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COMPARATIVE STUDIES ON CULTIVATED AND WILD ACCESSIONS OF
VIGNA VEXILLATA (L.) A. RICH.

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STATEMENT OF ACCESS
ABSTRACT

*Vigna vexillata* (L.) A. Rich is an underutilised species, but has attracted international interest because of several attractive multi-purpose features, such as its tuberous roots, its high protein content biomass and its potential as a source of resistance genes. However, despite these useful attributes, *V. vexillata* remains largely un-researched as a crop. Most studies have focussed on wild accessions from Africa and to a lesser extent Australia, with only very limited numbers of accessions from Southeast Asia. While there are reports of the occasional use of wild plants by indigenous peoples in several countries, there are remarkably few reported studies on cultivated varieties. Further, there are very few cultivated accessions in international germplasm centres. Several taxonomic varieties have been described, all but one of which are wild. The exception is var. *macroisperma*, a large seeded variety reported to be at least partially domesticated. Recently, a large seeded cultivated variety sharing some of the attributes of var. *macroisperma* was reported from Bali, Indonesia. The Bali accessions are currently the only accessions in major international germplasm collections that are specifically designated as “cultivated”.

To underpin future varietal improvement of *V. vexillata*, better understanding is required of the difference between the cultivated and wild varieties, their genetic compatibility and the inheritance of potentially useful traits. Several sets of germplasm were available for the present study: several cultivated accessions from Bali, a known accession of var. *macroisperma* from Africa, and wild accessions from Africa and Austronesia. Three objectives were set up for the study: (i) to examine genotypic diversity of cultivated accessions from Bali and compare them with var. *macroisperma* and the wild accessions; (ii) to explore the genetic compatibility among the Bali accessions, and with var. *macroisperma* and the wild accessions; and (iii) to explore the inheritance of qualitative and quantitative traits in hybrid populations. In addressing these objectives, emphasis was placed on cultivated vs. wild traits to gain information potentially relevant to assisting future breeding programs for *V. vexillata*.

Six cultivated accessions from two villages in Bali, the var. *macroisperma* accession and 12 wild accessions from Africa or Austronesia were evaluated for selected morphological, agronomic and phenological traits when grown in large pots on benches outdoors. For data analyses, genotypic variation was assessed between the three main classes of accessions, between provenances within classes, and between accessions within provenances. The experiment was conducted at James Cook University from spring 2007 to winter 2008. Weekly mean daily maximum temperatures ranged from a high of about 40°C in midsummer down to a low of about 10°C in winter. Photoperiod increased from 12.47 h at planting to a maximum of 14.10 h in late December, and then back to 11.77 h in June.
Most of the accessions flowered 50-100 days from sowing. The exceptions were the cultivated Bali accessions and a wild accession from Africa, which did not flower until autumn (April-May). It was apparent that flowering in these accessions was most likely delayed by the long photoperiods during midsummer. The cultivated Bali accessions and var. *macroisperma* exhibited several qualitative attributes, including ovate leaflet shape, broad leaflet width, non-black-speckled seed testa and non-dehiscent pods that distinguished them from the wild accessions. Likewise, the Bali accessions and var. *macroisperma* shared many quantitative traits. For example, leaflet width, peduncle length and pod and seed size were all generally larger than the wild accessions. The Bali accessions exhibited bigger seed size than var. *macroisperma* (6.9 vs. 4.6 g per 100 seeds, respectively) while the wild types averaged only 1.6 g per 100 seeds. The Bali accessions were unique in having an average of eight flowers per peduncle, whereas all other lines had around four or fewer. Generally, the largest source of variation in quantitative traits was between the three classes of accessions, with the exception of some tuber attributes, where the largest source of variation was between accessions within provenances.

A second study explored the genetic compatibility of the cultivated Bali accessions with the two other accession classes. Seventeen accessions, comprising eight cultivated Bali accessions, one var. *macroisperma* accession and eight wild accessions from Africa and Austronesia, were grown in pots in shade house facilities. Not all hybrid combinations were attempted because for some accession combinations, suitable matching flowers were not available at the same time. The main aim was to attempt enough crosses between accessions from the respective classes to establish whether the classes were genetically cross-compatible. Hybridisation was conducted by hand pollination in the morning, using newly-open flowers that had been emasculated before sunset on the day before. The cultivated Bali accessions initially flowered under the shorter photoperiods of spring, but then reverted to vegetative growth as photoperiods lengthened. Renewed flowering was successfully stimulated by placing black plastic covers over the plants each day to reduce the photoperiod to 12 hours.

Pods and viable hybrid seed were obtained from the Bali x Bali, var. *macroisperma* x wild and wild x wild combinations. However, there was difficulty in obtaining viable and/or fertile hybrids between the Bali accessions and the other two classes. Depending on the particular hybrid combination, different genetic breakdown mechanisms were observed with the Bali x var. *macroisperma* and Bali x wild combinations. In some instances, flowers failed to set pods and/or the young pods absceded before maturity; pods set but seed were shrivelled and/or non-viable; viable seeds were set but the hybrid seedling plants were short-lived; or, in a few instances (Bali x Austronesian hybrids), vigorous hybrid plants were obtained but were sterile.
A laboratory study was therefore undertaken to explore possible reasons for the observed hybrid breakdown. Cytological analyses were conducted for the cultivated Bali x wild Austronesian hybrids to examine chromosome numbers during meiosis and mitosis in the parents and hybrids. There was no difference in chromosome number between the Bali accessions, the Austronesian accessions and those hybrids that were viable but infertile. All exhibited 2n = 22. Pollen viability analyses using Alexander’s stain were also conducted to investigate the possible cause of the observed sterility of the Bali x Austronesian hybrids. The percentages of viable pollen and the numbers of pollen grains in the hybrids were significantly lower than both the cultivated and wild parental accessions. Consistent with this observation, a small number of viable seeds was obtained when viable pollen from the parents was backcrossed onto the sterile hybrids.

A final experiment was carried out to explore the inheritance of qualitative and quantitative traits using hybrid combinations combining putative wild and domesticated traits i.e. between a wild parent (P1) and either a Bali cultivated accession or var. macrosperma (P2). Three subsets of experiments were established: (i) two backcross populations (P1, P2, F1, BCP1, BCP2) from cultivated Bali x wild Austronesian crosses; (ii) two hybrid populations (P1, P2, F1) from var. macrosperma x wild African crosses; and (iii) one ‘complete’ population set (P1, P2, F1, BCP1, BCP2, F2) from a var. macrosperma x wild Australian cross. The populations within subset (i) were incomplete, because no F2 generation seed were obtained, and only limited numbers of backcross seeds were available. Subsets (i) and (ii) were grown in pots in the shade house, whilst subset (iii) was grown outdoors in pots on benches.

The backcross plants in subset (i) were either largely infertile, or particularly in the case of the backcrosses to the cultivated parent (BCP2), were strongly photoperiod-sensitive and like the cultivated parental plants, did not flower during the evaluation. Thus observations were possible on only a limited number of vegetative traits, e.g. leaflet shape, leaflet width and time to flowering. Based on Chi-Square analysis, it was evident that lanceolate leaflet shape and narrow leaflet width were each controlled by a single dominant gene. As the hybrid plants and the backcrosses all flowered later than the wild parent, but most flowered sooner than the cultivated parent, time to flowering was probably a quantitative trait controlled by several genes.

With subset (iii), lanceolate leaflet shape and narrow leaflet width were again each controlled by single dominant genes as were short leaflet hair length, black-speckled seed testa pattern and dehiscent pods. For most of the quantitative traits measured, the F1 generation exhibited mid-parent values, suggesting largely additive genetic variance. Some of the attributes often associated with domestication, e.g. seed size and stem thickness, exhibited high narrow sense heritability suggesting they would be easy to breed for. Likewise, tuber traits tended to have
high narrow sense heritability. However, narrow sense heritability for some important agronomic traits, like seed yield per plant and harvest index, were low to moderate. In general terms, the limited observations possible with the subset (ii) populations were consistent with those observed in the other subsets.

