Improving mango breeding efficiency through improved pollen storage, fruit retention and understanding of the heritability of quantitative tree architectural traits

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To my family:

My bliss & muse

STATEMENT OF CONTRIBUTION OF OTHERS

- All data chapters of the thesis include research work in collaboration with my associate advisors Dr Ian Bally and Dr Tony Page.
- Data collection for Chapter 4 was done in collaboration with Cheryldene Maddox, Department of Agriculture and Fisheries (DAF), Mareeba, Queensland.
- Statistical data analysis for Chapter 4 was carried out by Dr Joanne De Faveri, biometrician, Department of Agriculture and Fisheries (DAF), Mareeba, Queensland.
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GENERAL ABSTRACT

Mango is one of the most important tropical/subtropical fruit crops (Chapman, 2000), with a world production of 45.225 million tonnes (FAOSTAT, 2014b). Worldwide mango breeding programs aim to develop novel cultivars that fulfill the needs of modern mango industries (Campbell and Zill, 2009). The conventional breeding technique involves crosses between selected parents and evaluating the resulting progenies for desirable characters. A lack of pollen availability, poor selection, a low rate of fruit set and retention are major problems in conventional breeding for mango crop improvement (Iyer and Dinesh, 1997; Iyer and Degani, 1997; Roizman, 1986). The aim of this research is to address these issues and to improve the efficiency of traditional breeding techniques. This research focusses on higher breeding efficiency by extending pollen storage, and investigates the improved methods of assessing tree vigour via an understanding of the heritability of quantitative traits related to dwarf phenotypes. Furthermore, this work explores enhanced hybridised fruit set and retention through application of plant growth regulators (PGRs) and micronutrients.

Short-term storage and retrieval of mango pollen

Two storage media in combination with four different storage temperatures were evaluated to extend the storage and viability of mango cv. NMBP-1201 pollen. Subsequent evaluations revealed that pollen stored in hexane at room temperature and pollen stored alone at -20ºC and -80ºC retained the highest viability following one week of storage. Pollen viability was significantly reduced following one week of storage in all conditions. Analysis of pollen germination rates showed that, following storage for one and two weeks, the phytotoxic effect of paraffin oil on mango pollen was greater than that of hexane at all temperatures. However, paraffin oil exhibited phytotoxic effects at all storage temperatures. These findings suggest that paraffin oil has higher phytotoxic effects on mango pollen compared to that of hexane; however, pollen stored in hexane retained a significantly higher level of viability for one week at room temperature. Thus, hexane may be a suitable pollen storage option for routine mango breeding work.

Identification and heritability assessment of the most efficient method to assess tree vigour

From twelve analysed tree morphological traits, trunk cross-sectional area (TCA) displayed the highest correlation with tree vigour, and thus was determined to be the most suitable trait for rapid vigour assessment. The heritability of TCA was assessed across several mango breeding populations growing on two research stations. TCA was recorded in 1909 progeny across 41 mango breeding families. Subsequent assessment revealed poor heritability for TCA ($h^2 = 0.23$) among the tested mango breeding population. Poor heritability indicates that environment has a greater influence on TCA than genotype. However, a number of potential low-vigour families were identified that could serve as parental lines in future crosses to develop low-vigour mango plants.

The effects of foliar-applied PGRs on fruit set and retention

The effects of PGRs on fruit set, retention, and quality were determined in the two mango cultivars NMBP-1243 and Keitt during the 2014 and 2015 mango seasons at Walkamin Research Station, Department of Agriculture and Fisheries, Mareeba. Three PGRs, namely NAA (25 and 50 ppm), 2,4-D (25 and 40 ppm), and gibberellic acid (5 and 10 ppm), were sprayed alongside a control treatment (no spray) onto the inflorescences of selected mango trees at full bloom stage. Following treatment, 25 panicles per tree were selected and tagged to observe subsequent fruit set and retention until harvest. Results showed that, in both varieties in both seasons, 2,4-D (40 ppm) treatment either significantly reduced or did not affect fruit set as compared to untreated trees (mean fruit set per panicle for cvs. NMBP-1243 and Keitt is 7.5 and 12.9 respectively) observed 28 days following full bloom stage. However, trees treated with 2,4-D had significantly higher rates of fruit retention at harvest. Foliar spray of 2,4-D reduced fruit size and weight in cv. NMBP-1243, but did not affect cv. Keitt. Thus, it was concluded that foliar-applied PGRs administered at flowering do not increase early fruit set, but may increase fruit retention at harvest to twice that of untreated trees. An increase in fruit retention can improve breeding efficiency as well as mango tree productivity and farm profitability.

The effects of foliar-applied micronutrients on fruit set and retention

Fruit set and retention are important phenomena in commercial fruit production. Low fruit set and retention significantly affect mango breeding efficiency and contribute towards low yields

in commercial mango orchards. The process of fruit set and retention is multidimensional and is directly or indirectly affected by nutritional factors. This work aimed to improve fruit set, retention, and quality in the two mango varieties NMBP-1201 and R2E2 via the application of micronutrients. Foliar application of the two micronutrients zinc and boron at two concentrations was performed at the start of bloom. Results showed that these micronutrients did not improve fruit set, retention, or quality in either mango variety.

Summary

In summary, the findings of this research suggest that the tested techniques may have significant effects on classical mango breeding efficiency. Mango pollen was successfully stored for one week in hexane and maintained significantly higher pollen viability as compared to paraffin oil storage. TCA was identified as the most suitable candidate for rapid vigour assessment in large breeding populations; however, subsequent assessment revealed poor TCA heritability which indicates a low influence of genotype on the TCA phenotype. A number of mango breeding families with low TCA were identified as potential parental lines for future crosses to breed low-vigour trees. Foliar application of 2,4-D and NAA led to significantly higher levels of hybridised fruit retention compared to that in control trees, which may increase the amount of progenies available for further growth and assessment in mango breeding. Conversely, foliar application of micronutrients had no significant impact on fruit set and retention. Further research is required to refine these techniques to increase the efficiency of classical mango breeding projects.

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2,4-D	2,4-Dichlorophenoxyacetic acid
a.i.	Active ingredient
ANOVA	Analysis of variance
BLUP	Best linear unbiased predictions
B	Boron
cv(s)	Cultivar(s)
DAF	Department of Agriculture and Fisheries
DAFB	Days after full bloom
DM	Dry matter contents
Fig	Figure
GA	Gibberellic acid
h^2	Narrow-sense heritability
LSD/l.s.d.	Least significant difference
NAA	Naphthylacetic acid
PGRs	Plant growth regulators
RCD	Completely Randomised Design
REML	Residual Maximum Likelihood
SD	Standard deviation
SE	Standard error
SEM	Standard error of means
SERS	Southedge Research Station
TCA	Trunk cross-sectional area
TSS	Total soluble solids
WRS	Walkamin Research Station
Zn	Zinc

Chapter 1: General introduction

Mango (*Mangifera indica* L.) is an important tropical/subtropical fruit crop produced commercially in more than 80 countries (Saúco, 2004; Tharanathan *et al.*, 2006). Annually, over 45 million tonnes of mango are produced worldwide, which makes mango the 5th largest global fruit crop surpassed only by banana, apple, grapes, and orange (FAOSTAT, 2014b). In major mango producing countries, the industry makes use of few commercial mango cultivars (Bally *et al.*, 2000b; Honsho *et al.*, 2013; Negi, 2000; Pinto *et al.*, 2004a). These cultivars commonly exhibit various negative traits, such as high vegetative vigour, low or erratic yield, poor fruit quality, narrow maturity period, and susceptibility to physiological disorders and diseases (Human *et al.*, 2006). Despite these negative qualities, there is continued use of these selected cultivars, which originated in America or Southeast Asia (Saúco, 2004). The development of novel mango cultivars is important because the selection of cultivated varieties is primarily based on fruit quality rather than on modern horticultural and industrial requirements (Iyer and Dinesh, 1997). Current mango breeding programs are focused on reducing vegetative vigour, increasing productivity, increasing resistance to physiological disorders, diseases, and pests, and increasing fruit shelf life to compete in high-end markets (Campbell and Zill, 2009).

Mango breeding programs are typically founded on the classical breeding approach (Bally *et al.*, 2009a; Iyer and Dinesh, 1997; Mukherjee *et al.*, 1961). Despite the inefficiency of this time-consuming and costly process, classical breeding was historically the only available method for breeding cultivars and rootstock in fruit crops such as mango (Lavi *et al.*, 1993b). Classical breeding employs hand pollination technique that is laborious, costly, and time-consuming, and which results in low seed production (Roizman, 1986). Despite the inefficiency of hand pollination, the approach contributes towards successful mango breeding because diverse progeny can be produced in a relatively small hybrid population, which increases the liklihood of a unique combination of desirable parental alleles in individual progeny genotypes (Iyer and Dinesh, 1997). However, low fruit set and the subsequent high fruit drop that follow hand pollination reduce the efficiency of hand pollination and reduce the number of hybrids that are recovered from crosses (Lavi *et al.*, 1998). Breeding efficiency can be improved by a number of different approaches: increasing the amount of quality pollen available for hand

pollination, having knowledge of heritability and breeding value to assist parental selection, and improving fruit set and retention.

