LG7 (6)	
LG25	40.5	8			LG10 (8)
LG26	28.6	7			LG11 (7)
LG27	9	5	LG16 (5)		
Total	**762.2**	**759**	**384**	**157**	**292**

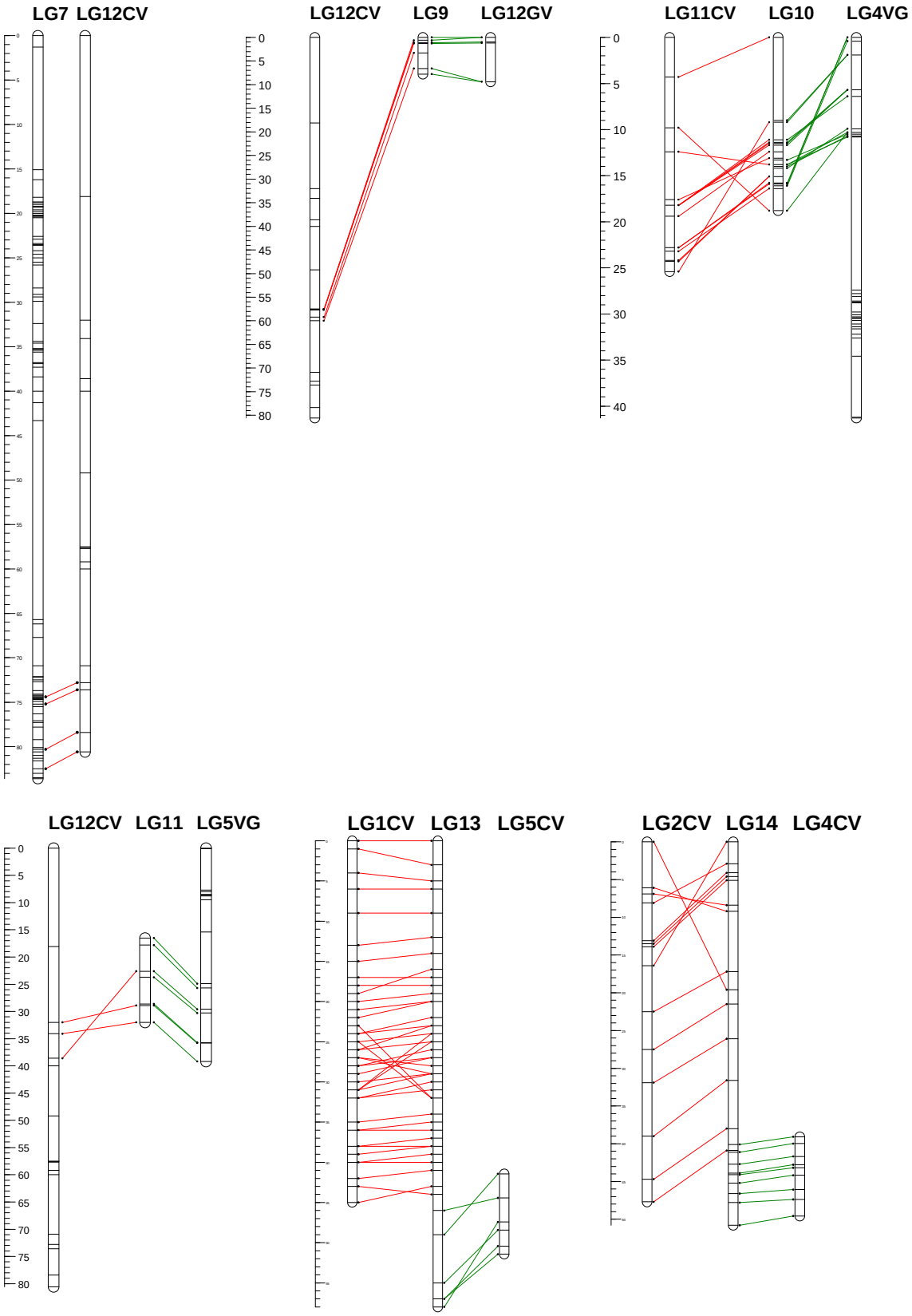
Appendix 6.4. Illustrations of the marker orders being maintained between corresponding LGs of the integrated map and the component maps, for the three soybean RIL populations derived from crosses between two oilseed cultivars (Valder and CPI 26671) and a landrace variety (G2120)

CV = CPI26671 x Valder; CG = CPI26671 x G2120; VG = Valder x G2120





Appendix 6.4. Continued…



Appendix 6.5. Locations and parameters of SML QTLs for physiological traits associated with drought stress responses in the three soybean RIL populations derived from crosses between two oilseed cultivars (Valder and CPI 26671) and a landrace variety (G2120)

RWC = Relative water content (%); R_RWC = Relative response relative water content; g_e = Epidermal conductance (mm/s); g_{e70} = Epidermal conductance (mm/s) at relative water content of 70%;

NS1/ NS2 = No. of stem nodes before/ after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets after re-watering; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score

LG = Linkage group; Pos = QTL position on LG (in cM); PVE = Phenotypic variance explanation (%) (+ve or –ve depending on whether the QTL increased or decreased the trait effect); Markers/ QTLs detected by the SML method are listed in the table in order of increasing *P*-value

♪: Underlined LG and Pos are positions assigned to SML QTLs based on the genotype correlation ($r > 0.84$) between markers linked to SML QTLs and markers mapped on the integrated linkage map; ‡: Markers highlighted in the same colour are overlapped markers/QTLs for a trait in a population, based on genotype correlation ($r > 0.84$) among markers. Underlined markers indicate common markers/ QTLs for a trait across populations.