To summarise, the studies here have generated substantial comparative information about the genotypic variation and genetic compatibility among cultivated Bali accessions, var. *macrosperma* and wild accessions from Africa and Austronesia. The data suggested that genotypic variation within the cultivated Bali accessions was small compared with the wild accessions. Despite some similarities between the Bali accessions and var. *macrosperma*, those two classes of accessions clearly belong to different gene pools given the genetic barriers between them. Even so, the inheritance of some of the qualitative traits common to both classes was apparently similar. It is concluded that development of improved varieties of *V. vexillata* using the cultivated Bali type would require access to greater genotypic variation than observed here, although it would be possible (if time-consuming) to backcross in important traits from wild accessions. A more conventional breeding approach could be followed using var. *macrosperma*, but again, a concerted attempt will be required to identify wider genotypic variation than appears to be available in international collections. Finally, a taxonomic revision is required for the cultivated Bali accessions, given their strong genetic incompatibility with all the other *V. vexillata* accessions evaluated in these studies.
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Seed of the most of the accessions of *Vigna vexillata* used in my research were sourced from the Australian Native *Vigna* Germplasm Collection curated by Professor Lawn. Seed of the var. *macrosperma* accessions, CPI 15452, CPI 17457 and CPI 114171, was provided by Dr Sally Dillon of the Australian Tropical Crops & Forages Germplasm Centre. Seed of the cultivated Bali accessions was sourced from my colleague Dr Agung Karuniawan. The assistance of Mr Paul McLennan of CSIRO Davies Laboratory in growing the Bali accessions through quarantine and arranging approval of their release by AQIS is acknowledged.

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STATEMENT OF SOURCES DECLARATION
1.0 INTRODUCTION

The gap between the human population and the quantity of the food supply has already become a global threat (Ekanayake et al. 2000). According to the U.S. Census Bureau, the world population had reached approximately 6.5 billion people at May 2007 and has been estimated at approximately 6.8 billion people at May 2009. Meanwhile, FAO estimated that about 854 million people in the world were chronically undernourished in the period 2001 - 2003, and about 96% of them live in developing countries (FAO 2006). The estimated number of undernourished people had increased by 150 million in 2009 due to higher food prices (FAO 2009).

As the major source of proteins for more than two billion people worldwide, grain legumes play a crucial role in food security, as well as the sustainability of agricultural systems, particularly in developing countries (Singh and Singh 1992; Popelka et al. 2004; Kaviraj et al. 2006). In addition, the world demand for grain legumes has been increasing because vegetarian diets have become a growing trend (Kaviraj et al. 2006). Grain legumes rank second behind cereals as a source of human food and animal feed and rank third for world crop production after cereals and oilseeds (Desai et al. 1997; Popelka et al. 2004). Pulses are two to three times richer in protein than cereals (Desai et al. 1997). Most of the proteins in legumes are generally high in lysine, but low in methionine, cysteine and / or tryptophan (Desai et al. 1997; Popelka et al. 2004). However, when they are combined with cereals, legume proteins provide a balanced diet. Furthermore, pulses supply variety of alternative food for people who have limited access to animal protein (Summerfield and Lawn 1987) or for cultural or religious reasons, cannot consume it.

Vigna Savi is a genus in the family Fabaceae that can be grown successfully in difficult environments (e.g. high temperature, low rain fall, poor soil) with few economic inputs (White 1972). In addition, many Vigna species produce multiple edible products, e.g. green seeds, pods, tubers and leaves as vegetables and dry seeds as edible pulses, and these products provide subsistence farmers with a food supply throughout the growing season as well as dry seeds that are easy to store and transport (Fery 2002). The genus contains several major tropical pulse crops, including V. radiata (L.) Wilczek (mungbean), V. unguiculata (L.) Walp. (cowpea), V. mungo (black gram), V. angularis (adzuki bean), V. umbellata (rice bean) and V. aconitifolia (mat bean). The genus also includes V. vexillata which has attracted some international interest as an underutilised crop (National Research Council (NRC) 1979; Williams and Haq 2002).

\[1 \text{www.census.gov/ipc/www/popclockworld.html} \]
Interest in *V. vexillata* is due to several factors: good nutritive value of its tubers and herbage (Vanderborght 1989); a potential source of resistance genes for the breeding of cowpea (*Vigna unguiculata*) and mungbean (*Vigna radiata*) (Thotappilly et al. 1994); reputed medicinal properties (Padulosi and Ng 1990; Chifundera 1998); a forage / cover crop (Milford 1967, Vanderborght 1989; Garba and Pasquet 1998a,b); and an erosion control plant (Padulosi and Ng 1990; Garba and Pasquet 1998a,b).

To date, *V. vexillata* has not been widely cultivated. Some parts of the plant, particularly the tuberous roots and sometimes the pods and seeds, are eaten by people in some areas of the tropics, for example, in Africa (Irvine 1952; Padulosi and Ng 1990), and India (Chandel et al. 1972; Bhattacharyya et al. 1984). In Australia, the tubers are a wild food source of the Aborigines (Lawn and Cottrell 1988). Meanwhile, in Bali Province, Indonesia, the storage roots are locally sold by street vendors to tourists in the locality of Kampial in the district of Jimbaran, and are a staple in four localities in the district of Tabanan (Karuniawan et al. 2006). The tubers of the Bali cultivated plants have a light brown skin, a creamy coloured flesh inside, are usually peeled and cooked and taste like potatoes with a small groundnut aroma (Karuniawan et al. 2006). The young tubers are eaten raw in some parts of Africa (Maxted et al. 2004). The leaves are eaten as a salad in hot weather in Kenya and also eaten in Tanzania and Zaire, Africa (Maxted et al. 2004). Due to its high palatability to herbivores, the use of the foliage as a tropical forage has been reported in Africa (Maxted et al. 2004), Australia (Lawn and Cottrell 1988) and East Indonesia (Karuniawan et al. 2006).

*V. vexillata* is characterised by a significantly higher protein content in the seeds than some other *Vigna* species, e.g. *V. luteola*, *V. reticulata*, *V. oblongifolia*, *V. unguiculata dekindtiana*, *V. racemosa*, *V. reticulata* and *V. ambacensis*. For example, its seeds comprise approximately 25% of protein both in wild accessions and var. *macrosperma*, but also contain some antinutritional compounds, e.g. tannin and trypsin inhibitors, as often observed in other legumes (Marconi et al. 1997; Singh et al. 2007). The protein content of tuberous roots of *V. vexillata*, at 15%, is about three times higher than that of potato (*Solanum tuberosum*) and yam (*Dioscorea alata*) and about six times that of cassava (*Manihot esculenta*) (Chandel et al. 1972). Tubers contain per 100 g dry matter: protein 3.4 g, fat 0.2 g, carbohydrates 18.9 g, Calcium 58.5 mg, Phosphorus 88.7 mg and Iron 1.0 mg (Chandel et al. 1972). Due to its high protein content in both seeds and tubers, Yadav et al. (2003) considered *V. vexillata* as a valuable crop to combat nutritional deficiencies in the Indian sub-continent. Per 100 g dry matter, an actively growing, flowering plant contains: crude protein 20.3 g, crude fibre 26.4 g, ether extract 6.1 g, nitrogen-
free extract 37.2 g and ash 10.1 g (Milford 1967). The high carbohydrate content of *V. vexillata* tubers has even led to the suggestion that they might form a useful source of fermentable feedstock for the production of liquid ethanol fuel (Saxon 1981).