Successful pollen storage and high viability of stored pollen contribute towards improved hand pollination efficiency. Mango flowering times vary greatly among genotypes and environments. Pollen storage extends pollen longevity, which allows pollen to be used in hand pollination that overcomes crossing barriers due to the asynchronous flowering of individual parents. Short-term pollen storage assists mango breeders by extending pollen availability within a flowering season (Chaudhury *et al.*, 2010; Dutta *et al.*, 2013a). For determining the viability of stored pollen before its use in hand pollination, it is essential to establish a working *in vitro* pollen germination system (Griffin, 1982; Khan and Perveen, 2009). The use of ultra-low temperature storage methods is restrictive because they are laborious and require sophisticated apparatus such as liquid nitrogen cryotanks (Dutta *et al.*, 2013a). Therefore, a more simple and effective method of pollen storage is needed to assist in classical breeding (Mishra and Shivanna, 1982).

Mango breeding efficiency may be enhanced by effective parental selection. The development of trees with reduced vegetative vigour is an important breeding goal. A reduced tree size phenotype is preferred over manual or chemical control of tree size because of the higher cost of manual/chemical approaches and the desire for low-level chemical use in specific markets (Campbell and Ledesma, 2013). The selection of parents for the breeding of low vigour/dwarf trees requires reliable vigour assessment techniques. The simplest measure of fruit tree vigour is trunk diameter (Guxiong *et al.*, 1987; Vargas and Romero, 1998); however, this technique lacks reliability. Vegetative vigour can also be assessed by measuring leaf, branch, stem, and root traits in different fruit crops (Abirami *et al.*, 2011; Saeed *et al.*, 2010; Srivastav *et al.*, 2009; Yanjun *et al.*, 2011). Best linear unbiased prediction (BLUP) is a standard technique used in animal breeding for calculating the random effects of a mixed model. This method was devised in animals to estimate breeding values that are used as a basis for selecting the most suitable family or individual progeny. The application of BLUP in plant breeding is novel. Two forms of BLUP are typically employed in plant breeding: the first is based on total genetic variance and the second is based on additive genetic variance. The former is used to estimate the phenotypic performance of a genotype in commercial testing. The latter determines the performance of a genotype as a parent in future crosses and is referred to as the breeding value (Piepho *et al.*, 2008). Breeding efficiency is improved by applying knowledge of heritability

or breeding values to parental selection in conventional fruit breeding systems, where the selection is based on phenotypic expression of the desired traits (Bauer and Léon, 2008).

Phytohormones influence fruit set and retention (Malik and Singh, 2003; Prakash and Ram, 1984), and micronutrients such as boron (B) and zinc (Zn) are important components of different enzyme systems that regulate fruit set and retention (Bernhard, 1961; Marschner, 2012). Foliar application of micronutrients, such as B and Zn, and synthetic plant growth regulators (PGRs), such as auxins and gibberellins, is widely reported to improve fruit set and retention in fruit tree crops (Chaplin and Westwood, 1980; Singh and Dhillon, 1987; Ward, 2004). NAA is a synthetic auxin documented to be effective in fruit drop management in many fruit crops including apple (Yuan and Carbaugh, 2007; Yuan and Li, 2008) and citrus (Anthony and Coggins, 2001). It is also established that gibberellic acid reduces fruit drop and increase fruit retention in fruit crops such as litchi (Singh and Lal, 1980) and grapefruit (El-Zeftawi, 1980). A further synthetic auxin, 2,4-D, also efficiently suppresses fruit drop in fruit crops such as citrus (Anthony and Coggins, 1999; Stewart *et al.*, 1951). NAA (Ram, 1992), 2,4-D (Ram, 1983), and gibberellic acid (Ahmed *et al.*, 2012; Singh, 2009) have been effectively used to reduce fruit drop in mango. Foliar B application is effective for improving fruit set in *Prunus*, *Malus*, and *Pyrus* species by enhancing bud and flower B concentrations (Callan *et al.*, 1978; Chaplin *et al.*, 1977; Chaplin and Westwood, 1980; Hanson, 1991b). B application has also been shown to increase fruit set in pear (Batjer and Thompson, 1949) and mango (Rajput *et al.*, 1976), and fruit retention in mango (Singh and Dhillon, 1987). B deficiency may lead to low fruit set in mango (de Wet *et al.*, 1989). Furthermore, foliar Zn application improves fruit set in mango (Daulta *et al.*, 1981). The foliar application of micronutrients and PGRs after hand pollination may improve fruit set and fruit retention. Considering the above, the present study was conducted with following aims.

1.1 Research aims

The general aim of the project is to develop techniques to increase the efficiency of the classical breeding approach in mango, specifically:

(i) Improving pollen availability throughout the duration of mango flowering by improving the viability of stored pollen.

(ii) Improving the efficiency of vegetative vigour assessment in mango breeding families by both identifying highly vigour-correlated trait and understanding its heritability.

(iii) Improving fruit set and retention in hybrid mango progeny by exogenous application of PGRs and micronutrients.

This thesis is structured into four data chapters (Chapter 3, 4, 5, and 6).

Chapter 3 deals with the factors influencing mango pollen viability following short-term storage. This work aims to determine the effect of different solvents and variation in storage period on pollen viability, with the intention of developing a practical short-term (i.e., within one season) pollen storage and retrieval protocol that can be used routinely by mango breeders.

Chapter 4 assesses the correlation between tree vegetative vigour and various morphological traits. This work aims to identify the potential tree trait that provides the most efficient measure of vegetative vigour and its heritability. These results will lead to an improved parental selection approach in crosses aimed at reducing vigour.

Chapters 5 and 6 evaluate the effect of exogenous PGR and micronutrient application on flowering responses that improve fruit set and retention of hybridised fruit, respectively. This work aims to improve breeding efficiency by extending the retention of hybridised fruit until fruit harvest. These results will also contribute towards improved tree productivity and farm profitability, and are therefore applicable in commercial mango production.

Chapter 2: Literature review

2.1 Introduction

2.1.1 Mango

Mango, *Mangifera indica* L., is an important fruit crop in many tropical countries but reliable yields are potentially affected by a tendency towards irregular or erratic bearing of fruit. This propensity for variable crop production is influenced by poor synchrony in canopy growth which affects flowering. Further factors that affect mango yield include climatic variations, temperature effects on flower sex ratios, poor pollination, pests, diseases (Bally *et al.*, 2009a; Iyer and Dinesh, 1997), inadequate nutrient and water availability, and poor cultivar and canopy management. In addition to affecting crop yield regularity, negative influential factors also lower the efficiency of mango breeding programs (Bally *et al.*, 2009a). Irregular bearing of fruit leads to significant annual yield fluctuations, which subsequently causes variation in fruit price and profit margins within commercial mango production (Monselise and Goldschmidt, 1982). Among the many potential factors that influence mango yeild, factors including pollination, fruit regulation, and effective parental selection based on heritability and breeding values are important because of their role in mango breeding, and hence they warrant further study.

2.1.2 Production and trade

World

Mango is an important tropical and subtropical fruit crop, with an annual production of 45.225 million tonnes worldwide; the 5th ranking global fruit crop following *Musa spp*. (banana and plantain), apple, grape, and orange (FAOSTAT, 2014b); Fig. 2-1, left panel). Mango is grown commercially in more than 80 countries (Tharanathan *et al.*, 2006), with the leading global producers being India, China, Thailand, Indonesia, Mexico, Pakistan, Brazil, Bangladesh, Egypt, and Philippines (FAOSTAT, 2014b); Fig. 2-1, right panel). Mango is a particularly important crop in Asia (Chapman, 2000), and known colloquially on the Indian subcontinent as ‘the king of fruits’.