Traits	CPI 26671 x G2120					CPI 26671 x Valder					Valder x G2120				
	LG♪	Pos	Marker‡	PVE %	*P*-value	LG	Pos	Marker	PVE%	*P*-value	LG	Pos	Marker	PVE%	*P*-value
RWC	-	-	mbPb-868411	3.8	0.0000	-	-	mbPt-876860	4.4	0.0000	-	-	soPb-855347	-2.3	0.0000
	-	-	soPb-825520	-1.7	0.0008	-	-	mbPt-869103	2.4	0.0021	7	70.9	soPb-855545	-1.5	0.0018
	-	-	mbPb-877505	1.1	0.0016	-	-	soPt-824183	1.9	0.0041	7	35.6	soPb-853608	-1.2	0.0036
	3	26.9	soPb-825841	-1.0	0.0024	-	-	soPb-853092	-1.7	0.0062	5	27.1	soPb-856104	-1.1	0.0055
	-	-	soPb-856220	0.9	0.0031	-	-	mbPt-868851	1.3	0.0083	7	66.2	soPb-855445	-0.9	0.0073
	-	-	soPb-854462	-0.9	0.0039	-	-	mbPb-871796	-1.3	0.0104	5	27.1	soPb-853611	-0.8	0.0091
	-	-	mbPb-867708	0.6	0.0047										
	-	-	mbPb-868567	0.6	0.0055										
	-	-	mbPb-877365	-0.6	0.0055										
	1	71.6	mbPb-871580	-0.5	0.0071										
	1	71.8	mbPb-869437	-0.5	0.0079										
	-	-	mbPb-870915	-0.4	0.0086										
	-	-	soPb-824011	-0.3	0.0094										
	-	-	mbPb-871053	0.3	0.0102										
R_RWC	25	29.7	soPt-854547	7.8	0.0000	-	-	soPb-855118	-3.1	0.0000	-	-	soPb-855967	1.2	0.0000
	-	-	soPb-854798	4.2	0.0008	-	-	mbPb-876469	2.1	0.0021	-	-	soPb-855549	-1.1	0.0018
	-	-	soPb-857441	-3.0	0.0016	-	-	soPb-856799	-1.7	0.0041	23	12.0	soPb-831556	0.8	0.0036
	-	-	mbPb-867968	-3.0	0.0024	13	48.9	soPt-853079	1.7	0.0062	-	-	mbPt-848956	0.5	0.0055
	-	-	mbPb-847486	-2.7	0.0031	-	-	soPt-825368	-1.5	0.0083	23	19.7	soPt-854142	-0.5	0.0073
	25	29.7	soPt-856082	2.6	0.0039	-	-	soPt-855439	1.2	0.0104	-	-	soPt-853101	0.5	0.0091
	-	-	soPb-824720	2.5	0.0047										
	-	-	soPb-857530	-2.5	0.0055										
	-	-	mbPb-869193	2.3	0.0063										
	25	0.0	soPb-854858	2.1	0.0071										
	23	6.5	soPb-853730	2.0	0.0079										
	-	-	mbPb-876843	1.7	0.0086										
	-	-	soPb-855123	1.7	0.0094										
	-	-	soPb-854350	-1.6	0.0102										
g_e	-	-	soPb-856646	-3.3	0.0000	-	-	soPt-824391	3.0	0.0000	-	-	soPt-857253	3.0	0.0000
	-	-	soPb-825530	2.9	0.0008	-	-	mbPt-868314	2.2	0.0021	-	-	soPt-854123	1.9	0.0018
	-	-	soPb-857280	-2.4	0.0016	-	-	soPb-853092	-2.0	0.0041	-	-	soPt-853791	1.1	0.0036
	-	-	mbPb-871053	2.4	0.0024	-	-	mbPt-869283	-2.0	0.0062	-	-	soPb-855667	-0.9	0.0055
	-	-	soPb-853727	2.1	0.0031	-	-	mbPt-846781	1.3	0.0083	7	70.9	soPb-855545	-0.8	0.0073
	-	-	mbPb-871011	-1.9	0.0039	-	-	soPt-856414	-1.3	0.0104	-	-	mbPt-848956	-0.6	0.0091
	-	-	mbPb-870915	-1.9	0.0047										
	-	-	soPb-853915	-1.8	0.0055										
	4	8.4	mbPb-877452	-1.7	0.0063										
	-	-	soPb-853985	1.3	0.0071										
	-	-	soPb-853488	-1.3	0.0079										
	-	-	mbPb-848410	1.1	0.0086										
	-	-	mbPb-867637	-0.9	0.0094										
	-	-	soPb-826118	0.8	0.0102										
g_{e70}	-	-	soPb-853767	4.6	0.0000	-	-	soPb-853062	2.7	0.0000	-	-	soPb-825520	3.0	0.0000
	-	-	soPb-855469	3.8	0.0008	-	-	mbPt-869283	-2.5	0.0021	-	-	soPt-825567	1.9	0.0018
	10	16.4	soPb-853348	-3.5	0.0016	-	-	soPb-855347	-2.2	0.0041	-	-	soPt-853101	-1.6	0.0036
	-	-	soPb-853488	-3.4	0.0024	-	-	mbPb-877518	-1.9	0.0062	-	-	soPt-856216	1.2	0.0055
	-	-	soPb-855967	-3.0	0.0031	-	-	soPt-855792	1.7	0.0083	-	-	soPb-853211	-1.1	0.0073
	-	-	soPb-856169	2.2	0.0039	13	12.1	mbPt-876684	-1.6	0.0104	20	0.0	soPb-853371	1.0	0.0091
	-	-	soPb-855633	-2.1	0.0047										
	-	-	mbPb-871360	2.1	0.0055										
	-	-	soPb-824120	2.0	0.0063										
	-	-	soPb-825017	-2.0	0.0071										
	-	-	soPb-825161	-1.9	0.0079										
	-	-	mbPb-868499	-1.5	0.0086										
	10	12.4	soPb-856842	-1.4	0.0094										
	10	16.1	soPb-831699	1.3	0.0102										