*V. vexillata* genotypes have been identified as having high levels of resistance to several cowpea insect pests (Fatokun 2002). The pubescence on the leaves, stems and pods of *V. vexillata* and the high content of para-aminophenylalanine are reported to be repulsive to major insect pests of cowpea including pod borers (*Maruca testulalis*) (Padulosi and Ng 1990) and pod sucking bugs (*Clavigralla tomentosicollis, Anoplocnemis curvipes*, and *Riptortus dentipes*) (Oghiakhe et al. 1992; Fatokun 2002). Wild accessions of *V. vexillata* also showed high levels of resistance to the parasitic plant *Striga gesnerioides* (Fatokun 2002). Anti-metabolites including trypsin inhibitors, tannins and lectins have been reported to be associated with bruchid seed weevil (*Callosobruchus maculatus*) resistance (Marconi et al. 1997). There is potential therefore for *V. vexillata* as a source of resistance traits for the improvement of cowpea (Maxted et al. 2004).

Production of mungbean and cowpea is also vulnerable to many diseases. For instance, cowpea mottle carmovirus (CPMoV) can cause grain yield loss of up to 75% (Thouvenel 1988). Even though several cowpea varieties, which have combined resistance to some viruses, e.g. cowpea yellow mosaic, blackeye cowpea mosaic, and cowpea aphid borne mosaic, have been developed (Singh et al. 2002), there is no report of a cowpea line that is known to be resistant to CPMoV (Ogundiwon et al. 2002). Meanwhile, a high level of resistance to CPMoV had been found in *V. vexillata* (Thottapilly et al. 1994), and it was reported to be controlled by a single dominant gene (Ogundiwon et al. 2002). While Karuniawan (2004) reported that cultivated *V. vexillata* from Bali province in Indonesia was susceptible to mosaic virus, some of the wild accessions may have useful disease resistance traits that could be used in breeding related crop species.

In Africa, *V. vexillata* tubers have been reported to be used as a remedy for sores and ulcers, whilst the pods are used to cure skin irritation (Maxted et al. 2004). Maceration of *V. vexillata* with several other plants was used as a treatment for the absence of lactation after child delivery in Congo (Chifundera 1998).

Generally, *V. vexillata* grows wild but occasionally it is also cultivated (Bhattacharyya et al. 1984; Karuniawan et al. 2003, 2006). In the wild, the species thrives in a wide range of conditions, e.g. in grassland and woodlands, in disturbed areas like the verge of roads and roadway cuttings (Lawn and Watkinson 2002). In Bali Province, Indonesia, the crop is
cultivated on poor and rocky soils, as well as on rich soils where it is sown after the rice harvest at the end of the rainy season (Karuniawan et al. 2006).

*V. vexillata* is also sometimes grown as a cover crop, soil improver and for green manure (Garba and Pasquet 1998a, 1998b), reflecting its capacity to fix its own nitrogen. In addition, owing to the ability to develop root nodules at an early stage, the species can be utilised to enrich the fertility of newly cultivated lands (Sasikumar and Sardana 1988). The plants grow fast and cover the ground quickly, and are thus useful for erosion control (Padulosi and Ng 1990; Garba and Pasquet 1998a, 1998b). Because of those qualities, Sasikumar and Sardana (1988) suggested that *V. vexillata* is suitable for a cover crop cum intercrop for rubber plantations. In India, Bhattacharyya et al. (1984) reported yields of *V. vexillata* tuberous roots were 7000 kg ha\(^{-1}\), which was comparable to the local sweet potato. A seed yield of 480 kg ha\(^{-1}\) was obtained from the same crop that yielded 7000 kg ha\(^{-1}\) of tuberous roots. Potential yield of tuberous roots in Bali Province, Indonesia was about 2000 to 3000 kg ha\(^{-1}\), whilst potential yield of seed was about 800-1200 kg ha\(^{-1}\) (Karuniawan et al. 2003, 2006).

Several aspects of *V. vexillata* that have been investigated include variation in agronomic, adaptive and taxonomic traits among Australian accessions (Grant et al. 2003); cytogenetic studies in wild types from Africa (Adetula et al. 2005); inheritance of selected traits in accessions of Australian and African origin (James and Lawn 1991); and genetic variation assessment of African and Australian accessions by isozyme and random amplified polymorphic DNA (RAPD) (Spinosa et al. 1998). Generally, *V. vexillata* accessions from Africa and Australia have received more research attention than accessions from South-east Asia. Even though some researchers have included some accessions from eastern Indonesia in their experiments (e.g. Karuniawan and Lawn 2007; Lawn and Watkinson 2002), the limited samples are inadequate to gain broad information for further crop improvement. Moreover, as a new crop, so far only limited use of cultivated *V. vexillata* has been reported (Bhattacharyya et al. 1984; Karuniawan et al. 2006; Asati and Yadav 2004).

To summarise, *V. vexillata* is an under-utilised legume species that appears to possess several multi-purpose attributes that make it a potentially valuable species for improving food security in village agriculture in the tropics. The present research was undertaken to explore the potential for genetic improvement of the species to develop varieties suited to cultivation. To that end, the current state of knowledge of the species is reviewed in the following chapter, with the specific objective of identifying the key areas where additional knowledge is needed to underpin future improvement research on the species.
2.0 LITERATURE REVIEW

2.1 Introduction

*V. vexillata* is considered an under-utilised legume crop by the International Plant Genetic Resources Institute (IPGRI) (Padulosi 1998). As outlined in the Introduction, the tubers and the seeds are consumed by people in several parts of the world (Padulosi and Ng 1990), but the crop is not considered a staple food, except in some localities in Bali, Indonesia (Karuniawan *et al*. 2006). However, the crop has attracted some international interest because it has characteristics of both a grain legume and of a root crop. Potentially, the species could be an important plant in the third world, both as a source of starch and protein for people, as well as a crop for marginal lands or areas that are often exposed to seasonal drought such as sub-Saharan Africa (Maxted *et al*. 2004).

This review investigates the biology, taxonomy and uses of *V. vexillata* in the context of identifying opportunities for improvement of this species. The aim is several fold: to summarise current understanding of the species as an under-utilized crop; to summarise the current status of improvement research with this species; to identify research priorities for crop improvement; and to identify suitable possible methods for improving *V. vexillata* and the major factors that might be involved in the process.

2.2 *Vigna vexillata* (L.) A. Rich.

2.2.1 Taxonomic relations

*V. vexillata* is a member of the family Fabaceae, subfamily Papilionoideae, tribe Phaseoleae DC, subtribe Phaseolinae Benth., genus *Vigna* Savi; subgenus *Plectotropis* section *Plectotropis* (Maxted *et al*. 2004).

The relationships between *Vigna* and *Phaseolus* historically have been controversial and recent taxonomic revisions of these groups have resulted in the transfer of numerous species from *Phaseolus* to *Vigna* and to other genera (Delgado-Salinas *et al*. 1993). One of the problems with the infra-generic taxonomy within the genus *Vigna* is that the majority of authors have created a classification based on small samples, which has resulted in much uncertainty and unnecessary synonymy (Maxted *et al*. 2004). However, the classification of the genus *Vigna* by Verdcourt (1970), as modified by Maréchal *et al*. (1978), is generally regarded as the accepted classification for the *Phaseolus-Vigna* complex (Maxted *et al*. 2004).

The genus *Vigna* is a large and diverse genus, and comprises about 75-80 species, distributed in tropical and subtropical regions of Africa, Asia and America and assigned to seven subgenera.
based on morphology (Maréchal et al. 1981; Maxted et al. 2004). The seven subgenera are further divided into several sections: *Vigna* (6 sections), *Plectotropis* (2 sections), *Ceratotropis* (3 sections), *Lasiospron* (1 section), *Sigmoidotropis* (5 sections), *Haydonia* (3 sections) and *Macrorhynchus* (1 section) (Maréchal 1982; Maxted et al. 2004).