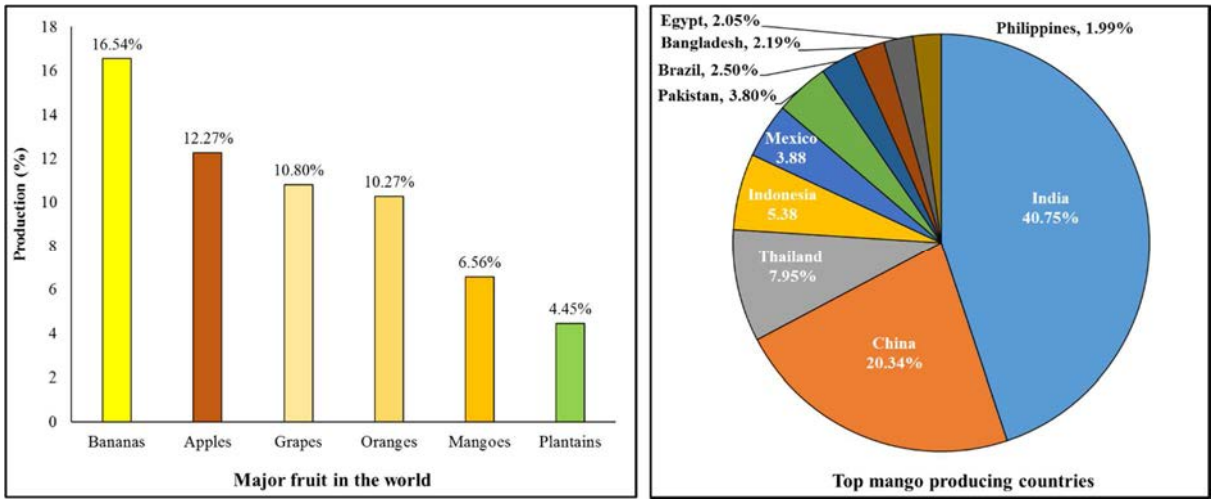

Fig. 2-1: Mango production status in the world and top mango producers

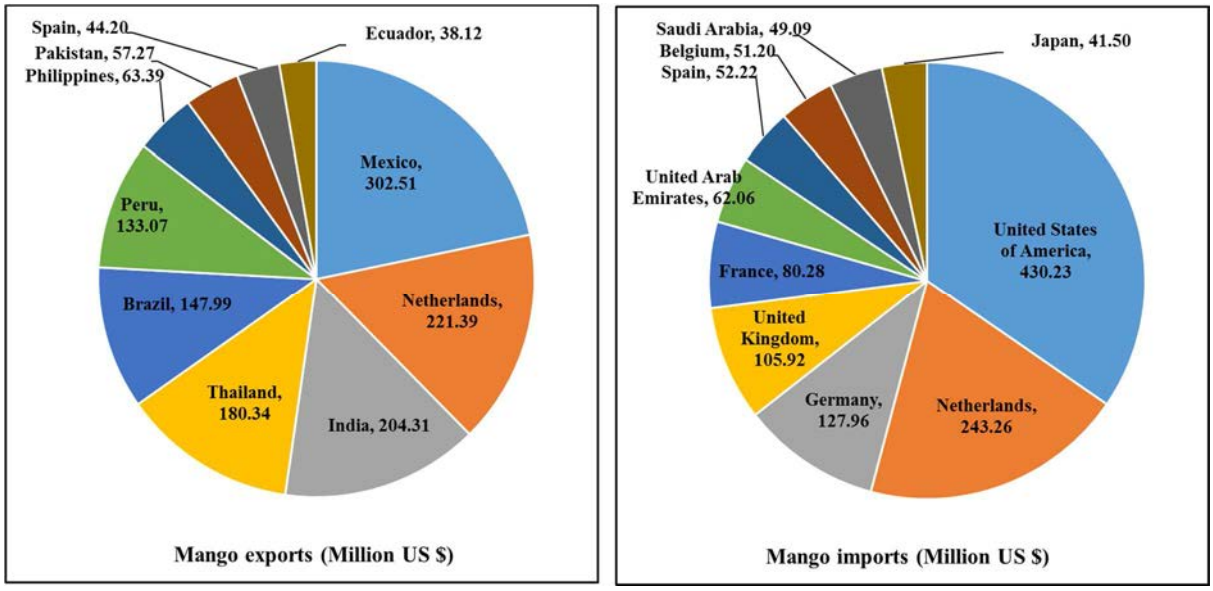

Fig. 2-2: Annual trade in mango (*Mangifera indica* L.; exports and imports) in the world

While worldwide production makes mango the 5th ranking global fruit crop, the vast majority of mango is traded and consumed within domestic markets (UNCTAD, 2010) and the international mango trade represents only 3% of global production (Fig. 2-2). Mexico is the largest mango exporter, followed by the Netherlands (as a re-exporter) and India. Combined, these three countries contribute more than 50% of world mango exports. The leading mango importers include the United States of America, the Netherlands, and Germany (FAOSTAT, 2014a); Fig. 2-2). Mango markets are now expanding because the fruit is gaining popularity with many consumers. The development of new varieties that exhibit higher yield, improved cosmetic quality, greater disease resistance, and higher shelf performance may help to ensure mango production rates can meet an increasing demand (Saúco, 2004).

Australia

The Australian mango industry represents only 0.09% of total world production (FAOSTAT, 2014a). Australian mango industry is a small but growing industry that predominantly serves a domestic market; however, the export market is expanding. Here, mango production extends over a wide environmental range (latitude 12°–39°S), under conditions found in the Northern Territory (dry monsoon tropical), the Kimberley region (tropical monsoon), Queensland (dry tropics), and New South Wales (arid to semi-arid areas) (Bally *et al.*, 2000b). The Australian mango industry is dominated by four cultivars: Kensington Pride, Calypso, R2E2, and Honey Gold. Combined, these varieties represent 95% of commercial mango production in Australia (http://www.industry.mangoes.net.au/mango-production; accessed: 28 April, 2017). The most popular mango in Australia's domestic market is Kensington Pride, which is valued by the consumer for its distinctive peachy flavour (Lalel *et al.*, 2003). However, this cultivar is associated with irregular bearing, susceptibility to anthracnose and black spot, and short post-harvest life as compared to that of other cultivars. Hence, the Australian Mango Breeding Program is working to develop novel cultivars that retain the flavour of Kensington Pride while maintaining higher productivity, increased disease resistance, improved skin colour, and longer post-harvest life (Bally *et al.*, 2009a; Kulkarni *et al.*, 2002).

Pakistan

Mango is the second largest fruit crop in Pakistan and represents 3.80% of annual mango global production worth US$57.27 million (FAOSTAT, 2014a) (Fig 2-2). The major mango producing provinces are Punjab and Sindh, which represent 76.7% and 22.6% of production volume, and 52.4% and 45.6% of production area, respectively (Khan *et al.*, 2008). The main mango varieties cultivated in Pakistan are Sindhri, Samar Bahisht Chaunsa, Kala Chaunsa, Sufaid Chaunsa, Dusehri, Anwar Ratole, and Langra (Rajwana *et al.*, 2011). United Arab Emirates, Saudi Arabia, United Kingdom, Oman, and Afghanistan are major importers of mangoes from Pakistan (Amin, 2012).

2.2 Breeding and domestication

2.2.1 Breeding goals

Mango breeding programs with clear objectives have been developed in many countries including Australia, Israel, Brazil, India, and Mexico (Bally *et al.*, 2009a; Carvalho *et al.*, 2004;

Chapman, 2000; Human *et al.*, 2006; Tomer *et al.*, 1997). The objectives of mango breeding programs vary in accordance with local environmental conditions and target markets. Primary objectives include development of enhanced tree dwarfing, regular fruit bearing, precocity, disease resistance, and attractive cosmetic and biochemical fruit quality (Bally *et al.*, 2009a). The main objectives of the Australian Mango Breeding Program are to develop cultivars with enhanced dwarf phenotypes, greater disease resistance, higher yields, and improved fruit quality (Bally *et al.*, 2009a; Dillon *et al.*, 2013).

2.2.2 Approach to mango breeding

Hand pollination

A classical breeding approach based on hand pollination has been adopted in many mango breeding programs for novel cultivar and rootstock development (Bally *et al.*, 2009a; Lavi *et al.*, 1993b; Lavi *et al.*, 2004). Hand pollination is an inefficient, laborious, costly, and time-consuming approach that results in a very low success rate concerning seed production (Roizman, 1986). Despite its inefficiency, significant progress has been made in developing new cultivars via hand pollination (Iyer and Dinesh, 1997).

Mango crop improvement is often carried out by means of recurrent selection, which involves performing controlled crosses between selected parents and evaluating the progeny for desirable characters. This selection procedure can take several years. Controlled pollination has proved to be successful in mango breeding because diverse progeny can be produced in a relatively small hybrid population. Compared to an open pollination method which produces a high number of self-pollinated progeny, controlled pollination increases the likelihood of obtaining a unique combination of desirable parental alleles in individual progeny genotypes. Controlled pollination also has the advantage of the ability to select both the ovule- and pollen-bearing parent. Controlled pollination can be easily performed on existing trees to facilitate crossing between cultivars (Bally *et al.*, 2009b), whether they are grown in an experimental plot or plantation, and does not require purpose-grown blocks of trees for pollen transfer. Controlled pollination can also be used to cross individual trees that are spatially or temporally separated (Bally *et al.*, 2009a). Successful controlled pollination requires an understanding of pollen biology, and its appropriate handling and storage.

Pollen storage

Long-term storage of pollen is important for plant breeding because it can ensure pollen availability outside of flowering seasons (Gill and Malik, 1992; Shivanna and Rangaswamy, 1992). Pollen longevity is influenced by its response to storage conditions. To determine the viability of stored pollen before its use in hand pollination, a working *in vitro* pollen germination system is essential (Griffin, 1982; Khan and Perveen, 2009).