Appendix 6.5. Continued 1…

Traits	CPI 26671 x G2120					CPI 26671 x Valder					Valder x G2120				
	LG	Pos	Marker	PVE %	*P*-value	LG	Pos	Marker	PVE%	*P*-value	LG	Pos	Marker	PVE%	*P*-value
NS1	-	-	soPb-825259	4.0	0.0000	-	-	soPt-824183	2.8	0.0000	8	1.2	soPb-856335	1.7	0.0000
	-	-	mbPb-848838	-1.8	0.0008	-	-	soPt-854003	2.0	0.0021	18	21.1	soPb-825062	-1.5	0.0018
	7	0.0	soPt-856127	-1.6	0.0016	-	-	soPb-825021	1.6	0.0041	5	27.1	soPb-853611	-1.2	0.0036
	-	-	soPb-826118	1.3	0.0024	21	24.9	mbPt-849130	1.3	0.0062	7	35.6	soPb-853608	-1.1	0.0055
	-	-	soPb-825639	1.3	0.0031	-	-	soPt-854925	-1.2	0.0083	-	-	soPt-856322	-0.9	0.0073
	-	-	soPb-825811	1.0	0.0039	14	47.8	mbPt-869415	1.1	0.0104	7	65.7	soPb-856137	-0.8	0.0091
	-	-	mbPb-868034	-0.8	0.0047										
	-	-	mbPb-848064	0.8	0.0055										
	-	-	soPb-832207	0.8	0.0063										
	-	-	soPb-824175	0.6	0.0071										
	-	-	soPb-831609	-0.6	0.0079										
	-	-	soPb-832024	0.5	0.0086										
	7	1.3	soPb-856183	-0.4	0.0094										
	5	21.0	soPb-853729	-0.4	0.0102										
rLL	-	-	soPb-825639	3.0	0.0000	-	-	mbPt-869390	5.4	0.0000	25	28.5	soPb-825425	-2.0	0.0000
	-	-	soPb-854462	-2.3	0.0008	25	40.5	soPb-854943	-5.2	0.0021	25	29.7	soPt-856082	1.9	0.0018
	-	-	soPb-856347	2.1	0.0016	-	-	mbPb-847253	-2.7	0.0041	7	34.6	soPb-855129	-1.5	0.0036
	-	-	mbPb-877441	-2.1	0.0024	13	16.9	soPt-832166	2.1	0.0062	7	34.4	soPb-855130	-1.5	0.0055
	-	-	soPb-832207	1.9	0.0031	25	40.5	soPt-854943	-2.1	0.0083	7	35.6	soPb-853608	-1.2	0.0073
	-	-	mbPb-871202	1.5	0.0039	-	-	soPb-831830	2.0	0.0104	18	21.1	soPb-825062	-1.1	0.0091
	-	-	mbPb-848410	-1.5	0.0047										
	-	-	soPb-855385	1.4	0.0055										
	-	-	soPb-832024	1.3	0.0063										
	-	-	mbPb-847371	1.3	0.0071										
	-	-	mbPb-848838	-0.9	0.0079										
	8	1.8	soPb-855377	0.8	0.0086										
	-	-	mbPb-847391	-0.7	0.0094										
	-	-	mbPb-847707	0.7	0.0102										
rLA	-	-	soPb-854462	-1.6	0.0000	-	-	mbPt-869390	3.5	0.0000	25	29.7	soPt-856082	1.7	0.0000
	-	-	soPb-856347	1.1	0.0008	-	-	soPt-854973	-2.1	0.0021	25	40.5	soPb-854943	-1.2	0.0018
	-	-	mbPb-877441	-1.0	0.0016	-	-	mbPt-847497	-1.9	0.0041	23	5.4	soPt-853730	1.0	0.0036
	-	-	soPb-825639	1.0	0.0024	13	16.9	soPt-832166	1.6	0.0062	-	-	soPb-825520	-1.0	0.0055
	-	-	mbPb-847707	1.0	0.0031	25	40.5	soPb-854943	-1.4	0.0083	7	34.4	soPb-855130	-0.9	0.0073
	-	-	mbPb-848410	-1.0	0.0039			mbPt-877082	-1.1	0.0104	-	-	soPb-857506	0.9	0.0091
	-	-	soPb-825138	0.9	0.0047										
	-	-	soPb-857449	0.8	0.0055										
	-	-	mbPb-847371	0.8	0.0063										
	-	-	mbPb-869177	-0.7	0.0071										
	-	-	soPb-853985	0.7	0.0079										
	-	-	mbPb-871202	0.7	0.0086										
	-	-	soPb-824175	0.7	0.0094										
	-	-	soPb-855385	0.7	0.0102										
NS2	7	0.0	soPt-856127	-3.5	0.0000	-	-	mbPb-848912	2.5	0.0000	-	-	soPt-824626	1.5	0.0000
	-	-	soPb-831538	3.4	0.0008	-	-	mbPt-847497	-2.4	0.0021	7	35.6	soPb-853608	-1.4	0.0018
	-	-	mbPb-848332	3.1	0.0016	13	16.9	soPt-832166	1.9	0.0041	-	-	soPb-855967	0.9	0.0036
	-	-	soPb-853488	-1.9	0.0024	-	-	mbPt-868387	-1.6	0.0062	5	27.1	soPb-856104	-0.9	0.0055
	7	0.0	soPb-856127	-1.8	0.0031	13	57.0	soPt-853631	-1.6	0.0083	-	-	soPt-825048	-0.8	0.0073
	-	-	mbPb-869181	1.5	0.0039	17	0.8	soPt-855577	1.5	0.0104	-	-	soPt-854205	-0.8	0.0091
	-	-	soPb-857343	-1.1	0.0047										
	7	41.3	soPb-831844	-1.1	0.0055										
	-	-	mbPb-877076	-1.1	0.0063										
	7	37.3	soPb-825453	-1.0	0.0071										
	-	-	soPb-855385	0.8	0.0079										
	7	40.0	soPb-825761	-0.7	0.0086										
	-	-	mbPb-848032	-0.7	0.0094										
	-	-	soPb-853201	-0.7	0.0102										
LL	-	-	soPb-825639	6.0	0.0000	-	-	mbPt-869390	2.1	0.0000	-	-	soPb-825520	-3.0	0.0000
	-	-	soPb-832207	3.6	0.0008	-	-	mbPt-847497	-1.8	0.0021	25	29.7	soPt-854547	1.8	0.0018
	-	-	soPb-854462	-2.9	0.0016	-	-	soPt-831546	1.7	0.0041	-	-	soPt-824626	1.7	0.0036
	-	-	soPb-832024	2.4	0.0024	25	40.5	soPb-854943	-1.5	0.0062	25	29.7	soPt-856082	1.3	0.0055
	-	-	soPb-856347	2.0	0.0031	13	32.5	mbPt-846998	1.4	0.0083	-	-	soPt-825048	-1.3	0.0073
	-	-	mbPb-846518	1.9	0.0039	-	-	mbPb-847253	-1.3	0.0104	7	35.6	soPb-853608	-1.2	0.0091
	-	-	soPt-832024	1.4	0.0047										
	-	-	mbPb-877441	-1.3	0.0055										
	-	-	soPb-855385	1.1	0.0063										
	-	-	mbPb-848410	-1.1	0.0071										
	-	-	mbPb-869177	-1.0	0.0079										
	-	-	soPb-825495	0.9	0.0086										
	-	-	mbPb-870560	0.8	0.0094										
	-	-	soPt-832207	0.7	0.0102										

Appendix 6.5. Continued 2…

Traits	CPI 26671 x G2120					CPI 26671 x Valder					Valder x G2120				
	LG	Pos	Marker	PVE %	*P*-value	LG	Pos	Marker	PVE%	*P*-value	LG	Pos	Marker	PVE%	*P*-value
LA	-	-	soPb-825639	7.7	0.0000	-	-	mbPt-869390	2.6	0.0000	-	-	soPb-825520	-2.3	0.0000
	-	-	soPb-854462	-5.2	0.0008	-	-	mbPt-847497	-2.0	0.0021	11	6.1	soPt-854224	-1.6	0.0018
	-	-	mbPb-877441	-3.6	0.0016	-	-	mbPt-877082	-1.5	0.0041	7	34.4	soPb-855130	-1.3	0.0036
	-	-	mbPb-848410	-3.0	0.0024	-	-	soPt-824724	-1.2	0.0062	-	-	soPt-824626	1.3	0.0055
	-	-	soPb-832207	2.7	0.0031	-	-	soPt-825682	-1.0	0.0083	7	34.6	soPb-855129	-1.1	0.0073
	-	-	mbPb-877396	2.4	0.0039	-	-	mbPb-847253	-1.0	0.0104	11	7.2	soPt-824436	-0.9	0.0091
	-	-	soPb-855385	2.2	0.0047										
	-	-	soPb-856347	1.7	0.0055										
	-	-	soPb-832024	1.6	0.0063										
	-	-	soPb-853727	-1.6	0.0071										
	-	-	mbPb-846518	1.5	0.0079										
	18	16.8	soPt-825083	-1.5	0.0086										
	-	-	soPb-855212	1.5	0.0094										
	-	-	mbPb-869177	-1.4	0.0102										
nNS	-	-	mbPb-847391	-9.1	0.0000	-	-	mbPt-847497	-3.3	0.0000	-	-	soPt-824626	2.8	0.0000
	-	-	soPt-853786	6.6	0.0008	17	0.8	soPt-855577	2.8	0.0021	16	0.0	soPt-856479	1.7	0.0018
	-	-	mbPb-846565	-4.8	0.0016	-	-	soPb-853062	2.3	0.0041	-	-	soPb-825048	-1.1	0.0036
	-	-	soPb-855385	2.5	0.0024	-	-	mbPb-848912	2.3	0.0062	-	-	soPb-853502	1.1	0.0055
	-	-	mbPb-870330	2.5	0.0031	-	-	soPt-853508	2.1	0.0083	16	0.0	soPt-855872	1.0	0.0073
	-	-	soPb-831538	1.9	0.0039	13	16.9	soPt-832166	1.9	0.0104	26	28.6	soPb-853334	-0.9	0.0091
	-	-	soPb-855967	1.8	0.0047										
	7	74.4	soPb-857058	1.0	0.0055										
	-	-	soPb-854637	1.0	0.0063										
	-	-	mbPb-867637	0.6	0.0071										
	7	74.1	soPb-854948	0.6	0.0079										
	-	-	soPb-854798	0.6	0.0086										
	-	-	mbPb-870784	-0.5	0.0094										
	-	-	mbPb-877540	-0.5	0.0102										
nLL	-	-	mbPb-867968	4.2	0.0000			soPt-831546	2.8	0.0000	-	-	soPb-825520	-3.7	0.0000
	-	-	mbPb-869190	-2.3	0.0008	-	-	mbPt-847497	-1.6	0.0021	-	-	soPt-824626	1.5	0.0018
	-	-	soPb-831714	2.0	0.0016	25	40.5	soPb-854943	-1.3	0.0041	-	-	soPt-825048	-1.4	0.0036
	-	-	soPb-825495	1.8	0.0024	13	32.5	mbPt-846998	1.3	0.0062	25	29.7	soPt-854547	1.4	0.0055
	-	-	mbPb-870560	1.7	0.0031	-	-	soPt-824724	-1.2	0.0083	25	29.7	soPt-856082	1.2	0.0073
	-	-	mbPb-846518	1.2	0.0039	-	-	soPt-853550	-1.2	0.0104	26	28.6	soPb-853334	-1.2	0.0091
	-	-	soPb-854462	-1.1	0.0047										
	5	21.0	soPb-853729	-1.0	0.0055										
	18	16.8	soPt-825083	-0.9	0.0063										
	-	-	soPb-825639	0.8	0.0071										
	-	-	mbPb-877396	0.7	0.0079										
	7	74.1	soPb-854948	0.7	0.0086										
	-	-	soPb-825811	0.6	0.0094										
	-	-	soPb-855212	0.5	0.0102										
nLA	-	-	soPb-854462	-4.6	0.0000	-	-	mbPt-877082	-1.9	0.0000	11	6.1	soPt-854224	-2.3	0.0000
	-	-	mbPb-846691	-2.4	0.0008	-	-	soPt-831546	1.7	0.0021	-	-	soPb-825520	-2.0	0.0018
	-	-	mbPb-870560	2.0	0.0016	-	-	soPt-824724	-1.7	0.0041	-	-	soPt-824626	1.1	0.0036
	-	-	soPb-825495	1.7	0.0024	-	-	mbPt-847497	-1.6	0.0062	-	-	soPt-825048	-1.1	0.0055
	18	16.8	soPt-825083	-1.6	0.0031	-	-	soPt-854973	-1.2	0.0083	7	34.4	soPb-855130	-1.0	0.0073
	-	-	soPb-854083	1.4	0.0039	-	-	mbPt-871253	1.1	0.0104	11	7.2	soPt-824436	-0.9	0.0091
	-	-	soPb-855385	1.3	0.0047										
	-	-	soPb-832207	1.0	0.0055										
	-	-	mbPb-848699	-1.0	0.0063										
	23	6.5	soPb-853730	0.8	0.0071										
	-	-	mbPb-871105	0.8	0.0079										
	-	-	mbPb-867968	0.8	0.0086										
	-	-	soPb-825639	0.7	0.0094										
	-	-	mbPb-869527	-0.7	0.0102										
Rc	-	-	mbPb-847391	-4.9	0.0000	-	-	mbPt-847497	-3.1	0.0000	-	-	soPt-824626	2.8	0.0000
	-	-	mbPb-876802	-3.2	0.0008	-	-	mbPt-869390	2.6	0.0021	-	-	soPt-853101	2.2	0.0018
	-	-	soPb-825639	3.2	0.0016	17	0.8	soPt-855577	2.4	0.0041	23	5.4	soPt-853730	1.8	0.0036
	-	-	soPb-854462	-2.8	0.0024	13	32.5	mbPt-846998	2.0	0.0062	25	29.7	soPt-854547	1.4	0.0055
	-	-	mbPb-871105	2.2	0.0031	21	24.9	mbPt-849130	1.4	0.0083	7	0.0	soPt-856127	1.2	0.0073
	-	-	mbPb-846315	-1.6	0.0039	21	3.0	mbPt-849106	1.4	0.0104	-	-	soPt-854382	1.0	0.0091
	-	-	soPb-823933	1.6	0.0047										
	-	-	mbPb-848410	-1.4	0.0055										
	7	74.1	soPb-854948	1.1	0.0063										
	-	-	soPb-832207	1.1	0.0071										
	-	-	soPb-824011	1.1	0.0079										
	7	0.0	soPt-856127	-1.0	0.0086										
	-	-	soPb-857540	1.0	0.0094										
	-	-	soPb-855385	0.9	0.0102										