*V. vexillata* is classified under section *Plectotropis* along with *V. kirkii*, whilst *V. nuda* and *V. longissima* are classified under the other section, *Pseudoliebrechtsia* (Maxted et al. 2004). Morphologically, subgenus *Plectotropis* is considered to link the African (subgenus *Vigna*) and Asian (subgenus *Ceratotropis*) species of *Vigna* (Maréchal 1982). Based on phylogenetic trees as the result of the internal transcribed sequences (ITS) of nuclear ribosomal DNA from 29 *Vigna* species, Goel et al. (2002) suggested that subgenus *Plectotropis* appeared closer to subgenus *Vigna* rather than forming a link between African and Asiatic species. In addition, that study suggested that *V. kirkii* was closer to *V. longifolia*, which is classified under subgenus *Lasiospron*, than to *V. vexillata*. A relatively distant relationship between *V. kirkii* and *V. vexillata*, classified in the same subgenus and section, was also indicated by Jaaska (1998) using isoenzyme analysis of 29 *Vigna* species.

Due to its great variability and wide distribution, many varieties and forms of *V. vexillata* are recognized, including var. *vexillata*, var. *angustifolia*, var. *davyi*, var. *dolichonema*, var. *linearis*, var. *lobatifolia*, var. *macrosperma* and var. *ovata*. These are distinguished on the basis of leaflet size and shape, pods and seed size. The species shows a wide range of variation across its distribution which makes varietal separation difficult (Garba and Pasquet 1998a; Maxted et al. 2004). Indeed, as discussed below, Garba and Pasquet (1998a) classify Australian forms of var. *angustifolia* as var. *linearis*.

Many *V. vexillata* varieties are widely distributed throughout Africa, which is one of the major centres of diversity for the species. There are significant ex situ collections from throughout its range, with the exception of var. *dolichonema*, which has been identified as a Tanzanian endemic (Maxted et al. 2004). Among the different botanical varieties of *V. vexillata*, var. *macrosperma* is the only variety that has been characterised by traits usually associated with at least partial domestication (Garba and Pasquet 1998a, 1998b). The pods, seeds and leaflets of var. *macrosperma* are larger, and the plants are more robust, than in the other varieties (Maréchal et al. 1978), attributes that are among the first that change during the process of domestication of food legumes (Smartt 1978).

*V. vexillata* var. *macrosperma* has 12-15 cm pod length and 7-9 mm pod width, large unicolour seed c. 3.5-5 mm in diameter, ovate-elliptical leaflets and most plant parts are hirsute. The
The morphological diversity of the wild Australian *V. vexillata* material is less than that found in Africa (James and Lawn 1991; Grant *et al.* 2003). Australian wild forms of *V. vexillata* are typically fine-stemmed and relatively narrow-leaved, twining or compactly-erect herbaceous plants, as are those of the nearby Indonesian islands, and Papua New Guinea. Based on the leaf shape, Australian *V. vexillata* accessions have been classified into two varieties, var. *angustifolia* (also known from Africa), and a locally described variety, var. *youngiana* (Grant *et al.* 2003). Based on morphological and isozyme similarities, Garba and Pasquet (1998a, 1998b) classify the Australian, Austronesian and SE Asian forms as *V. vexillata* var. *linearis* Craib, a variety first described in the Flora of Siam. While Garba and Pasquet (1998a, 1998b) found that *V. vexillata* from South East Asia and Australia was morphologically close to var. *angustifolia* from West Africa, isozyme patterns for accessions from Australia were clearly distinguishable from the West African var. *angustifolia*. Even so, those Australian accessions were apparently more closely related to African forms of var. *angustifolia* than to three Indian accessions.

Cultivated *V. vexillata* has been found in Bali and Timor, Indonesia and several accessions were collected from two regions, Jimbaran and Tabanan, in Bali (Karuniawan *et al.* 2003; Karuniawan *et al.* 2006). These accessions exhibit large leaflets, pods and seeds, the pods are non-dehiscent and the seeds are uniform in colour. Seed samples were sent to the National Botanic Garden of Belgium, and based on the plant and seed morphologies, it was confirmed as *V. vexillata* (Karuniawan *et al.* 2006). Eight of these accessions were successfully introduced through quarantine into Australia, and were available for study in the context of this thesis. Based on their large seed size, and broad leaflet size, these accessions are broadly consistent with the key descriptors used by Maréchal *et al.* (1978) to define the cultivated variety *macrosperma*. However, their precise taxonomy remains to be clarified.

### 2.2.2 Origin, domestication, and distribution

*V. vexillata*, sometimes known as “wild cowpea”, is considered to be closely related to *V. unguiculata* (L.) Walp. (cowpea) (Delgado-Salinas *et al.* 1993; Jaaska 1999; Bisht *et al.* 2005). It is a pan tropical, widely distributed species, found throughout the tropics and subtropics of Africa, Asia, and Australia (Verdcourt 1970; Lawn and Cottrell 1988; Garba and Pasquet 1998a, 1998b). Two centres of intra-specific variability of *V. vexillata* can be recognised, one in Africa (from Tanzania to South Africa) and the other in Asia (from Yunnan, China to Indonesia).
including northern and eastern Australia (Maréchal et al. 1981; Wong 1997; Garba and Pasquet 1998b). It most probably originated in the Old World tropics, although collections have been made from the New World tropics.

In Africa, *V. vexillata* is generally found between 1 and 2300 m altitude, except for var. *vexillata*, which is found as high as 3860 m altitude (Maxted et al. 2004). In Australia, *V. vexillata* is restricted to the higher rainfall (> 700-800 mm) isohyet zones of the tropics and subtropics (Lawn and Cottrell 1988). In India, *V. vexillata* grows wild in western Ghats, central Peninsular hills, western Himalayas and Meghalaya (Bisht et al. 2005; Vimala and Nambisan 2005). Meanwhile in Indonesia, *V. vexillata* can be found at 50-600 m altitude (in Bali) and 400-500 m altitude (in West Timor) (Karuniawan et al. 2006). Many herbarium samples of wild *V. vexillata* from East Indonesia are shown at the Bogor Herbarium, Indonesia, an indication that wild *V. vexillata* is widely distributed in East Indonesia. On Java Island, this species can be found only in the region south of Bandung (Karuniawan et al. 2006).

After Poaceae, Fabaceae is the most domesticated family of plants, but the proportion of the species that has been domesticated varies greatly between the families (Evans 1993). Within the genus *Vigna*, according to Evans (1993), *V. radiata* (L.) Wilczek was reported to be domesticated in Madhya Pradesh 3700 BP, whilst *V. unguiculata* (L.) Walp. was domesticated in Kintampo, West Africa 3400 BP. However, where *V. unguiculata* (L.) Walp. was first domesticated is still uncertain and different centres of diversity and origin of the cowpea have been proposed, e.g. northeast Africa (Pasquet 2000), west Africa (Vaillancourt and Weeden 1992), and eastern and southern Africa (Coulibaly et al. 2002). Meanwhile, there is no exact information about when and where *V. vexillata* was first grown as a crop. As noted previously, it is currently grown as a crop in Bali and Timor, Indonesia (Karuniawan et al. 2006) and India (Asati and Yadav 2004).

In addition to the documented reports noted above, Dr Rémy Pasquet (Personal communication to RJ Lawn, 2009) indicated that he has seen material in Sri Lanka similar to the cultivated *V. vexillata* from Bali described by Karuniawan et al. (2006). It is possible, given past cultural ties between southern India, Sri Lanka and Bali, that similar forms of *V. vexillata* to that found in Bali are also grown in parts of tropical India.

### 2.2.3 Morphology and biology

*V. vexillata* is usually a perennial herbaceous plant with climbing or trailing stems up to 6 m long, with sparse to dense brownish hairs, a cylindrical stem, with erect and divergent leaflets (Pienaar and Kok 1991; Wong 1997; Karuniawan et al. 2006). James and Lawn (1991)
reported there are two basal branching habits in this species in Australia. First, a twining habit which has branches with internodes of approximately equal length and emerging at an acute angle from the main stem ('twining' habit). Second, a 'rosette crown' habit has branches with an initial short internode, arising almost horizontally from the base of the plant. Generally the rosette trait was associated with an erect plant type with shortened internodes (James and Lawn 1991; Grant et al. 2003). James and Lawn (1991) suggested the rosette crown habit might be more amenable to mechanised harvesting of seed if the crop were developed for that purpose.