Successful pollen storage is crucial for crop improvement programs that rely on the hybridisation of individuals which is normally hampered by asynchronous flowering caused by spatial or geographical separation (Mishra and Shivanna, 1982). An efficient pollen storage system that results in pollen with improved post-storage viability is essential for the ultimate success of breeding programs. A considerable amount of research has been undertaken on pollen storage for improved hand pollination in diverse fruit crops (Chaudhury *et al.*, 2010; Cohen *et al.*, 1989; Dutta *et al.*, 2013a; Ganeshan, 1986; Imani *et al.*, 2011; Parfitt and Almehdi, 1983). In general, these studies have shown that low-temperature storage leads to the highest retention of pollen viability in tree crops (Sedgley and Griffin, 1989). For example, cherimoya pollen maintained at -20°C, -80°C, and -196°C for three months maintained 10.4%, 14.2%, and 13.6% viability, respectively (Lora *et al.*, 2006). However, successful pollination in the field was not significantly different when using either stored or fresh cherimoya pollen, the latter of which had 57% pollen viability (Lora *et al.*, 2006). Apple (*Malus domestica*) pollen maintained higher viability (90.66%) following seven months of -80°C storage compared to that of pollen stored at -4°C (Imani *et al.*, 2011).

2.2.3 Reproductive biology

Inflorescences and flowers

The mango inflorescence is a cluster of flowers that, depending on cultivar and environmental conditions, forms a primarily terminal panicle approximately 45 cm long. Panicle colour varies between varieties, ranging from yellow-green to light green (Litz, 2009). An individual panicle can contain between 500 and 6000 flowers, out of which typically <50% are hermaphrodite and the remainder are functionally male. The proportion of perfect to male flowers is dependent on the cultivar and its interaction with the environmental conditions (Bally *et al.*, 2009a; Mukherjee and Litz, 2009).

Perfect/hermaphrodite flowers have one or two fertile stamens and functional female organs whereas male flowers have one or more stamens and staminodes and an abortive pistil (Kostermans and Bompard, 1993). Both flower types are comparable in size, which varies from 6 to 8 mm in diameter (Bally *et al.*, 2009a). The calyx consists of five ovate-oblong, concave sepals. The corolla is twice the length of the calyx and consists of five pale yellow petals (Singh, 1960a).

Natural pollination

As for many other subtropical fruit species, pollination is an important phenological event for mango fruit development (Ramírez and Davenport, 2016). Mango is pollinated primarily by insects (Anderson *et al.*, 1982; Singh, 1988; Sung *et al.*, 2006), although wind and gravity also play a minor role (Davenport, 2009; Mallik, 1957; Singh, 1961). Successful insect pollination is important for high fruit set and yield in mango, and ineffective pollination is one of the leading causes of poor cropping (Anderson *et al.*, 1982; Dag and Gazit, 2000; Singh, 1997). The major insect groups that facilitate mango pollination are the orders Diptera (Anderson *et al.*, 1982; Dag and Gazit, 2000; Singh, 1988), Hymenoptera (Anderson *et al.*, 1982; Dag and Gazit, 2000; Singh, 1997), and Coleoptera (Dag and Gazit, 2000; Singh, 1997). The use of insecticides during flowering periods decreases pollinator activity and fruit set in turn (Singh, 1997).

Pollen biology and storage

Mango flowering times vary greatly among genotypes and environments, and on occasion the flowering periods of individual cultivars have little to no overlap. Pollen storage is used to extend its longevity and allows its use in pollination performed at a later date, thus overcoming crossing barriers due to asynchronous flowering between individuals. Short-term pollen storage provides a breeder with viable pollen within a flowering season and allows pollination of a late emerging flower with an earlier flowering genotype (Chaudhury *et al.*, 2010; Dutta *et al.*, 2013a; Mishra and Shivanna, 1982; Sedgley and Harbard, 1993). Pollination of early flowering genotypes with pollen from a late emerging flower requires pollen storage from one season to the next.

Critical external factors that affect pollen viability include relative humidity (RH) and temperature. Ultra-low temperature (−196°C) storage methods are laborious and require

sophisticated apparatus such as liquid nitrogen cryotanks (Dutta *et al.*, 2013a), which make them impractical in developing countries. A simpler and more effective pollen storage method is needed to facilitate accessible controlled cross-pollination (Mishra and Shivanna, 1982). Studies of pollen viability following storage in either organic solvents or low temperature are required to determine if organic solvents or mineral oils can maintain pollen viability similarly to low temperature storage.

A pollen storage method commonly employed in different fruit crops is based on controlled temperature and humidity. In almond (*Prunus dulcis*), Martinez-Gomez *et al.* (2000) concluded that pollen viability did not decrease significantly following 4°C storage for two months. For long-term storage (up to 12 months), storage temperatures ranging from 0°C to -20°C were found to be more reliable, maintaining 70–75% pollen viability. In papaya (*Carica papaya*), pollen grains stored at 5°C for two months germinated at relatively lower rates compared to those stored at 18°C (30% vs 45% germination, respectively(Cohen *et al.*, 1989). In another study, Ganeshan (1986) successfully stored papaya pollen for 300 days in liquid nitrogen (-196°C) and observed that stored pollen retained a similar viability compared to that of fresh pollen (58% vs 54% viability, respectively).

In addition to low temperature storage, organic solvents, such n-Hexane and Cyclohexane, can be used to extend pollen longevity from weeks to months (Dhingra and Varghese, 1990; Iwanami and Nakamura, 1972; Liu and Cao, 1984). Organic solvents preserve pollen viability by providing anhydrous storage conditions and limiting oxygen supply (Jain and Shivanna, 1988b), while simultaneously preserving pollen membrane integrity (Iwanami, 1984). This method has been reported to be effective in some leguminous taxa (Mishra and Shivanna, 1982), *Chrysanthemum spp.* (Ikeda and Numata, 1998; Iwanami, 1975), *Camellia japonica* (Iwanami, 1972), *Vitis coignetiae* Pulliat (Honma *et al.*, 2003), *Crotalaria spp.* (Jain and Shivanna, 1988a; Jain *et al.*, 1990) and *Vitis vinifera* (Agarwal, 1983). Jain and Shivanna (1990) successfully stored pollen grains of *Crotalaria retusa* in mineral and vegetable oils. Pollen storage in organic solvents is cheaper than cryopreservation and does not ultimately depend on stable infrastructure, which therefore may be a more practical approach in developing countries.

2.2.4 Parental selection

Vegetative vigour

Tree vigour can be described as the intensity of vegetative growth (Nesme *et al.*, 2005). Vigour is negatively related to productivity and yield of fruit trees (Jerie *et al.*, 1989). Control of tree vigour improves tree architecture and canopy size which consequently improves orchard productivity by reducing pesticide usage (Olmstead *et al.*, 2006) and reducing labour required for harvesting, pruning, and thinning operations (Fideghelli *et al.*, 2003). The increasing popularity of high density and ultra-high density planting systems is associated with high costs and intensive labour requirements for fruit tree pruning in a high-density plantation (Byrne, 2012; Horton, 1985).

The development of fruit trees with improved tree architecture appropriate for competitive high-density fruit production holds great potential. Tree characteristics such as vigour, habit, and fruiting type are considered most often by breeders in fruit breeding programs (Laurens *et al.*, 2000). Fruit trees with an innate dwarf phenotype have been exploited for many years (Fideghelli *et al.*, 2003). However, genetic control of tree size and shape has only recently become an objective of mango fruit breeding programs (Bally *et al.*, 2009b; Bally *et al.*, 2009a).

Vegetative vigour assessment

The appropriate selection of parents is a critical step in fruit breeding programs. For the development of low vigour/dwarf trees, parental selection requires reliable vigour assessment techniques. The simplest measure of fruit tree vigour is trunk diameter (Guxiong *et al.*, 1987; Vargas and Romero, 1998); however, trunk diameter is a particularly crude measure of vegetative vigour and is not widely used except for in mango. Alternate methods for measuring fruit tree vigour are listed in Table 2-2.

An early study found that a higher phloem to xylem ratio was linked to dwarfing rootstocks in apple (Beakbane and Thompson, 1939). Later work reported that vegetative growth is associated with anatomical features of the roots (Beakbane, 1953), stems (Beakbane, 1941), and leaves (Beakbane, 1967) in apple. Trunk cross-sectional area (TCA) is often used to assess tree vigour in apple and other tree species (Barden *et al.*, 2002; Khatamian and Hilton, 1977). In mango, tree vigour may be assessed by stem growth, bark percentage of root, and area of

root vessels (Majumdar *et al.*, 1972). Low-vigour mango trees exhibit a higher phloem to xylem ratio (Kurian and Iyer, 1992).