Appendix 6.6. Common and co-localized SML QTLs detected in the three soybean RIL populations derived from crosses between two oilseed cultivars (Valder and CPI 26671) and a landrace variety (G2120)

RWC = Relative water content (%); R_RWC = Relative response relative water content; g_e = Epidermal conductance (mm/s); g_{e70} = Epidermal conductance (mm/s) at relative water content of 70%;

NS1/ NS2 = No. of stem nodes before/ after re-watering respectively; nNS = No. of new stem nodes; rLL = No. of remnant live leaflets; LL = No. of leaflets after re-watering; nLL = No. of new leaflets; rLA = Remnant live leaf area; LA = Leaf area after re-watering; nLA = New leaf area; Rc = Recovery score

LG = Linkage group; Pos = QTL position on LG (in cM);

♪: Underlined LG and Pos are positions assigned to SML QTLs based on the genotype correlation ($r > 0.84$) between markers linked to SML QTLs and markers mapped on integrated linkage map; ‡: Highlighted markers are those selected for sequencing

CPI 26671 x G2120

LG♪	Pos	Traits	Marker‡
1	71.6	RWC	mbPb-871580
1	71.8		mbPb-869437
3	26.9	RWC	soPb-825841
4	8.4	g_e	mbPb-877452
5	21.0	NS1	soPb-853729
5	21.0	nLL	
7	0.0	NS1	soPb-856127
	0.0	NS2	or soPt-856127
		Rc	
	1.3	NS1	soPb-856183
7	37.3	NS2	soPb-825453
	40.0 or 41.3	NS2	soPb-825761 or soPb-831844
	74.1 or 74.4	nNS	soPb-854948
		nLL	or soPb-857058
		Rc	
8	1.8	rLL	soPb-855377
	12.4		soPb-856842
10	16.1	g_{e70}	soPb-831699
	16.4		soPb-853348
		LA	
18	16.8	nLL	soPt-825083
		nLA	
23	6.5	R_ RWC	soPb-853730
23	6.5	nLA	
	0.0	R_ RWC	soPb-854858
25	29.7	R_ RWC	soPt-854547
			or soPt-856082
-	-		soPb-854798
-	-	R_ RWC	or soPb-855123
-	-	NS1	
-	-	rLL	soPb-825639
-	-	rLA	or soPb-832024
-	-	LL	or soPb-832207
-	-	LA	or soPb-856347
-	-	nLL	
-	-	nLA	
-	-	Rc	
-	-	LL	
-	-	LA	mbPb-846518
-	-	nLL	
-	-	rLL	mbPb-847371
-	-	rLA	
-	-	rLL	
-	-	nNS	mbPb-847391
-	-	Rc	
-	-	rLL	mbPb-847707
-	-	rLA	
-	-	g_e	

CPI 26671 x Valder

LG	Pos	Traits	Marker
	12.1	g_{e70}	mbPt-876684
		rLL	
	16.9	rLA	soPt-832166
		NS2	
13		nNS	
		LL	
	32.5	nLL	mbPt-846998
		Rc	
	48.9	R_RWC	soPt-853079
	57.0	NS2	soPt-853631
14	47.8	NS1	mbPt-869415
		NS2	
17	0.8	nNS	soPt-855577
		Rc	
	3.0	Rc	mbPt-849106
21	24.9	NS1	mbPt-849130
		Rc	
		rLL	
25	40.5	rLA	soPb-854943
25	40.5	LL	or soPt-854943
		nLL	
-	-	LL	
-	-	nLL	soPt-831546
-	-	nLA	
-	-	R_ RWC	soPb-855118
-	-		or soPb-856799
-	-	rLL	
-	-	LL	mbPb-847253
-	-	LA	
-	-	NS2	mbPb-848912
-	-	nNS	
-	-	rLA	
-	-	NS2	
-	-	LL	
-	-	LA	mbPt-847497
-	-	nNS	
-	-	nLL	
-	-	nLA	
-	-	Rc	
-	-	g_e	mbPt-869283
-	-	g_{e70}	
-	-	rLL	
-	-	rLA	
-	-	LL	mbPt-869390
-	-	LA	
-	-	Rc	
-	-	rLA	
-	-	LA	mbPt-877082