The stems often exhibit anthocyanin pigmentation. In some accessions, anthocyanin pigmentation extends throughout the mature stem and into the peduncle and pod tissue. In other accessions, pigmentation is limited to areas adjacent to stem nodes (James and Lawn 1991; Grant et al. 2003). Stem hairs are broad-based and ferruginous (Maxted et al. 2004).

Leaves in *V. vexillata* are commonly trifoliolate with a few unifoliate accessions (Maxted et al. 2004). Leaflet shape is narrow-lanceolate to broad ovate, 3-16 cm long, 5-15 mm wide, with apex acute to acuminate, base rounded, cuneate or truncate, usually entire but rarely slightly lobed, and silky hairs on both surfaces (James and Lawn 1991; Wong 1997). Petioles are 1.5-11.5 cm long, whilst the stipules are lanceolate with cordate bases, mostly 0.5-1.3 mm long (Wong 1997). Although there is some variation for leaf size and shape, all of the Australian and West Timor Indonesia collections of wild *V. vexillata* are characteristically more gracile and narrow-leaved than some African and south Asian forms of the species (Lawn and Watkinson 2002), which are large-leaved, thick stemmed, scrambling vines.

Flowers of *V. vexillata* may vary in colour from yellow, pink to purple (Wong 1997; Maxted et al. 2004), with an asymmetric shape due to a keel curved to the left and a unilateral pocket facing the tip of the style (Baudoin and Maréchal 1985). The keel morphology is one of the key traits to distinguish *V. vexillata* from *V. unguiculata* (Barba and Pasquet 1998a, 1998b). Morphological data of *V. vexillata* flowers are as follows: inflorescences are 2-6 flowered; axillary racemes; peduncles 4.5-36 cm long; pedicel 1-2 mm long; calyx tubular 5-7 mm long (Wong 1997). Pods are linear-cylindrical, 4-15 cm x 2.5-9 mm, 10-18 seeded, with brown bristly hairs (Wong 1997). Seeds of *V. vexillata* are globose to oblong reniform, 2.5-5 mm x 2-5 mm, plain brownish-green to black or dark red with black speckles (James and Lawn 1991; Wong 1997).

The plant usually produces a large, fusiform, edible, tuberous root. However, two tubers or branched tubers are also observed in some plants (Chandel et al. 1972). Tubers are 5-11 cm long, 1.5-2.0 cm broad and they have an easily peeled skin (Vimala and Nambisan 2005). Large variation in tuber size and shape was observed in plants in the field, but the variation was less
apparent when different accessions were cultivated in similar soil or grown in similar potting media in the glasshouse (Lawn and Watkinson 2002).

*V. vexillata* is a self-pollinated species and is usually propagated by seed, but it is also possible to propagate by stem cuttings. Most accessions exhibit hypogeal germination, but some accessions are reported to have epigeal germination (Vanderborght 1989). Depending on variety and seed source, seed takes about 3-10 days to germinate.

Most *Vigna* species have a diploid chromosome number of 2n=22, with a few 2n=20 species (Maxted *et al.* 2004). Lavania and Lavania (1982) used the Giemsa C-Banding technique to study somatic chromosome banding patterns of eight important pulse crops, including *V. radiata* (2n=22) and *V. unguiculata* (2n=22). Adetula (2006) also found that *V. unguiculata* has 11 pairs of chromosomes (2n=22), whilst the number of chromosomes in *V. vexillata* was identified as 11 pairs (2n=22) (Adetula *et al.* 2005). To analyse the genomic organization, variability and evolution within species of *Vigna*, Galasso *et al.* (1997) investigated the genomic organization and distribution of Ty1-copia type retro-transposons in seven different species and subspecies of *Vigna*, including *V. vexillata*, and five different species from related legume genera (*Glycine*, *Phaseolus*, *Pisum*, *Vicia*, and *Cicer*). All the subspecies of *V. unguiculata* (section Catjang and section Vigna) and *V. vexillata* showed minor within-group variations and major differences with the related legume genera in terms of band characteristics.

### 2.3 Varietal improvement through plant breeding

A pre-requisite for an effective plant breeding program aimed at genetic improvement of *V. vexillata* cultivars for food crop production is that breeders have access to a wide range of genotypes from which to choose parental types with desirable attributes. Only through the ability to identify and hybridise parental types with complementary desirable traits can hybrid plants combining the desired elements of both parents be developed and selected.

#### 2.3.1 *V. vexillata* germplasm resources

A brief overview of the germplasm resources of *V. vexillata* in key international and national seed banks is summarised in Table 2.1. The largest *Vigna* germplasm collection, mainly of African wild *Vigna*, is held at the International of Tropical Agriculture (IITA), Ibadan, Nigeria. This organization holds the CGIAR world mandate to conserve *V. unguiculata* (cowpea), *V. subterranea* (bambara groundnut) and their relatives (Maxted 2004). Other genebanks that hold accessions of *Vigna* within the CGIAR system include ILRI, CIAT, AVRDC (mostly Asiatic *Vigna* spp) and ICARDA. The total number of *Vigna* accessions held under the CGIAR system at March 2009 was 32,003. Of these, only 466 were *V. vexillata*. In contrast, the number of *V.*
unguiculata accessions conserved within the CGIAR system was 18,254. Meanwhile, as at March 2009, the number of *V. vexillata* accessions held in the IITA genebank was 187. There are no explicitly-listed cultivated accessions in the IITA collection, but there are 4 var. macrosperma accessions (TVNu 64, TVNu 240, TVNu 719 and TVNu 1616), two of which were listed as coming from Africa. According to Maxted et al. (2004), further collection is a priority for this variety.

Table 2.1 Numbers of accessions of *V. vexillata* and related species in key international and national germplasm collections.

<table>
<thead>
<tr>
<th>Institution</th>
<th>All <em>Vigna</em> spp</th>
<th><em>V. vexillata</em></th>
<th>Cultivated</th>
<th>var. macrosperma</th>
</tr>
</thead>
<tbody>
<tr>
<td>IITA <em>a</em></td>
<td>19,613</td>
<td>187</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>CGIAR <em>b</em></td>
<td>32,003</td>
<td>466</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>USDA <em>d</em></td>
<td>13,141</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AusTRCFC <em>e</em></td>
<td>2,985</td>
<td>186</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

* http://genebank.iita.org/browse/default.aspx
* http://www.singer.cgiar.org
* http://www.ars-grin.gov

The largest *Vigna* collection outside the CGIAR system is held in the Phaseolinae genebank at the National Botanical Gardens of Belgium (Jardin Botanique National de Belgique) at Meise, Brussels. As at March 2009, the total number of accessions was 872, of which c. 90% were wild species. 120 accessions of *V. vexillata* are held at Meise, with only two accessions of var. macrosperma (NI 339 and NI 111). NI 339, from Costa Rica, is the accession used by Maréchal et al. (1978) to describe the variety. Only three of the *V. vexillata* accessions at Meise (NI 1857, 1858 and 1859) are explicitly described cultivated forms, and all were large-seeded accessions collected from Bali, Indonesia by Karuniawan et al. (2006).

The United States Department of Agriculture (USDA) also holds germplasm of several *Vigna* species. At May 2007, of a total 13,141 accessions, 7,107 accessions were *V. unguiculata* and only 15 accessions were *V. vexillata*. In Australia, the Australian Native *Vigna* Collection included a set of 77 wild *V. vexillata* accessions collected from four states in Australia, mostly (c. 82%) from Queensland (Lawn and Watkinson 2002). A subset of that collection was duplicated at the Australian Tropical Crops & Forages Collection (AusTRCFC) at Biloela (R.J. Lawn, personal communication, 2009). There are a further c. 186 exotic accessions of *V. vexillata* in the Biloela collection, mostly wild accessions from Africa, introduced as potential pasture legume plants. Only one accession, CPI 69030, is explicitly listed as var. macrosperma.
To summarise, the number of *V. vexillata* accessions in national and international seed collections is small relative to those of related species like cowpea (Table 2.1). The overwhelming majority are wild accessions, with very few accessions of *V. vexillata* either explicitly described as “cultivated” or as var. *macrosperma*. The latter variety was considered to be at least a semi-cultivated variety by Garba and Pasquet (1998b) although it is described as a “weedy” type in the National Botanical Garden of Belgium database and as a “wild” type in the IITA database. While it is likely that there are small numbers of accessions in other national or regional seed collections, it is also likely that there is a degree of duplication of accessions among the major collections.