Stomatal density is also used to assess the vigour of fruit trees such as citrus (Guxiong *et al.*, 1987), pear (*Pyrus spp.*; (Liang *et al.*, 2010; Ying *et al.*, 2010), and peach (*Prunus persica*; (Niu *et al.*, 2008). Citrus plant vigour is significantly correlated with leaf stomatal density, internode length, bark percentage, leaf thickness, seedling height, and trunk diameter (Guxiong *et al.*, 1987). The tree traits that are associated with vigour in other tree crops can be used to assess mango tree vigour. Several leaf traits such as leaf length, leaf fresh weight, Chlorophyll fractions and leaf stomatal density (Abirami *et al.*, 2011; Guxiong *et al.*, 1987; Liang *et al.*, 2010; Pal *et al.*, 1983; Yanjun *et al.*, 2011; Ying *et al.*, 2010), branch and stem traits such as shoot diameter, internode length, stem xylem percentage, shoot dry weight and twig length (Fen-xue *et al.*, 2008; Ma *et al.*, 2011; Saeed *et al.*, 2010; Shi *et al.*, 2000; Srivastav *et al.*, 2009; Wang and Li, 2006; Wang *et al*., 2006; Yanjun *et al.*, 2011), and root traits such as root diameter and root length (Srivastav *et al.*, 2009) are also reported to correlate with tree vigour in different fruit crops

One aim of this research is to develop fast and efficient ways of measuring canopy architecture and dwarfing traits in breeding populations, which can be used to identify individual progeny and families with these desirable traits. Analysis of the identified traits that characterise a breeding pedigree improve the understanding of quantitative trait heritability and help to identity candidate parents for incorporation of desirable tree traits in the breeding population. This research explores tree traits that are important for parental selection in respect to promoting dwarfing in mango.

Heritability assessment

Narrow sense heritability is defined as the proportion of trait variance that is caused by additive genetic factors, and specifically refers to the ratio of additive variance (transmissible to next generation) to phenotypic variance (Griffiths *et al.*, 2015). Narrow sense heritability is used to predict how traits are manifested in progeny following the selection in comparison to their parents and can be determined using best linear unbiased predictions (BLUP), which is a technique for calculating the random effects of a mixed model. This method was devised to estimate breeding values in animals and is commonly employed to select the most suitable progeny for future crosses; however, it has only recently been applied in plant breeding (Piepho

et al., 2008; Purba *et al.*, 2001). Two forms of BLUP are typically employed in plant breeding: the first is based on total genetic variance and the second is based on additive genetic variance. The former is used to estimate the phenotypic performance of a genotype in commercial testing. The latter determines the performance of a genotype as a parent in future crosses, and is also known as the breeding value (Piepho *et al.*, 2008). Knowledge of breeding values is important for effective parental selection and improves breeding efficiency by avoiding unproductive crosses (Bauer and Léon, 2008; Hardner *et al.*, 2012). Parental selection based on breeding values increases the proportion of progeny with superior phenotypic expression of a desirable trait (Hardner *et al.*, 2012).

2.3 Regularity of bearing

2.3.1 Fruit set and retention

Low fruit set and high fruit drop significantly affect breeding efficiency (Singh *et al.*, 2005), and are among the major causes of low yield in mango orchards (Malik and Singh, 2003; Prakash and Ram, 1984). Depending upon the cultivar, the fruit set percentage in mango is less than 0.1% of perfect flower number, and is affected by flower sex ratio, pollen viability, and environmental conditions during the pollination process such as temperature (Bally *et al.*, 2009a; Guzman-Estrada, 1997; Prakash and Ram, 1984). The pollen donor parent contributes greatly to mango fertilisation success because of compatibility issues with the ovule donor parent, which determines the fruit set success rate following crossing (Ram *et al.*, 1976). Pollen viability is one of the major factors limiting the success rate of crossing in mango breeding, as well as mango orchard yields because of its effect on fruit set percentage (Davenport, 2009; Singh, 1954).

Fruit set

Fruit set and micronutrients

The understanding of exogenous micronutrient application such as boron (B) and zinc (Zn) which plays major role in mango fruit set is very important. Micronutrient application is essential for successful commercial fruit production (Rossetto *et al.*, 2000). Spraying foliage with micronutrient solutions is a common strategy to overcome plant deficiencies and improve fruit quality (Martens and Westermann, 1991; Swietlik, 2002). Both macro- and micronutrients

are more rapidly absorbed via foliar application compared to soil application (Bahadur *et al.*, 1998; Stampar *et al.*, 2002; Wojcik, 2004).

Boron is an essential microelement required for regular growth in higher plants (Marschner, 2012). It plays an important role in pollen germination, pollen tube growth, and successful fruit set (Stanley and Lichtenberg, 1963). Foliar application of B before flowering is effective for improving fruit set in *Prunus, Malus,* and *Pyrus* species by enhancing bud and flower B concentrations (Callan *et al.*, 1978; Chaplin *et al.*, 1977; Chaplin and Westwood, 1980; Hanson, 1991b). Boron application to flowers in the form of boric acid sprays also increases fruit set in pear (Batjer and Thompson, 1949). In mango, leaf B deficiency leads to low fruit set (de Wet *et al.*, 1989). Foliar application of B has been shown to increase fruit set (Rajput *et al.*, 1976) as well as fruit retention (Singh and Dhillon, 1987) and pollen germination rates (de Wet *et al.*, 1989).

Foliar application of Zn can also improve fruit set in mango (Daulta *et al.*, 1981). Zinc in the form of foliar zinc sulphate ($ZnSO_4$) application significantly increased leaf Zn concentration in mango, and Zn uptake following foliar application was higher compared to Zn uptake following soil application (Bahadur *et al.*, 1998). Some general recommendations for foliar application of these micronutrients are provided for mango in different countries. In Pakistan, application of Zn (200-240 g $ZnSO_4$/plant) and B (60-80 g borax/plant) to the soil around the root zone is recommended for mango in March and April (corresponding to the end of the winter season (FVDP, 2011). In Australia, three foliar applications of 1% B is recommended four weeks before flowering and bud break (Meurant and Kernot, 1999).

Fruit set and plant growth regulators

In fruit tree crops, fruit set is regulated by endogenous phytohormones such as auxins, gibberellins, and cytokinins (Kumar *et al.*, 2014). Foliar application of synthetic plant growth regulators (PGRs) has been used to improve fruit set in several fruit tree crops (Anthony and Coggins, 1999; Anthony and Coggins, 2001; El-Otmani *et al.*, 2000; Singh and Lal, 1980). The lower fruit set in Dusehri mango trees sprayed with 2,4-D compared to those trees sprayed with NAA and GA_3 (Ahmed *et al.*, 2012). Aliyu *et al.* (2011) also showed lower rates of fruit set in cashew trees sprayed with 2,4-D compared to that observed following application of other PGRs. However, some studies in fruit crops report higher fruit set as a result of foliar auxin

application, including application of 2,4-D (García-Martínez and García-Papí, 1979; Tuan and Chung-Ruey, 2013b; Tuan and Chung-Ruey, 2013a).

Fruit retention

A key issue in mango production is low fruit retention due to premature fruit drop. Despite sufficient flowering and early fruit set in mango, a significant level of fruit drop occurs during various fruit development stages. Fruit drop contributes towards low fruit yield in mango resulting in a substantial economic loss. High-level fruit drop also hinders mango breeding programs by reducing the availability of mature fruit/seed for further crossing and evaluation (Singh *et al.*, 2005).

Dahsham and Habib (1985) divided fruit drop into three separate phases: post setting drop (occurring in the first two months following pollination), June drop (occurring when fruits are 60–75 days old), and pre-harvest drop (occurring at fruit maturity). High rates of fruit drop are related to periods of relatively low fruit auxin contents. Ram (1983) attributed high-level fruit drop due to the high level of an abscisic acid-like inhibitor present in the first 21 days following pollination, which is a period of slow fruit growth. Another study revealed that major fruit drop occurs 25–50 days after fruit set (Guzman-Estrada, 1997). Singh (1960b) proposed that immature fruit drop occurs during the first four weeks following pollination.

Fruit retention and micronutrients

Plant nutrients, along with plant hormones, also affect fruit retention (Teaotia and Luckwill, 1956). Some reports suggest that Zn deficiency contributes to pre-harvest fruit drop (Davenport, 2009; Jirón-Porras and Hedström, 1985). In one experimental study on Kinnow mandarin, Razzaq *et al.* (2013) showed that foliar Zn application (0.6% $ZnSO_4$) at the fruit set stage resulted in significantly higher fruit retention compared to that in unsprayed control trees. In the same variety, Ullah *et al.* (2012) observed no such effect with B, reporting that foliar application of 0.1%–0.4% boric acid at the fruit set stage did not significantly affect fruit retention. In mango cv. Amrapali, foliar application of Zn (0.5% $ZnSO_4$) at the pea and marble stages of fruit development resulted in the highest level of fruit retention at marble and harvest stage (Bhowmick *et al.*, 2012). Conversely, some studies do not report a significant impact of foliar spray on fruit retention. Masroor *et al.* (2016) found no significant change in fruit retention rates at harvest in 'Summer Bahisht Chaunsa' mango trees observed over two years

following Zn application (0.5% and 1.0% $ZnSO_4$) repeated in November and March. Similarly, in olive, trees were sprayed with Zn (2.5 kg m^{-3} $ZnSO_4$) and B (2.5 kg m^{-3} boric acid) both at the mature flower stage and 15 days after, and, in two of three experimental olive varieties, there were no significant differences in fruit retention rates (Saadati *et al.*, 2016).