Valder x G2120

LG	Pos	Traits	Marker
5	27.1	RWC	soPb-853611
5	27.1	NS1	or soPb-856104
		NS2	
	0.0	Rc	soPt-856127
		RWC	
		NS1	soPb-855130
		rLL	or soPb-855129
		rLA	or soPb-853608
7	34.4 or 34.6 or 35.6	NS2	
		LL	
		LA	
		nLA	
	65.7	NS1	soPb-856137
	66.2	RWC	soPb-855445
	70.9	RWC	soPb-855545
		g_e	
8	1.2	NS1	soPb-856335
11	6.1	LA	soPt-854224
	or 7.2	nLA	or soPt-824436
16	0.0	nNS	soPt-855872
			or soPt-856479
18	21.1	NS1	soPb-825062
		rLL	
20	0.0	g_{e70}	soPb-853371
	5.4	rLA	soPt-853730
23		Rc	
	12.0	R_ RWC	soPb-831556
	19.7	R_RWC	soPt-854142
	28.5	rLL	soPb-825425
		rLL	
		rLA	
25	29.7	LL	soPt-854547
		nLL	or soPt-856082
		Rc	
	40.5	rLA	soPb-854943
26	28.6	nNS	soPb-853334
		nLL	
-	-		soPt-853791
-	-	g_e	or soPt-854123
-	-		or soPt-857253
-	-	g_{e70}	soPt-825567
-	-		or soPt-856216
-	-	g_{e70}	soPb-853211
-	-	nNS	or soPb-853502
-	-	R_ RWC	mbPt-848956
-	-	g_e	
-	-	NS2	
-	-	LL	
-	-	nNS	soPt-825048

-	-	rLL	
-	-	rLA	mbPb-848410
-	-	LL	
-	-	LA	
-	-	Rc	mbPb-848410
-	-	NS1	mbPb-848838
-	-	rLL	
-	-	g_e	mbPb-867637
-	-	nNS	
-	-	R_ RWC	
-	-	nLL	mbPb-867968
-	-	nLA	
-	-	rLA	
-	-	LL	mbPb-869177
-	-	LA	
-	-	LL	
-	-	nLL	mbPb-870560
-	-	nLA	
-	-	RWC	mbPb-870915
-	-	g_e	
-	-	RWC	mbPb-871053
-	-	g_e	
-	-	nLA	mbPb-871105
-	-	Rc	
-	-	rLL	mbPb-871202
-	-	rLA	
-	-	LA	mbPb-877396
-	-	nLL	
-	-	rLL	
-	-	rLA	mbPb-877441
-	-	LL	
-	-	LA	
-	-	RWC	soPb-824011
-	-	Rc	
-	-	NS1	soPb-824175
-	-	rLA	
-	-	LL	
-	-	nLL	soPb-825495
-	-	nLA	
-	-	NS1	soPb-825811
-	-	nLL	
-	-	g_e	soPb-826118
-	-	NS1	
-	-	NS2	soPb-831538
-	-	nNS	
-	-	g_e	
-	-	g_{e70}	soPb-853488
-	-	NS2	
-	-	g_e	soPb-853727
-	-	LA	
-	-	g_e	soPb-853985
-	-	rLA	
-	-	RWC	
-	-	rLL	
-	-	rLA	
-	-	LL	soPb-854462
-	-	LA	
-	-	nLL	
-	-	nLA	
-	-	Rc	
-	-	LA	soPb-855212
-	-	nLL	
-	-	rLL	
-	-	rLA	
-	-	NS2	
-	-	LL	soPb-855385
-	-	LA	
-	-	nNS	
-	-	nLA	
-	-	Rc	
-	-	g_{e70}	soPb-855967
-	-	nNS	
-	-		mbPb-867708

-	-	nLA	
-	-	g_{e70}	soPb-853062
-	-	nNS	
-	-	RWC	soPb-853092
-	-	g_e	
-	-	RWC	soPt-824183
-	-	NS1	
-	-	LA	
-	-	nLL	soPt-824724
-	-	nLA	
-	-	nLA	soPt-854973
-	-	rLA	
-	-		mbPb-871796
-	-	RWC	mbPt-868851
-	-		mbPt-869103
-	-		mbPt-876860
-	-		mbPb-876469
-	-	R_ RWC	soPt-825368
-	-		soPt-855439
-	-		mbPt-846781
-	-	g_e	mbPt-868314
-	-		soPt-824391
-	-		soPt-856414
-	-		mbPb-877518
-	-	g_{e70}	soPb-855347
-	-		soPt-855792
-	-		soPb-825021
-	-	NS1	soPt-854003
-	-		soPt-854925
-	-	rLL	soPb-831830
-	-	NS2	mbPt-868387
-	-	nNS	soPt-853508
-	-	LA	soPt-825682
-	-	nLL	soPt-853550
-	-	nLA	mbPt-871253

Total no. QTLs **47**

-	-	nLL	
-	-	nLA	
-	-	g_{e70}	soPb-825520
-	-	rLA	
-	-	LL	soPb-825520
-	-	LA	
-	-	nLL	
-	-	nLA	
-	-	R_ RWC	soPb-855967
-	-	NS2	
-	-	NS2	
-	-	LL	
-	-	LA	soPt-824626
-	-	nNS	
-	-	nLL	
-	-	nLA	
-	-	Rc	
-	-	R_ RWC	
-	-	g_{e70}	soPt-853101
-	-	Rc	
-	-	RWC	soPb-855347
-	-	R_ RWC	soPb-855549
-	-	g_e	soPb-855667
-	-	NS1	soPt-856322
-	-	rLA	soPb-857506
-	-	NS2	soPt-854205
-	-	Rc	soPt-854382

Total no. QTLs **34**

-	-		mbPb-868411
-	-		mbPb-868567
-	-	RWC	mbPb-877365
-	-		mbPb-877505
-	-	RWC	soPb-825520
-	-		soPb-856220
			mbPb-847486
-	-		mbPb-869193
-	-	R_ RWC	mbPb-876843
-	-		soPb-824720
-	-		soPb-854350
-	-		soPb-857441
-	-		soPb-857530
-	-		mbPb-871011
-	-		soPb-825530
-	-	g_e	soPb-853915
-	-		soPb-856646
-	-		soPb-857280
-	-		mbPb-868499
-	-		mbPb-871360
-	-		soPb-824120
-	-		soPb-825017
-	-	g_{e70}	soPb-825161
-	-		soPb-853767
-	-		soPb-855469
-	-		soPb-855633
-	-		soPb-856169
-	-		mbPb-848064
-	-	NS1	mbPb-868034
-	-		soPb-825259
-	-		soPb-831609
-	-	rLA	soPb-825138
-	-		soPb-857449
-	-		mbPb-848032
-	-		mbPb-848332
-	-	NS2	mbPb-869181
-	-		mbPb-877076
-	-		soPb-853201
-	-		soPb-857343
-	-		mbPb-846565
-	-		mbPb-870330
-	-		mbPb-870784
-	-	nNS	mbPb-877540
-	-		soPb-854637
-	-		soPb-854798
-	-		soPt-853786
-	-		mbPb-869190
-	-	nLL	soPb-825639
-	-		soPb-831714
-	-		mbPb-846691
-	-	nLA	mbPb-848699
-	-		mbPb-869527
-	-		soPb-854083
-	-		mbPb-846315
-	-	Rc	mbPb-876802
-	-		soPb-823933
-	-		soPb-857540
Total no. QTLs			**106**

Appendix 6.7. Sanger sequences of 19 selected DArT markers linked to QTLs for physiological traits associated with drought stress responses in the two soybean RIL populations involving G2120 as a parent