For example, research into the records of the Australian Commonwealth Plant Introduction database and the online databases of IITA and the Jardin Botanique National de Belgique has shown that the sole accession of *V. vexillata* var. *macrosperma* listed in the Australian Tropical Crops and Forages Collection database is duplicated in the Australian collection as well as the IITA and Meise collections (R.J. Lawn, personal communication, 2009). Further, the evidence that this accession is a “wild” or “weedy” type as listed in all three collections is questionable. The putative duplicate accessions are listed in Table 2.2.

It appears that there are at least four accessions of *V. vexillata* var. *macросperma* in the AusTRCFC seed collection, but all four may be duplicates (Table 2.2). The first accession, CPI 15452 (JFM 134), was provided by the Agricultural Research Office at Malakal, Sudan, and entered Australia on 3 December 1951. The second accession, CPI 17457 (JFM 174), was provided by the Ministry of Agriculture at Wad Medani, Sudan. The local name was given as “Babun”. It entered Australia on 1 October 1952. At the time, both Malakal and Wad Medani were the locations for major agricultural development projects led by the British (both remain important agricultural centres in 2009). The third accession, CPI 69030, provided by IITA under the accession code TVNu 64, was received at CSIRO Brisbane in April 1975. The fourth accession, CPI 114171 was introduced from the National Botanical Garden of Belgium as NI 111 by the Queensland Department of Primary Industries and Fisheries on August 1987. Interestingly, both TVNu 64 and NI 111, and thus CPI 69030 and CPI 11471, trace directly to the original accession CPI 15452 received from the Sudan by CSIRO Australia. Whereas the original accession had arrived as part of consignment of legume cultivars then being evaluated in the Sudan, however, NI 111 had been labelled as a “weedy type” at Meise and as a “wild type” at IITA. These descriptions are retained at AusTRCFC for CPI 69030 and CPI 11471.
Table 2.2 Accessions of *V. vexillata* var. *macrosperma* duplicated in several seed collections.
(Source: R.J. Lawn, personal communication, 2009).

<table>
<thead>
<tr>
<th>Institution (Accession Number)</th>
<th>Provenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AusTRCF (CPI 15452)</td>
<td>Agricultural Research Office, Malakal, Sudan. Forwarded to CSIRO Plant Industry Australia in 1951, as <em>V. vexillata</em>, along with numerous legume crop cultivars then being evaluated in the Sudan.</td>
</tr>
<tr>
<td>AusTRCF (CPI 17457)</td>
<td>Ministry of Agriculture, Wad Medani, Sudan. Forwarded to CSIRO Plant Industry Australia in 1952, along with numerous legume crop cultivars then being evaluated in the Sudan.</td>
</tr>
<tr>
<td>Jar. Bot. Nat. Belg. (NI111)</td>
<td>Received from CSIRO Australia as CPI 15452. Identified as var. <em>macrosperma</em>. Given local number NI 111. Described as “weedy type”.</td>
</tr>
<tr>
<td>IITA (TVNu 64)</td>
<td>Received from CSIRO Canberra as CPI 15452 (possibly via Meise, since it is also recognized as NI111). Described as “wild type”.</td>
</tr>
<tr>
<td>AusTRCF (CPI 69030)</td>
<td>Received from IITA in 1975 as “var. <em>macrosperma</em> TVNu 64, wild type”.</td>
</tr>
<tr>
<td>AusTRCF (CPI 114171)</td>
<td>Received from Jar. Bot. Nat. Belg. in 1987 as “var. <em>macrosperma</em> NI111”. Entered as “wild type”.</td>
</tr>
</tbody>
</table>

In 2008-2009, seeds from CPI 15452, CPI 17457, CPI 69030 and CPI 114171 were grown out in Townsville to test the suggestion that all may be duplicates (R.J. Lawn, personal communication, 2009). The four accessions were morphologically and phenologically indistinguishable. Further, all four accessions shared attributes consistent with cultivated origin (thick stems, large leaves, robust viny bush habit, non-dehiscent pods). The fact that CPI 15452, CPI 69030 and CPI 114171 are the same would be expected (Table 2.2). The fact that the second accession from Sudan, CPI 17457, obtained from a different research station, appears to be the same as CPI 15452, is consistent with it being a cultivated plant being evaluated in Sudan at that time.

Interestingly, TVNu 64 has been supplied to several researchers as part of a subset of *V. vexillata* accessions that have been evaluated for various purposes (e.g. Jackai et al. 1996; Marconi et al. 1997; Spinosa et al. 1998). Its provenance is variously listed as “Australia” or “unknown”. Despite successful identification of its pre-Australian provenance as “Sudan”, the origin of this accession remains unclear. Few if any of the other crops sent to Australia from the
two research stations in Sudan (Table 2.2) were indigenous cultivars. Given that the original seed used by Maréchal et al. (1978) to describe var. macrosperma was sourced from Costa Rica, a New World origin for CPI 15452 (= TVNu 64 = NI 111) is possible, although two other var. macrosperma accessions in the IITA seed bank (Table 2.1) were sourced from Africa.

Presumably, the limited numbers of accessions of *V. vexillata* currently available in *Vigna* germplasm collections (Table 2.1) reflects the fact that a key reason for collecting the species has been to augment the germplasm resources available for varietal improvement of more important species like cowpea and mungbean. Likewise, the very limited numbers of cultivated or semi-cultivated accessions of *V. vexillata* presumably reflect the limited extent that the species has been cultivated.

### 2.3.2 Evolutionary processes during domestication

Domestication can be defined as “a selection process conducted by humans, consciously or unconsciously, to adapt plants and animals to cultivation or rearing and utilization by humans, whether as farmers or consumers” (Gepts 2002; Gepts and Papa 2002; Gepts 2004).

According to Evans (1993), domestication of plants involves three elements. First, it refers to genetic changes in plants which did not occur as a result of various environmental conditions. Secondly, these genetic changes occurred in a series of evolutionary processes. Thirdly, these changes often, but not always, began with cultivation (Ladizinsky 1985; Evans 1993). In addition, Gepts (2004) suggested that domestication will proceed only if the conditions are satisfied in the three areas (human, plant and environment) and that there are interactions between these three aspects.

One of the most important determinants in the evolution of crop plants is the level of genetic diversity within the domesticated gene pool, particularly with the reference to the wild ancestral gene pool (Gepts and Papa 2002). Genetic diversity is important as a necessary condition to further evolution, not only in the wild but also in breeding programs (Gepts and Papa 2002), because the domestication of plants mostly initially involved the selection of genotypes from wild populations that exhibited attributes that were useful to mankind (Sleper and Poehlman 2006). Rates of evolutionary change itself can obviously be accelerated by selection under domestication (Smartt 1978).

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1 Commonwealth Plant Introduction Database records, CD ROM.
Various researchers have conducted comparative studies of the processes and patterns of domestication in cereals and legumes, the two biggest domesticated family groups (e.g. Zohary 1989; Abbo et al. 2009). In addition, numerous studies of domestication processes and evolution have been conducted, both in the cereals (e.g. Harlan et al. 1973; De Wet 1981; Salamini et al. 2002; Paterson et al. 2003) and the legumes (e.g. Zohary and Hopf 1973; Smartt 1978; Kislev and Bar-Yosef 1988). Generally, the cereals have received more comprehensive focus than the legumes. Interestingly, there has been considerable similarity between the way that the cereals and legumes, while quite different plant species, both have been changed through the process of domestication (Donald and Hamblin 1984). The result is remarkable similarity between species in terms of which wild traits have been eliminated and the cultivated ones that have replaced them.