Fruit retention and plant growth regulators

Naturally occurring plant hormones, or PGRs, play an important role in fruit growth, development, and abscission (Ram, 1992). The signals that are responsible for fruit drop have a hormonal basis (Singh *et al.*, 2005). There are numerous classes of hormones which each have specific roles in the regulation of fruit drop/retention. Two such classes, namely auxins and gibberellins, are discussed below. Ram (1992) suggested that high-level fruit drop in mango could be the result of auxin, gibberellin, and cytokinin deficiency.

Synthetic PGRs have been used to investigate hormonal control of pre-harvest fruit drop in fruit crops such citrus, apple, and mango (Arteca, 1996; Nawaz *et al.*, 2008; Yuan and Carbaugh, 2007). These PGRs include naphthaleneacetic acid (NAA), 2,4-D, and gibberellic acid (Ward, 2004). NAA is a synthetic auxin proven to be effective in fruit drop management in apple (Yuan and Carbaugh, 2007; Yuan and Li, 2008) and citrus (Anthony and Coggins, 2001). A further synthetic auxin, 2,4-D, also efficiently suppresses fruit drop in fruit crops such as citrus (Anthony and Coggins, 1999; Stewart *et al.*, 1951). Gibberellic acid has been demonstrated to enhance fruit retention in fruit crops such as litchi (*Litchi chinensis*; (Singh and Lal, 1980) and grapefruit (El-Zeftawi, 1980).

Auxins

Indole-3-acetic acid (IAA) is considered as the primary auxin in plants and has many important functions (Arteca, 1996). IAA influences cell division and elongation, and hence regulates plant growth and development (Thimann, 1977). The main sources of auxins in the plant are young leaves and seeds. The maintenance of abscission zones is related to maintenance of auxin supply (Poorter *et al.*, 2006). Decreased concentrations of auxins lead to fruit drop (Arteca, 1996). Prakash and Ram (1984) reported low-level auxin in the developing fruit of mango. Increased levels of auxins induce rapid fruit growth, whereas high auxin inhibitor levels lead to fruit drop (Prakash and Ram, 1984).

Numerous studies have reported improved fruit retention in mango following the application of auxins. Gokhale and Kanitkar (1951) were the first to investigate the relationship between mango fruit drop and auxin by using the synthetic auxins NAA and 2,4-D. They established that foliar applications of 20 mg L^{-1} of either NAA or 2,4-D were slightly effective in reducing fruit drop. The highest rate of fruit retention was attained by foliar application of NAA (40 mg L^{-1}) both at the pea fruit development stage and one month later (Maurya and Singh, 1979). In Pakistan, the same results were achieved by application of NAA (20 mg L^{-1}) at pea and marble fruit development stages in Sindhri, Langra, and Dashehari mangoes (Naqvi *et al.*, 1990). Sharma *et al.* (1990) reported that NAA sprays significantly increased fruit retention. In Samar Bahisht Chaunsa mango, foliar application of NAA (20 ppm) reduced fruit drop in sprayed trees compared to that of control (65.39% vs 84.93%, respectively; (Chattha *et al.*, 1999).

Gibberellins

Gibberellins are naturally occurring compounds in plants that belong to a group known as terpenoids. Gibberellins affect physiological processes such as plant growth, dwarf phenotypes, flowering, seed dormancy, and germination (Arteca, 1996). The direct role of gibberellins in fruit drop has not been characterised till to date (Davenport, 2009; Ram, 1983); however, indirect involvement has been reported, with a decreased endogenous gibberellic acid (GA_3) level associated with the stimulation of ethylene production which facilitates fruit abscission (Arteca, 1996). Ram (1992) reported that a reduction of endogenous gibberellins induced fruit drop in Dashehari, Chausa, and Langra mangoes. A study by Bains *et al.* (1997b) revealed that near-to-drop fruit and their pedicels displayed decreased levels of GA_3. Improved mango fruit retention following foliar application of GA_3 was demonstrated by Singh (2009) and Ram (1983). Singh (2009) reported that the exogenous application of gibberellins (both GA_3 and GA_4) on panicles significantly increased the rate of fruit retention compared to that resulting from other treatments.

Mango fruit drop is also influenced by plant stress responses caused by various environmental conditions e.g. high/low temperatures and drought (Roemer *et al.*, 2008). These stress conditions induce an abnormal hormonal balance, based on both auxin and ethylene, which leads to fruit abscission (González-Carranza *et al.*, 1998). The interaction of ethylene and auxin governs formation of the abscission zone, which is a process that ultimately leads to fruit drop (Roemer, 2011). Synthetic PGRs such as NAA (Ram, 1992), 2,4-D (Ram, 1983), and GA_3

(Ahmed *et al.*, 2012; Singh, 2009) have been used to mitigate fruit drop in mango; however, fewer studies have been reported concerning the effect of PGRs on Australian varieties in tropical Queensland.

Chapter 3: Evaluation of novel methods for short-term storage and retrieval of mango pollen.

3.1 Abstract

This study investigated the effects of different storage conditions on pollen viability in mango cv. NMBP-1201. Two storage media (hexane and paraffin oil) were tested in combination with four different storage temperatures, (ambient temperature, 4ºC, -20ºC, and -80ºC) for their effects on maintaining viability in stored mango pollen. Results showed that, following one-week storage, pollen in hexane at ambient temperature and pollen alone at -20ºC and -80ºC retained the highest viability. Viability was significantly reduced in pollen stored for more than one week. Following two-week storage, the phytotoxic effects of hexane on mango pollen were greater at -20ºC and -80ºC; however, paraffin oil showed comparable phytotoxic effects at all storage temperatures. These results suggest that both hexane and paraffin oil have phytotoxic effects on mango pollen, but ambient-temperature hexane storage for one week maintains significantly high pollen viability, and thus may be used as a pollen storage option for routine mango breeding work. The results of this study provide the mango breeder with a suitable method to store mango pollen for one week. Furthermore, an effective pollen viability test is described that can be used to assess the quality of stored pollen before its use in crossing, which will improve the crossing success rate in mango.

3.2 Introduction

Breeding has been successfully employed for mango crop improvement in many countries including Australia, Israel, Brazil, India, and Mexico (Bally *et al.*, 2009b; Brettell *et al.*, 2004; Carvalho *et al.*, 2004; Chapman, 2000; Human *et al.*, 2006; Tomer *et al.*, 1997). Mango breeding objectives vary between programs, as they reflect local environmental conditions and target markets. Common breeding objectives include dwarf phenotypes, regular fruit bearing, higher disease resistance, attractive cosmetic qualities such as fruit size, fruit skin colour, improved fruit quality and longer shelf life (Bally *et al.*, 2009a; Usman *et al.*, 2001). The main objectives of the Australian Mango Breeding Program are to develop cultivars with dwarf

phenotypes, adequate disease resistance, high yields, and improved fruit quality (Bally *et al.*, 2009b; Dillon *et al.*, 2013).

A classical breeding approach based on hand pollination has been adopted in many mango breeding programs for novel cultivar and rootstock development (Bally *et al.*, 2009a; Brettell *et al.*, 2004; Mukherjee *et al.*, 1961; Mukherjee *et al.*, 1968). Hand pollination is an inefficient, laborious, costly, and time-consuming approach that results in very low seed production (Roizman, 1986). Despite this inefficiency, significant progress has been made in developing novel mango cultivars using hand pollination (Bally, 2013). Hand pollination has contributed towards the generation of great diversity in relatively small hybrid populations, which enhances the likelihood of a unique combination of desirable alleles in individual genotypes. Therefore, hand pollination is a more successful approach compared to open pollination, which produces a higher proportion of progeny from self-pollinated parents (Honsho *et al.*, 2012).

Controlled crosses can be performed via hand pollination or via the enclosure of parental lines with suitable pollinators in an otherwise insect-proof cage (Bally *et al.*, 2009a). Controlled crosses offer an advantage over open pollination crosses in respect to the selection and identification of both ovule- and pollen-bearing parents. Hand pollination can be easily performed on existing trees (Bally *et al.*, 2009b), regardless of if they exist within an experimental plot or a plantation, and does not require purpose-grown blocks of trees to facilitate pollen transfer and crossing between cultivars. Hand pollination can also be used to cross individual trees that are spatially or temporally distant (Bally *et al.*, 2009a). Successful hand pollination requires an understanding of pollen biology and appropriate pollen handling and storage.

Mango flowering times vary among genotypes and environments, which occasionally results in little to no overlap in flowering periods between individual cultivars. Pollen storage can be used to extend pollen longevity, thus overcoming crossing barriers due to asynchronous flowering times between individuals. Short-term pollen storage provides a breeder with viable pollen throughout a flowering season (approximately four weeks) and allows hand pollination of a late flowering variety with pollen from earlier flowering variety (Chaudhury *et al.*, 2010; Dutta *et al.*, 2013b; Mishra and Shivanna, 1982; Sedgley and Harbard, 1993). The hand pollination of early flowering varieties with pollen from a late flowering variety requires pollen storage from one season to the next.