>mbPb-877452
AGCGTATGCGGGTTCCGAATCTCCGAAGACTCTGTTACCTTTTCCTGTCTCGTTGACGAG
GTCAGGTTATCCCATGACTTTCTTATGAGTCCTGTGGCATTTAGGTTTCCTATCTACAAT
TCATTAAACACCAGAAAAGCATTGGAGTCTTTCTTTTTTTATGATCTCCGACCCCGCAAG
GNACTACTGGGCGGAGNTNCTCAATCCAATTCCTTACCACAGCTGCTTTAAGCTTTCGTT
CCTTTCGTATAGTGGAAAAGGGCTGNNTTTCCCTAGACGGAAGAAATAAGAAAGAAACTT
ACTAAATTAAGAAGACCAAAGAAAGCCATAAGCTACAGCTTCCCCTGCCGGAGTTGTGCT
TGGTGGAATTAAGGCCTCAAATCATCACTTTCTCACCGACNTTTACCTATGCTAAACGTG
TCCCAGTTTACTAACGANCCGGTGACGTAAAGCTTCTATTCTCAATTGATCCTTTTTTCT
TCCCCTCTGCACTGNATCCATCAN

>soPb-825017
CAGATGAAACAAATATCTTCTAGCCTGCAAGTGTTAAAATGACTTATATTCTATAAATAT
TGCTAAGCTTTTTGCTTCAAAACCTATTAGAATAGACTCTAGATTGATGGTATGTTTTCA
TGGTAATTGGTTCTTTAAACATCGCACTCTACTTTAACTGTAACTGTCTGCTTTTTGTTT
GTAGGTGAGAAGGGCATTGGAGAATCAACGGGGAAACCTTTGCATTATAAAGGGACAAGC
CTTCATCGTATAATTAGAGGTTTTATGGCTCAAGTAAGTTGCCCTTCATGGTTACTCTAT
TCATGATTGTCACTAAGGGTGACCTGACCCAACACATGAGGCTCTCACCTAGTGGGATTT
AGGAATGGTAGATTATGTAGCCTTACCCACACAAGTGGAGATGTTGTTTCTAGAATTTGA
ACTCATGACCTCCAGATCATAAGGCTGCA

>soPb-825062
TGCAGACGNATCACTGAAATACAAAAAGATGTAGTAGCCAACATCCTTTTCATTGTAAGT
CGTCAAGGAGATAACATAAATGACCATTGTTACCCAAGGGGTCTAGCTCAGTTGGTTGAG
TTGTTGTAAACCCCCAAATACCTAAGTTTGATTCATATGGATAAAAAAAATGACCAGAGT
TTCATTTGTTTCACCACACTAAACACTTGACTGCA

>soPb-825639
TGCAGAGGAATGAGTCGCCGCCTCTTGCTGCGTAATTAGAAGGTGGAGCTTTCGGCTTTT
TGACATGAGAGGAAACTGAAAGAAACGGGTATCAGAGACAAATTTCAGAGAATGAAGTAC
GGAATGTTGTCGCTACTGGAAGTGGAAATGGACCCACCCATGTAAGTGAGAATTGAGAAA
ATTTGAAAATTGATTCTCTGTGTTTTGAGGCTTACCTTGTGGAGATGGAATGTCATGTCA
TCAACTTCCACCACAATGTCGCTTGGCAATCCCGTTGTGCAGAACCTGCA

>soPb-832207
TGCNGAGGAATGAGTCGCCGCCTCTTGCTGCGTAATTAGAAGGTGGAGCTTTCGGCTTTT
TGACATGAGAGGAAACTGAAAGAAACGGGTATCAGAGACAAATTTCAGAGAATGAAGTAC
GGAATGTTGTCGCTACTGGAAGTGGAAATGGACCCACCCATGTAAGTGAGAATTGAGAAA
ATTTGAAAATTGATTCTCTGTGTTTTGAGGCTTACCTTGTGGAGATGGAATGTCATGTCA
TCAACTTCCACCACAATGTCGCTTGGCAATCCCGTTGTGCAGAACCTGCA

>soPb-853348
CAGAGGTTGTCTGCGCGGAAGAGGACGTAAATCTTCGTGTGTCAACGGACTTGTTGGCCG
TGATTGACAAAGGGTGCAGAAAATGACGTTTGTCTTTGCATGGTATCATGCGTTGAATCT
TAGAGATAGCGAAAGAATGTTTATACGGATAACCACTTGGGTATTTCCGCTGGCCCCCTA
ACTTCACGAGTTAGTATTGCAGAGGTTGTCTGCGCGGAAGACGACGTAAATGTCCGCATG
TGAACGGACATGTTAGCTGCGATTGACAAAGGGTGCAGAAGACGACGTTTGTCTCTGCAT
GGTATCATGCGTTGAGTCTTACAGATAGCAAAAGAATGTTTATAGGCATAACCACTTGGG
TATTACCGTCGGCCCCCTAACTTCACGAGTTAGTACTGCA

>soPb-853488
TGCAGGTCGTAGAATTTTAAACACTCACCCAAGACACTTGTGCACAGAGAGAAACACTAA
AACCTTGTGAGGAAAAGTGAGGCAAGCCGACCTGATTGATTTGTACTAGTAACCAATTCT
GTCTAAAACGTTTGTTTATGCTTCCATTGCAAGATCATGGCAATTGCAAAAAGTTGCAGT
TGAGGGAATTTGCAGCTATTGAAAGTGTTGGAGGATTCAAAGCCTGCTCTTATTTTGTTT
TACTTGAGGACAAGCAAAGTTTTTAATTGAGAGATTTTGATAACTGCAAATTAAATGTGT
TTTTGATAATGAGAAATAAAGTGAAATTGTCTTTAAAAATTATTATTTAGCAGTTATTTG
TGCTTAATCGTTAAGAATTGATTTTCTTTTCCAAATTATGGCTGCA

>soPb-853729
TGCAGCATCTTCGGTTTCGAAGCGCATCCTGAACCTCATTCCAGCTGAATAAGAATAATC
AAGGCTCTTTAAGAATCTGTGGATAGGTATGATGAACTCAGATGTACTGACCCTACAAAG
ACAGTGGAATATCAAAGACACTGATCAGATGCCATACTTTGTAAGAAGGAAAGAAGAAAA
AAAAAACAGGTATTACAAAAAAAACCATGAAAACTTAGACCACAATAGTACATACNTGTA
AAATCATCAACTGAAGAAAACCATTTCAAGATTCAAACGTTGTGCNAAAANAAAAGAACA
ATGTAGTCTAATTCTAGATTTATTGTCATCAAGAATCTGTTTGTTTGAAAAAGTAACCCA
ACTATTGGNACACTCAAGTATTAGCAATAACAGGAGCAATAGTCATTGCACTGCA

>soPb-855130
TGCAGAGAAAGATGACCGGTCCAAGTCTCCCAACAAGAATCACAGGATGACGTTAACCGA
ATAGTTCCTAACCGATTGATTATTCAGGGATTGAGGAAAACAACCATAGGCGACATCGGT
CGTAAAATACACCCGACTGATATTTGTCGAGAAACATCACCGGAAATCATTTACCAATAA
CGGTTGTTTTTCGGCCGACATTGTGCAACGTTTTTGTAATTTTCCAACGCCGGGCGATAA
TATATTTTTACGGTTGAGAAAGTTTTATTGTCTGATGTCACCCATAATTAGTAGACTCGA
GGTCGGGTAGGGTTTTTCGTGTGAATTTAACCGAGCTTGTGTTTCGACCAAAAAGTGTCG
GCTCTAATTTTATCGGCTGAGAAAACACTGGCAAACCTTGGCGAAAAAATATGACTGATG
GGAGATTGATCGGAAAAAATCCTGCA