There are many plant traits which are changed by domestication, such as seed retention, reduced seed coat thickness, greater size of the harvested organs, correlative changes in size, polyploidy and DNA content, more rapid and uniform germination, synchronization of flowering and maturation, life cycle and breeding system, and the loss of bitter and toxic substances (Smartt 1978; Donald and Hamblin 1984; Ladizinsky 1985; Smartt and Hymowitz 1985; Evans 1993; Ladizinsky 1998; Hancock 2004). While the most highly evolved forms are not necessarily the most suitable for all agricultural systems, in general the important crops, e.g. soybean, groundnut and cowpea, exhibit most of these traits (Smartt 1986).

The following traits, which are frequently changed by domestication, were consistently observed in the cultivated accessions of *V. vexillata* described by Karuniawan et al. (2006) and the semi-cultivated var. *macrosperma* accessions variously described by Maréchal et al. (1978), Garba and Pasquet (1998a, 1998b) and Maxted (2004):

(i) **Seed retention.** Karuniawan et al. (2006) reported no pod dehiscence in the cultivated form of *V. vexillata* from Bali. This trait is one of the earliest and most consistent differences between domesticated and wild types in legumes (Evans 1993). Ladizinsky (1979) reported that pod indehiscence in lentil was controlled by a single recessive gene.

(ii) **Greater size of seed.** *V. vexillata* var. *macrosperma* (TVNu 64, TVNu 72 and TVNu 73) have bigger seed (c. 4.9 g per 100 seeds weight) compared to its wild relatives (c. 2.1 g per 100 seeds weight) (Marconi et al. 1997). Likewise, the cultivated accessions described by Karuniawan et al. (2006) had large seed.

(iii) **Change in non-harvested organs, such as leaflet shape and size.** *V. vexillata* var. *macrosperma* accessions have larger leaflets (Garba and Pasquet 1998a, 1998b), compared to wild accessions from Austronesia and Africa, most of which have lanceolate shape as the
dominant form (James and Lawn 1991; Grant et al. 2003). However, there are some wild African forms with large, broad ovate leaflets (James and Lawn 1991).

(iv) Loss of seed dormancy. Occasionally, seed dormancy is caused either by hard seed coats (Ladizinsky 1985) or by an intrinsic requirement for a period of post-harvest maturation. No scarification was required for the Bali cultivated accessions of *V. vexillata* to germinate rapidly (Karuniawan et al. 2006). In contrast, scarification was needed to obtain germination from wild accessions (James and Lawn 1991; Grant et al. 2003; Karuniawan and Lawn 2007). Ladizinsky (1987) argued that changes of seed dormancy in lentil occurred even before cultivation took place.

(v) Loss of bitter and toxic substances. Much lower levels of tannin were observed in var. *macropersoma* (TVNu 64, TVNu 72 and TVNu 73) (c. 3.6 g kg$^{-1}$) compared to its wild relatives (c. 20.2 g kg$^{-1}$) (Marconi et al. 1997).

Even so, the process of domestication of *V. vexillata* has only been partial at best (Garba and Pasquet 1998a, 1998b; Karuniawan et al. 2006), with some traits that are more typically found in wild progenitors still observed in cultivated *V. vexillata* accessions. For instance, the cultivated accessions described by Karuniawan et al. (2006) were viny in habit, whereas most domesticated legumes are compact bushes (Smartt 1978).

Knowledge of evolutionary relationships between crops and their wild progenitors is important, not only in plant conservation but also in plant improvement (Henry 2005; Abbo et al. 2009). Evolutionary relations are important in assessing the practicality of breeding objectives and in evaluating different approaches to breeding (Smartt 1986). The determination of relations between wild and cultivated forms is greatly facilitated by experimental studies, e.g. experiments based on comparative morphological observation and hybridisation (Smartt 1978).

The use of wild relatives as sources of new traits is well established in breeding programs for crop improvement, but the efficiency with which wild germplasm is utilised for introducing specific characters into elite cultivars varies greatly (Bisht et al. 2005). Genetic barriers often occur in hybridisation between a crop and its wild relatives, inhibiting the ease with which fertile progeny combining desired attributes can be recovered. Even so, it is evident that the use of wild relatives can play an important role in the development some crop species particularly in the improvement of resistance to environmental stresses (Zamir 2001).

Less extensive research and the information assembled on the genetic variability in the primary gene pool of cultivated *V. vexillata* as outlined in the previous section, appear to be limitations to its development as a useful crop. Therefore, it is likely that any serious program to develop
improved cultivars of *V. vexillata* will need to draw heavily on the relatively larger pool of weedy or even wild accessions of the species.

### 2.3.3 Barriers to exploiting wild relatives in plant breeding

Plant breeding can be defined as “the art and science of improving the heredity of plants for the benefit of humankind” (Sleper and Poehlman 2006). The aim of plant breeding has always been the improvement of existing cultivars with valued attributes by adding to them variations of importance found in other, less-valued lines, to create elite cultivars (Ladizinsky 1998). The strategy of plant breeding involves several steps, and the three first steps identified by Sleper and Poehlman (2006) are identifying morphological, physiological, and pathological traits in cultivated plants that are important for crop improvement; searching out genes that encode useful traits from cultivated species and their close relatives; and combining genes of the useful traits into cultivated plants using conventional breeding or new biotechnology techniques.

The sexual hybridisation process starts with the germination of pollen of one plant on the stigma of another plant, with the basic aim of combining the desirable traits from two different parent plant genotypes into the one genotype (Ladizinsky 1998). This technique has been used successfully to achieve many different objectives e.g. to improve yield potential and stability, to transfer biotic and abiotic resistances, and to improve product quality (Sleper and Poehlman 2006; Timko and Singh 2008).

According to Ladizinsky (1998), the successful introgression of specific genes to one species from a related species whether wild or cultivated by hybridisation depends upon: cross-compatibility, production of hybrid seeds, normal development of the F₁ hybrids, a reliable amount of seed production and no hybrid breakdown in the segregating generations. In relation to introgression by hybridisation, in order to provide a genetic perspective and genetic focus for cultivated plants, Harlan and De Wet (1971) proposed three gene pool concepts:

(i) **Primary gene pool (GP-1).** Among members of this gene pool, crossing is easy and the hybrids are generally fertile with normal chromosome pairing;

(ii) **Secondary gene pool (GP-2).** Gene transfer between the species in GP-1 and the species from this gene pool is possible, but the hybrids tend to be sterile or weak and the chromosomes pair poorly or even not at all;

(iii) **Tertiary gene pool (GP-3).** Crossing between the species in GP-1 and the species from its tertiary gene pool is still possible, but the hybrids tend to be non-viable or completely sterile. Gene transfer is almost impossible, unless another technique, such as embryo culture, can be performed to obtain viable hybrids.
Timko and Singh (2008) suggested that *V. vexillata* may constitute a tertiary gene pool for cowpea. Singh et al. (2007) modified the Harlan and De Wet (1971) concept by proposing a quaternary gene pool (GP-4), where the introgression of specific genes from this gene pool into a domesticated species may be feasible using somatic hybridisation and genetic transformation technology.