Mango pollen exhibits a short period of viability (<6 hours) and is sensitive to desiccation (Dutta *et al.*, 2013a; Issarakraisila and Considine, 1994). Various terms have been used to describe pollen viability, including pollen quality, stainability, sterility, viability, and germinability (Akond *et al.*, 2012; Dafni and Firmage, 2000; Dafni *et al.*, 2005; Dutta *et al.*, 2013a; Dutta *et al.*, 2013b). Pollen viability varies significantly among plant species and in response to different storage conditions (Akond *et al.*, 2012; Dafni and Firmage, 2000; Hanna and Towill, 1995). *In vitro* pollen germination is an effective method to assess pollen vigour and is a more accurate indicator than pollen staining methods (Sedgley and Griffin, 1989). A successful *in vitro* pollen germination system is a prerequisite for pollen research and is necessary for testing the capacity and viability of stored pollen before it is used for hand pollination (Griffin, 1982). High pollen viability is important for successful breeding and the profitability of commercial fruit crops (Hanna and Towill, 1995; Shivanna and Rangaswamy, 1992).

Short and long-term pollen storage is depended on critical external factors that affect pollen viability, including relative humidity and storage temperature. In general, lower storage temperatures are needed for longer storage periods (Siregar and Sweet, 2000). Ultra-low temperature storage methods are laborious and require sophisticated apparatus such as liquid nitrogen cryotanks (Dutta *et al.*, 2013b), which reduces their suitability for routine work. Therefore, a more simple, effective method of pollen storage is needed to facilitate classical breeding (Mishra and Shivanna, 1982). This study aims to investigate simple and cost-effective methods for short-term mango pollen storage (i.e., weeks) that would suit the routine hybridization activities within the flowering season of a mango breeding program. Furthermore, this work intends to develop a suitable *in vitro* pollen germination protocol to test stored pollen viability.

In the current experiment, organic solvent and mineral oil, in combination with ambient or low temperatures, were used as media to maintain pollen viability during extended storage. The viability of mango pollen grains was evaluated following different combinations of storage media, temperature, and duration. For the various storage durations, the effectiveness of media use was compared to pollen stored alone at low temperatures. Experimental treatments were designed so that pollen viability following short-term storage could be monitored at one-week intervals. Before pollen was stored, different pollen germination protocols were also tested to optimise post-storage *in vitro* pollen germination assay. The current study was based on the

hypothesis that organic solvent and mineral oil maintain mango pollen grain viability regardless of temperature during short-term storage, as has been reported in other crops such as crotalaria, lilies, peach, and apple (Agarwal, 1983; Iwanami and Nakamura, 1972; Jain and Shivanna, 1988b; Jain and Shivanna, 1988a; Jain and Shivanna, 1990; Liu and Cao, 1984).

3.3 Materials and methods

3.3.1 Pollen collection and drying

The pollen used in this experiment was collected from freshly opened flowers of the mango cultivar NMBP-1201 between 8:00 and 10:00 (≤23°C) am in September 2016 at Walkamin Research Station (17°8′17″S 145°25′41″E; Elevation: 599.23 m), Department of Agriculture and Fisheries (DAF), Mareeba. Flowers were collected prior to anthesis, a stage characterised by pink anther colour. The harvested flowers were transported to the laboratory at the Department of Agriculture and Fisheries (DAF), Mareeba, and placed in the sun to prompt anther dehiscence. Freshly dehisced anthers, with pollen grains visible on the anthers as a greyish powder, were collected in a petri dish and dried in a desiccator for two hours.

3.3.2 Pollen storage

Storage media

Dried pollen grains from 20 flowers were resuspended in the different storage media in a 2.0 ml screw-cap free-standing microtube (Astral Scientific Pty Ltd, Gymea, NSW, Australia) and stored at room temperature in a controlled growth room (27ºC) and at low temperatures in a cold-storage facility (4ºC, -20ºC, -80ºC) at the Department of Agriculture and Fisheries (DAF), Mareeba. The experimental mango pollen storage treatments are listed in Table 3.1.

Table 3-1: Media used for experimental pollen storage

Storage Media	**Details**
Control	No solvent, pollen grains stored alone in microtube.
Hexane	n-Hexane, 95% anhydrous; Sigma-Aldrich Pty. Ltd., Sydney, Australia; one of the most favourable organic solvents for *Crotalaria retusa* L. pollen storage (Jain and Shivanna, 1988b; Jain and Shivanna, 1988a).
Paraffin oil	Paraffin oil, viscous liquid; Sigma-Aldrich Pty. Ltd., Sydney, Australia; commercially available mineral oil, effective in *Crotalaria retusa* pollen storage (Jain and Shivanna, 1990)

Storage temperature

Pollen grains were stored at one of four experimental storage temperatures: (i) 25–27ºC (ambient temperature); (ii) 4ºC; (iii) -20ºC; and (iv) -80ºC.

Storage duration

Pollen storage was maintained for one of four storage durations: (i) 1 week; (ii) 2 weeks; (iii) 3 weeks; and (iv) 4 weeks.

3.3.3 Pollen removal from storage and sample preparation for germination analysis

The pollen grains were removed from storage each week for up to four weeks. Pollen grains were separated from oil/organic solvent via a filtration process through Whatman No. 5 filter paper (Sigma-Aldrich Pty. Ltd., Sydney, Australia) before they were assessed in an *in vitro* germination test (Iwanami, 1984). Whatman No. 5 filter paper was used in oil treatments to remove the paraffin oil from the pollen surface. Paraffin oil along with pollen grains was poured onto the filter paper directly from the screw cap microtubes and allowed to filter through, leaving pollen grains on the paper. The pollen grains on the filter paper were then rinsed 4–5 times with hexane to remove residual oil, before they were dried for 10–15 minutes.

3.3.4 Data collection

In vitro *pollen germination*

Pollen viability was tested following removal from storage media using the *in vitro* germination test described by Iwanami (1984).

Pollen germination test protocol development

A previously described germination test (Chaudhury *et al.*, 2010) did not result in mango pollen germination due to the lack of important nutrients in the germination media (calcium, magnesium, and potassium). Thus, several alternative pollen germination protocols were tested in 2014 and 2015 (Table 3-2 and 3-3).

To test each protocol, 2–3 drops of germination medium was placed onto the germination surface. Mango cv. 1201 pollen grains were transferred to the germination surface and, for

microscope slide germination surfaces, covered with coverslip. Germination tests were incubated at 27±2ºC for 6 hours, after which a light microscope (model BH2, Olympus, Eagle Farm, QLD, Australia; 10X magnification) was used to view and quantify germinated and non-germinated pollen grains. Pollen grains were considered as germinated when pollen tube length surpassed their diameter. Three microscope slides were prepared and quantified per germination test, and germination data were used to calculate average pollen germination rate per treatment.

Table 3-2: Germination media used in mango pollen *in vitro* germination tests

No.	**Germination media recipe**
1	15% Sucrose + 20 ppm H_3BO_3 + 20 ppm IAA (Chaudhury *et al.*, 2010)
2	0% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3
3	2.5% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3
4	5% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3
5	7.5% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3
6	10% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3 (Brewbaker and Kwack, 1963)
7	15% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3
8	25% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3

Table 3-3: Germination surfaces used in mango pollen *in vitro* germination tests

No.	**Germination surfaces**
1	Simple microscope slide
2	Single concave microscope slide
3	Petri dish (hanging drop method)
4	24-well polystyrene plate

In vitro *pollen germination test*

Microscope slides were prepared for *in vitro* pollen germination by placing 2–3 drops of germination medium (10% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3) on the slide. Pollen grains (~400–500) were transferred by dipping segments of filter paper that held pollen recovered from storage, which had been filtered and rinsed, in the germination medium contained on a slide. Pollen grains in germination medium were covered with a cover slip and incubated at 27±2ºC for 6 hours. Following incubation, a light microscope (model BH2, Olympus, Eagle Farm, QLD, Australia; 10X magnification) was used to view *in vitro* pollen germination and quantify germinated and non-germinated pollen grains. Four slides were assessed per storage treatment and germination rate data were used to calculate average pollen germination rate per treatment.

3.3.5 Experimental layout and statistical data analysis

The experimental design was a completely randomized design (CRD) having factorial arrangements with three factors (storage media, storage temperature, and storage duration), replicated four times. Data were subjected to square root transformation to meet the assumption of ANOVA that data should have normal distribution. The experimental data were analysed by ANOVA using SAS/STAT software version 9.0 to test overall data significance. Treatment average values were compared with the least significant difference test (LSD) to determine the presence of significant differences among treatment average values. Analysed data average values were back-transformed for biological interpretation.

3.4 Results

3.4.1 Pollen germination test protocol development

At the commencement of this study, pollen germination was tested on several different surfaces using germination media described by Chaudhury *et al.* (2010). No pollen germination was observed except for a low pollen germination rate on simple microscope slides. Therefore, the simple microscope slide as an appropriate germination test surface was selected for further testing. The highest germination rate (83.33%) was observed in pollen incubated in germination medium #6 containing 10% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3, and no pollen germination was apparent in the other tested germination media (Table 3-4). Therefore, the combination of simple microscope slides and

germination medium #6 was used for the remaining mango pollen *in vitro* germination tests in this study.