>soPb-855545
AGAGAGAGCTGGTGCTTGTCACAGGGCTTTGGAGGATGGAGGATCTAGGGAATTTAATGC
ATGGATTGAAGAGTTGGAGGTAGTGGAACCAGCATGGGTGGGCAGCAAGTTCACTTGGGT
TAGACCTAATGGTTCATCTAGAAGCAAACTAGATAGGTTCCTCGTATCTGCGGATTGGCT
TACACGATGGCCTGATACAGCTCAATATACACTAGAGAGAAATTTTTCCGACCATTGCCC
AATTCTGCTCAAATCGAGAAGGTCTGATTGGGGACCTAAGCCTTTCAAAATAATGGAGTG
TTGGCTAGCAAATGCTTCTTTCAAGAAAGCTGTGGTGGAAAGTTGGTCATCTAACCAGCT
AAGTGGATGGGGAGGGTATGCATTAAAAATGAAAATAAAAGCACTCAAATTCAGGTTGAG
GGAGTGGAATAGAGACCATTATGGGGATACACTTAAGAAATTCCAGAAGATTGAGCAAGA
GTTGAATAAGCTGGAGGAGATCACTGCTGATAGACAACTATCTCATCAGGAGACATTAGG
CAGGAAACAGCTGCA

>soPb-855967
GAGGCGTATGTCAAAAGAACCAGCCAAGTTGATAGTCCTACCCTGTGGCATGCTAGACTT
GGGCATGTAGACTATCAAATGCTACAAATTATAGCATCTAATAAGATTATTGGTGGGCTT
CCAGAATTGAAATAGGTGAAGGAGGAAGTTGTTTGCCAAGGATGTCAATTTGGTAAGGCT
TACAAGCTGCCATTTAAAAGTTCCAACAGAGAAAGAACCATTCCTTTCGAACTTATACAC
ACAGATCTAATGGGGTCGACAAAAACACCTAGTTATAGTGGATGTAAATATACTATGGTA
CTAGGGGATGATTACACTCAATATACTTGGTTGTATTTCCTAAAGGAGAAAAGTGAAGTT
ACACATAAGTTTGTTATTTTCTCTGACATGGTGGAGAAGGAGTTTGATGCAAAAATCAAA
TGCTTGAGGAGTGACAATGGAGGTGAGTTCAACTCAACAGATTTCACAGTTTTTTGTCAT
GAAAAAAAGGATCCAAAGGCAATTTACTTGTTGAGATACACTGCA

>soPb-856104
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GGTGCCCACTGAATACTGAGCTGACATGAGATCTCTTTGAAAATTCAATGCCTTTCAGAT
TCAAAGTTTACCCAGCAAGAACTTCCAGCGTGTAAGCCGATTCTCACACCACGAGCGGTA
ACACTATCTACTTTTTTGTAATACTTTGCATTGTTGCTCGTTATTAAATGTGTACCTCAA
TGCATCTTGGTGTCGTTATTTATGTGGTGGTATAGCTTGTACTTAGTTTAATTTTTTCCA
TGCAGGTTATTTCAGCATTCTTGCTTGTTAGCATTGTATTCGTTCCTATTGGAGTTGCTT
CACTAATTGCTTCAAGGAAGGTAATGTTGAGTGCTATAATGTGTTGAATTTATGCTTACA
GTTGTCTATAATGCTGAGTGCAACTTTCACTTTCTTGTATCTATCAGGTTGTTGAAATTG
TTTTTAGGTATGAATCAACATGCATACCACATGAAGTTACTGACAAGGTAGCATACATTC
AGAGTCCTGCA

>soPb-857280
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TTCATTAAACACCAGAAAAGCATTGGAGTCTTTCTTTTTTTATGATCTCCGACCCCGCAA
GGCACTACTGTAGAGCACTACTGGGCGGAGNTNCTCAATCCAATTCCTTACCACAGCTGC
TTTAAGCTTTCGTTCCTTTCGTATAGTGGAAAAGGGCTGGCTTTCCCTAGACGGAAGAAA
TAAGAAAGAAGAAACTTACTAAATTAAGAAGACCAAAGCCATAAGCTACAGCTTCCCCTG
CCGGAGTTGTGCTTGGTGNAATTCAGGCCTCAAATCATCACTTTCTCACCGACCTTTACC
CATGCTAAACGTGTCCCAGTTTACTAACGAACCGGTGACGTAAAGCTTCTATTCTCAATT
GATCCTTTTTTCTTCCCCTCTGCACTGNATC

>soPt-854142
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TTGAATAAAAGATATAACAATATAAAGCCAGTTCCTATGTAGCAGAAAAAATTTATAAGA
AAACATCACATTACCATTGAAATAGGATGCATACATATAAAACAAATTGTAGCAGTAGAA
TCAATATCAGAAAAAAAAATTATATGCCTATCCATAACATTTTTGAGAGATTATATATAT
GTTTGAAACAATGAGACAATGAGTCCGGATAAATACGATCAAGCCCACATAAAAAATCCC
CTCCAATACAGGGAAATGGCCAAAAAAAAAACATCCATAAAAAATTCCGGCAATTTAAGT
TCCNAACTTCCGGTTAAATGAATAACACATTACACAAGCATGATTATTACTGAAAATACT
TCCTTAGCTATCCTAAAAAACACAAAAGAGTAATATTCACATAAGTGGCAATACCAGAGT
GTCTGGCATTGACATTCTCAAATGCAAGAAAACAAACCCATCCTACCCACTTGACCATGA
TCGTCTTCAAAGTAGGACTAAAACTAAATATAATCATTAATAGCCATTCATAGGGCATGT
GAGANAACACTTGTTAGAACAAATTGATAAAAAAATTCAAAAG

>soPt-854224
NAGCTCGGTACCAAGCTTGGNGTAATCATGGTCATAGCTGTTTCCTGAGNNNAGGCCATA
GCTATNAGCAAGTGAGCNACANNCNAAAAATTGTTGGAATGTTTATAGTTAGAGATTTAA
TTGGTTTGCATGATTTTGGTATTTCTATTTATTCATAATTTGGAGACACGGATCATATAA
CTTTAGGATTTAGATCAAGTAGAGTGGTTTTAAAATAAATCAGGAATTGAATTTAATTAG
TTGTAGATCAAATTTAGTTGGGGTTCTTGCACTAGTAAAGTTATGATTCTTATGGTTTTC
AGTAGGAGCTAATCCTGTATTTTGAATTTACTTATATAGAATTTGATCATTTCAGAATGA
TCTGTTAGGTTTCATTTACCACCCCTTACATACCATTTTTTATCTTCACATCTAAATTCT
TCTTCCTCTTTGGCGCATATCAACAGGACAGACAAAATAAAATTTAGCCTGCCTTTTTTA
GAAGATAAGTTTTGAGGGCTATTAATAGTACTCTCAACGGTTATGCTTTTGACTTAGTTG
AACATTTGTTCATTGCTTTTGATTGGCTCCCCCAGTCTTCTCAATAGCAGTATCATCATC
AACACAATCATTCTCCTCTGCCACTACCAACTAGTGCTGAGACTACTCCTAACTCTCCTT
CGCCAACTCCATTTCCTCCGACTTTGTTTCCACTTGATGATCTCGCCATTGAACTTCATA
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>soPt-854382
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GAAATCTCACCCTATACAAGATCATTGCTCTCTGGTTCACCTCCACTCTCCTTATAAGGA
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>soPt-856082
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TCACAGAACCGGGAAGGGAATCAAGCAGATCTTTAGGATTTGATGAAACTAATGAAAGTT
CCACTGTGGAAAGTATTGAGATATCAAATAATGGTTCTGCAACTGAGCAGACAGGAAAGT
TTGGACTGAGCATTTACAACAATACTATTTCTAGTTCCCCTTCTCATGCAATAATACCAA
CTAATTTAGCTCCACCTCTAAGTCCAACAGAAGTTAGCCCAAATATTACATCTCCAATGT
CATCAAATGATTATGATGAAACTGATTTCGCCGAGGAAGAGCGGTTCAAGCCATCAAAAG
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GTTCTCAAATACCACTTCCAGAAGTAACAACAATTTCTGAAATGAATGAGTTATTACTTC
AAAACCGTGCCTCATATCGTTCTATGGTAAATGATTTAATGACATTTTTCTTTTCATTTT
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CCTAGGTGGTCTTCGGNTGTTGATCAAGAGCTACTGCACTGGATC