Inter-specific hybridisations within the genus *Vigna* have been attempted by various researchers for different reasons. Through inter-specific hybridisation, potentially useful adaptive traits from one species can be transferred to other species within the same genus. Hybridisation within genus *Vigna* has been done generally to transfer genetic resistance to pests and diseases. For instance, Chen et al. (1989) conducted hybridisation between *V. radiata* x *V. glabrescens* in order to transfer genetic resistance to pests from *V. glabrescens* to *V. radiata*. Hybridisation between *V. unguiculata* x *V. vexillata* has been attempted by Gomathinayagam et al. (1998) and Fatokun (2002) in order to transfer to *V. unguiculata* genetic resistance to diseases (cowpea aphid borne mosaic virus and cowpea yellow mosaic virus) and to pests (*Callosobruchus maculatus*, *Maruca vitrata*, and *Striga gesnerioides*) from *V. vexillata*. Only Gomathinayagam et al. (1998) reported a successful attempt to obtain hybrid plants between *V. vexillata* and *V. unguiculata*, but so far there is no further information about those hybrids.

James et al. (1999) conducted hybridisation between *V. radiata* spp. *sublobata* x *V. mungo*. The lack of cross fertility between those two accessions confirmed the inappropriateness of the original attribution of the Australian accessions of *V. radiata* spp. *sublobata* to *Phaseolus mungo*. Hybridisation involving four indigenous species of *Vigna* in Australia (*V. vexillata*, *V. lanceolata*, *V. luteola* and *V. marina*) and two sub-species of mungbean (*V. radiata* spp. *sublobata* and *V. radiata* spp. *radiata*) was done by Palmer et al. (2002) with limited success. Hybridisations within the genus *Vigna*, especially inter-specific hybridisations, frequently face problems of genetic barriers. Most of the inter-specific hybridisations fail to produce hybrids because pods fail to grow and abscise prematurely.

There are two mechanisms whereby genetic barriers express: pre-zygotic and post-zygotic barriers (Pickersgill 1993; Ladizinsky 1998; Rieseberg and Carney 1998). Pre-zygotic barriers often relate to reproductive isolation, for instance pollen-pistil incompatibility (Pickersgill 1993; Ladizinsky 1998). Other causes include habitat, temporal isolation, and gametic incompatibility (Niklas 1997; Rieseberg and Carney 1998). With post-zygotic barriers, fertilisation followed by a zygote forming occur normally, but the barrier to introgression may take place at various
stages of hybrid development, which include all stages of embryo development, seed maturation and germination, and normal growth and fertility of the hybrid plants (Ladizinsky 1998).

The most common post-zygotic barriers include hybrid embryo abortion, hybrid weakness/inviability and hybrid sterility, chromosome elimination, and hybrid breakdown (Ladizinsky 1998; Rieseberg and Carney 1998). Hybrid weakness/inviability and hybrid sterility are often caused by meiotic irregularities. The term hybrid breakdown was used when the first generation (F₁) of hybrids are robust and fertile but the later generations are weak or non-viable (Ladizinsky 1998; Rieseberg and Carney 1998). Additionally, Pickersgill (1993) also suggested that post-zygotic barriers in the development of a viable seed and abnormalities in the development of hybrid seeds may be caused by maternal tissue and endosperm development, rather than the embryo itself. In a recent study of hybrid breakdown mechanisms in rice, Yamamoto et al. (2007) reported that hybrid breakdown was induced by the interaction of two recessive genes (hbd2 in rice cultivar Habataki and hbd3 in cultivar Koshihikari).

Intra-specific hybridisation of V. vexillata was successfully done by James and Lawn (1991) to investigate the inheritance of selected traits among wild accessions from Australia and Africa. This type of hybridisation also was done by Ogundiwin et al. (2002) to identify lines among V. vexillata germplasm that are susceptible to cowpea mottle carmovirus (CPMoV) and to explain the inheritance of resistance to the virus in V. vexillata. The study showed that resistance to CPMoV in V. vexillata is heritable and is controlled by a dominant gene.

Due to limitations to transferring useful genes caused by species barriers using conventional hybridisation techniques, several new technologies, such as tissue culture and embryo rescue, biochemical and molecular marker technologies, molecular cloning and genetic transformation have been developed to overcome the problem in conventional breeding (Chawla 2002). Nonetheless, embryo culture remains the first choice for overcoming genetic barrier problems when hybrid seeds are formed but fail to grow. Although this technology has not always been successful in Vigna inter-specific hybridisation, several researchers have successfully used it to obtain hybrids from Vigna inter-specific hybridisation (Chen et al. 1989; Gomathinayagam et al. 1998; Palmer et al. 2002).

2.3 Conclusions and implications for thesis research objectives

The preceding review has highlighted that V. vexillata is an underutilized legume species that has potential for development in the future as a crop plant. Almost every part of the plant can be used, and for a range of different purposes. Firstly, as a crop in its own right, the shoots, green pods, seeds, and tubers of V. vexillata provide a potential alternative protein source for regions
with low income and limited access to animal protein. Secondly, as part of an agriculturally important genus containing several important cultivated crop species, *V. vexillata* represents an important germplasm resource of potential use for crop improvement in other *Vigna* species, especially in relation to resistance to pests and diseases. Thirdly, because of its high protein content, *V. vexillata* has potential as a forage species suitable for grazing or for cutting for animal feed. Fourthly, because of its vegetatively-vigorous, scrambling habit, *V. vexillata* has potential as a nitrogen-fixing cover crop for intercropping with tree species or for the rehabilitation of marginal land. The latter role may be facilitated by the fact that the species is tuberous rooted and is a short lived perennial.

To date, most of the (very limited) research emphasis on the utilisation of this species is as source of genetic resistance to the pests and diseases that constrain cowpea and mungbean production. There has been some limited research and varietal development for forage varieties for grazing animal production, mainly in Africa and Australia.

As was outlined in the review, there is very limited published knowledge about the cultivation of this species, or the varieties that have been utilized to date. It is clear that wild plants, usually the tubers but sometimes the seed, have been collected opportunistically as a food source in some parts of the tropics. There is evidence that in village agriculture in several tropical areas, semi-domesticated varieties have been used as a source of both tubers and seed for food. In the few instances where detailed information is available, the cultivated forms appear to be large-seeded, broad-leaved types, similar to var. *macrosperna*. Surprisingly, however, there are remarkably few accessions of either known cultivated varieties or of var. *macrosperna* available in international germplasm repositories, or in those national repositories for which information is readily available.

As a prerequisite to future breeding research to underpin varietal improvement of cultivated *V. vexillata*, it was considered that an important first objective of this thesis would be to examine the genotypic diversity of known accessions of cultivated *V. vexillata* from Bali and to compare the variation in selected traits with that of a known accession of var. *macrosperna*, and in wild accessions of Austronesian and African origin. Given the partial domestication of the species, it was considered important that some emphasis be placed on a comparison of wild traits and those typically associated with, or useful in, domesticated plants. The second key objective of this thesis would be to explore the genetic compatibility of the cultivated *V. vexillata* accessions from Bali with themselves and with var. *macrosperna*, and the genetic compatibility between these two types and wild accessions of Austronesian and African origin. Where possible, the inheritance of qualitative traits and the heritability of quantitative traits would be explored.
Again, the emphasis would be placed on cultivated vs. wild traits, in order to generate information potentially relevant to future breeding programs. If any genetic incompatibilities were observed, it was considered important that the nature of the incompatibility be described, again in order to assist future breeding and genetic studies on *V. vexillata*.

To address the first objective outlined above, it was decided that an experiment be designed aimed at documenting the genotypic diversity among the available Bali cultivated accessions, the accession of var. *macroesperma* putatively sourced from the Sudan in northern Africa, and several wild accessions of *V. vexillata* from Africa, Australia and Indonesia. The aim of the experiment would be to measure a range of cultivated and wild type morphological, agronomic and phenological traits likely to be of agronomic, adaptive or taxonomic importance. A second set of experiments was designed to address the second objective of determining the level of crossability within the Bali cultivated accessions; between those accessions with the other accessions, both the African cultivated accession and the wild accession from Africa, Australia and Indonesia; between the African cultivated accession and wild accessions from Africa and Australia; and within the wild accessions. In a third set of experiments, populations involving hybrids between cultivated and wild type accessions would be used to exploit the inheritance of specific traits.

The conduct of these experiments and their outcomes are described in the following chapters.