>soPt-856127
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>soPt-856479
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AACAACAACGGTAAGATAGAGCGATGCAATGAGTTAGGCGACGACAGCGGCGGACAATAG
CAGGAAGATGGAGTAGCACTTGGTAATTCAAACGTTAAGCCTCTTAACCACTAGGGTCAC
GTGCAAAGATTTAAATGGTTAATCTCATGCATTAATCTTAAAATAAAACAAAGGTTTTAG
ATTGAAAATAACTCTTTAAAAAGTTATTCTTTGAGTAAGTGGATCTCCTTCCATATATGT
ATTTGCTACATAAGCATAATAATCAAATGACTCCCTCGGCCATGGAAAAATTATGCTCTG
ATCTCTAGCTACTAGCATTTGTCTTTTCGCAATTTTTTCCCAACTCATTATTAGATAGTT
TGTTGAGCAGGACTGCA

Appendix 6.8. BLAST search results of 19 selected DArT markers linked to QTLs for physiological traits associated with drought stress responses in the two soybean RIL populations involving G2120 as a parent

LG = Linkage group; Pos = Position; Chr./LG = Soybean chromosome/assigned corresponding linkage group (http://www.soybase.org/sbt/) that each DArT marker was aligned with; Ave.Rec.Rate = Average recombination rates on respective linkage groups (Hyten *et al*. 2010b; Table 2.1);

♪: E-value indicates the probability that the match is by chance alone and e is the exponent; ‡: Approximate position of DArT markers/QTLs on the Soybean Consensus Map 4.0

DArT markers				BLAST search results						Soybean Consensus Map 4.0	
Marker name	LG	Pos (cM)	Size (bp)	Chr./LG	Alignment position Start	Alignment position End	Query coverage (%)	E-value♪	Identities (%)	NCBI Accession number	Ave. Rec. Rate (cM/Mb)	App. Pos (cM)‡
soPb-855130	7	34.4	446	1/D1a	16527748	16528194	100	0	95.0	NW 003722731.1	1.8	29.5
soPt-856479	16	0	437		1910264	1909828	100	0.	99.3			3.4
soPb-825639	-	-	290	2/D1b	5509655	5509944	100	7e-147	100.0	NW 003722732.1	2.7	15.0
soPb-832207	-	-	290		5509655	5509944	100	9e-146	99.7	NW 003722732.2		15.0
soPb-825017	-	-	449	3/N	39351908	39352355	100	0	99.6	NW 003723081.1	2.1	82.5
soPb-855967	-	-	525		10040464	10040988	100	0	100	NW 003722733.1		21.0
soPt-854142	23	19.7	703	5	31717962	31718606	91	0	99.1	NW 003722735.1	2.1	66.0
soPt-854224	11	6.1	755	6/C2	38081314	38082006	92	0	99.1	NW 003722736.1	2.7	102.7
soPt-856082	25	29.7	705		6537540	6538237	99	0	99.9			17.6
soPb-853729	5	21	415	7/M	37222035	37222449	100	0	98.6	NW 003722737.1	3.0	113.2
soPb-825062	18	21	215	8/A2	10515522	10515736	100	2e-102	98.6	NW 003722738.1	3.1	32.9
soPb-856104	5	27.1	551	15/E	48863699	48863156	99	0	99.6	NW 003722745.1	2.0	96.7
soPt-856127	7	0	335		10306768	10306434	100	1e-169	99.7			20.4
soPb-853348	10	16.4	400	16/J	13516810	13516411	100	2e-161	91.8	NW 003722746.1	2.5	33.5
mbPb-877452	4	8.4	504	17/D2	23954984	23954493	97	0	94.2	NW 003722747.1	2.9	68.3
soPb-857280	-	-	511		4729	4231	98	0	98.2			0.0
soPb-853488	-	-	406	18	45639736	45639331	100	0	100	NW 003722748.1	1.8	80.6
soPb-855545	7	70.9	555		61819021	61819575	100	0	99.8			109.1
soPt-854382	-	-	344	20	41882090	41882433	100	3e-176	100	NW 003722750.1	2.4	101.3

Appendix 6.9. QTLs for physiological traits associated with drought stress responses in previous studies i. Mian *et al.* 1996, 1998a; ii. Charlson *et al*. 2009; iii. Du *et al*. 2009; iv. Abdel-Haleem *et al*. 2012; v. Carpentieri-Pipolo *et al*. 2012; vi. Virginia *et al*. 2012

Chr./LG = Soybean chromosome/assigned corresponding linkage group (http://www.soybase.org/sbt/); Pos = Position on the Soybean Consensus Map 4.0 (Hyten *et al*. 2010b)

References	Traits	Chr./LG	Pos (cM)	Marker/Marker interval	References	Traits	Chr./LG	Pos (cM)	Marker/Marker interval
i	Water use efficiency	4/C1	66.8	A063_1	iv	Slow canopy wilting	2/D1b	61.4	Satt296
		12/H	14.0	A381_1			4/C1	46.0	Satt646
		12/H	32.2	A089_1			5/A1	18.9	Satt276
		16/J	-	Cr497_1			12/H	78.9	Satt302
		16/J	72.5	K375_1			14/B2	68.4	Satt066
		16/J	86.7	A233_1			17/D2	25.5	Satt135
		18/G	-	B031_2			19/L	37.2	Satt462
		19/L	84.3	A489_1					
ii	Slow canopy wilting	8/A2	21.9	Sat_319	v	Limited hydraulic conductance	3/N	60.2 - 64.9	Satt339 - Sat_091
		13/F	64.4	Satt362			5/A1	31.9 - 41.6	Satt300 - Sat_356
		14/B2	5.5	Satt577			10/0	66.0 - 95.6	Satt478 - Satt581
		17/D2	36.1	Satt372			12/H	85.5 - 86.2	Satt317 - Sat_175
iii	Drought susceptibility index	1/D1a	50.0 - 53.3	Sat_343 - Sat_345	vi	Relative water content	2/D1b	116.5 - 126.2	ss107912689 - ss107929181
		5/A1	41.6 - 50.3	Sat_356 - Satt648			3/N	66.3 - 73.2	ss107912938 - ss107913615
		5/A1	51.6 - 58.5	Sat_171 - Satt385			11/B1	83.9 - 93.9	Satt604 - Satt546
		6/C2	103.3 - 103.9	Sat_312 - Satt134			18/G	52.0 - 75.0	ss107921048 - ss107923024
		7/M	103.6 - 125.6	Satt210 - Satt336					
		12/H	78.9 - 83.8	Satt302 - Satt142					
		16/J	10.6 - 12.2	Satt249 - Satt287					
		17/D2	24.4 - 46.8	Satt458 - Satt154					
		17/D2	56.7 - 62.9	Satt669 - Sat_292					
		20/I	55.5 - 66.8	Satt650 - Sat_418					