Table 3-4: The effects of germination media on mango cv. NMBP-1201 pollen germination rates. Germination tests were replicated three times with a sample size of ~400–500 pollen grains. Data were subjected to ANOVA and LSD to highlight statistical differences among germination rates resulting in various germination media. Different letters associated with germination rates indicate statistically significant differences.

Germination media	**Germination rate (%)**
(1) 15% Sucrose + 20 ppm H_3BO_3 + 20 ppm IAA	0.00^b
(2) 0% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3	0.00^b
(3) 2.5% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3	0.00^b
(4) 5% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3	0.00^b
(5) 7.5% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3	0.00^b
(6) 10% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3	83.33^a
(7) 15% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3	0.00^b
(8) 25% sucrose + 100 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3	0.00^b
l.s.d. ($p \leq 0.0001$)	0.71

3.4.2 *In vitro* germination test of fresh pollen

A germination test of fresh mango cv. NMBP-1201 pollen was performed according to the *in vitro* germination method described in section 4.3.1, which revealed an 87.22% germination rate (Table 3-5).

Table 3-5*:* *In vitro* germination test of fresh mango cv. NMBP-1201 pollen incubated at 27±2ºC for 6 hours. The germination test was replicated three times with a sample size of ~400–500 pollen grains.

Pollen type	**Germination rate (%)**
Fresh pollen	87.22

3.4.3 Effect of storage conditions on pollen germination rate

The ANOVA indicated that storage medium, duration, temperature, and the interactions between these factors had highly significant effects (*P*=0.001) on pollen germination rates (Table 3-6).

Table 3-6: ANOVA describing the effects of storage medium, duration, temperature, and their interactions on the *in vitro* germination rate of mango cv. NMBP-1201 pollen.

Effect	Degrees of Freedom	F-Value	Significance P level
Storage medium (M)	2	100.87	<0.0001
Storage duration (D)	3	148.61	<0.0001
Storage temperature (T)	3	6.56	0.0003
M × D	6	5.37	<0.0001
M × T	6	38.09	<0.0001
D × T	9	2.59	0.0085
M × D × T	18	3.87	<0.0001

Effect of storage media on pollen germination rate

The type of storage media had significant effects on pollen germination rates (Table 3-7). Those pollen grains subject to the control treatment (pollen grains stored alone) displayed the highest pollen germination rate (11.91%), and pollen stored in paraffin oil displayed the lowest germination rate (1.84%; Table 3-7).

Table 3-7: Effect of storage media type on overall pollen germination rate.

Storage medium	Germination rate ™ (%)	Germination rate BTM (%)
Control (No medium)	3.45[a]	11.91
Hexane	2.70[b]	7.30
Paraffin oil	1.36[c]	1.84

™ = square root transformed means; BTM = Back-transformed means; n = 64; Different letters associated with germination rates indicate statistically significant differences.

Effect of storage temperature on pollen germination rate

Pollen germination was significantly affected by storage temperature (Table 3-8). Pollen stored at -80ºC maintained the highest germination rate; however, the pollen germination rate following -80ºC storage was not significantly different to that observed for pollen stored at

both 4ºC and -20ºC. Pollen stored at ambient temperature displayed the lowest germination rate (Table 3-8).

Table 3-8: Effect of storage temperature on overall pollen germination rate.

Storage temperature	Germination rate ™ (%)	Germination rate BTM (%)
Ambient (25–27ºC)	2.04[b]	4.15
4ºC	2.62[a]	6.85
-20ºC	2.65[a]	7.04
-80ºC	2.71[a]	7.32

™ = square root transformed means; BTM = Back-transformed means; n = 48; Different letters associated with germination rates indicate statistically significant differences.

Effect of storage duration on pollen germination rate

Storage duration had a considerable impact on pollen germination rates, in that germination rates were reduced with increasing storage duration (Table 3-9). Aside from that observed for fresh pollen, the highest and lowest germination rates were apparent following one and four weeks of storage, respectively. No significant difference was observed between the germination rates of pollen stored for either two or three weeks (Table 3-9).

Table 3-9: Effect of storage duration on overall pollen germination rate.

Storage duration	Germination rate ™ (%)	Germination rate BTM (%)
1 week	4.55[a]	20.68
2 week	2.30[b]	5.29
3 week	2.18[b]	4.75
4 week	0.99[c]	0.97

™ = square root transformed means; BTM = Back-transformed means; n = 48; Different letters associated with germination rates indicate statistically significant differences.

Effect of storage medium in combination with storage temperature on pollen germination rate

The effect of pollen storage displayed the same trend during all storage durations (Table 3-10). Storage of pollen alone (control) resulted in the maintenance of significantly higher germination rates compared to that observed for pollen stored in hexane and paraffin oil. The highest and lowest germination rates were measured in the control treatment following one-week storage and in paraffin oil following four-week storage, respectively (Table 3-10).

Table 3-10: Effect of storage medium and duration on pollen germination rate.

Storage medium	Storage duration (weeks)							
	1		2		3		4	
	TM	BTM	TM	BTM	TM	BTM	TM	BTM
Control[+]	6.02[a]	36.19	2.70[cd]	7.26	3.24[c]	10.48	1.86[e]	3.44
Hexane	4.58[b]	20.94	2.53[d]	6.39	2.71[cd]	7.33	1.00[f]	0.99
Paraffin oil	3.05[cd]	9.31	1.68[e]	2.83	0.59[fg]	0.35	0.11[g]	0.01

[+] = Pollen stored alone; TM = square root transformed means; BTM = back-transformed means; n = 16; Different letters associated with germination rates indicate statistically significant differences.

Effect of storage temperature and duration on pollen germination rate

No significant differences were observed among the germination rates of pollen stored at different temperatures for one week (Table 3-11). Furthermore, there were no significant differences between the germination rates of pollen stored at ambient temperature following two-, three-, and four-week storage. Pollen stored at -20ºC for two weeks and -80ºC for three weeks had comparable pollen germination rates. The highest and lowest pollen germination rate was recorded in pollen stored at -80ºC for one week and in pollen stored at 4ºC for four weeks, respectively (Table 3-11).

Table 3-11: Effect of storage temperature and duration on pollen germination rate.

Storage temperature	Storage duration (weeks)							
	1		2		3		4	
	TM	BTM	TM	BTM	TM	BTM	TM	BTM
Ambient (25–27ºC)	4.51[a]	20.38	1.39[c]	1.94	1.30[c]	1.68	0.95[c]	0.90
4ºC	4.50[a]	20.24	2.31[b]	5.33	2.76[b]	7.62	0.90[c]	0.82
-20ºC	4.34[a]	18.86	2.82[b]	7.92	2.42[b]	5.86	1.04[c]	1.08
-80ºC	4.84[a]	23.39	2.69[b]	7.22	2.24[b]	5.00	1.06[c]	1.13

TM = square root transformed means; BTM = back-transformed means; n = 12; Different letters associated with germination rates indicate statistically significant differences.

Effect of storage medium and temperature on pollen germination rate

Experimental results showed that storage media type had significant effects on germination rates of pollen stored at different storage temperatures (Table 3-12). Pollen grains stored alone (control) and in paraffin oil displayed comparably higher germination rates when combined with -20ºC and -80ºC storage. In contrast to control and paraffin oil storage conditions, pollen stored in hexane at ambient temperature possessed the highest germination rate. Statistically

comparable low germination rates were observed for pollen stored in hexane at -20ºC and -80ºC. The highest pollen germination rate was measured in control storage at -20ºC (21.34%); however, this rate was statistically similar to that resulting from control storage at -80ºC (19.43%) and hexane storage at ambient temperature (17.61%; Table 3-12).

Table 3-12: Effect of storage medium in combination with storage temperature on pollen germination rate.

Storage medium	**Storage temperature**							
	Ambient (25–27ºC)		**4ºC**		**-20ºC**		**-80ºC**	
	TM	**BTM**	**TM**	**BTM**	**TM**	**BTM**	**TM**	**BTM**
Control[+]	1.46[c]	2.12	3.32[b]	11.04	4.62[a]	21.34	4.41[a]	19.43
Hexane	4.20[a]	17.61	2.89[b]	8.33	1.82[c]	3.30	1.91[c]	3.64
Paraffin oil	0.46[d]	0.22	1.64[c]	2.70	1.53[c]	2.33	1.80[c]	3.24

[+] = No medium; TM = square root transformed means; BTM = back-transformed means; n = 16; Different letters associated with germination rates indicate statistically significant differences.

Effect of storage medium in combination with both storage temperature and duration on pollen germination rate

The highest pollen germination rate in this study was recorded for pollen stored alone (control) at -80ºC for one week (46.28%), which was statistically comparable to pollen stored in control conditions at 4ºC and -20ºC for one week (36.12% and 35.44%, respectively), pollen stored in control conditions at -20ºC for three weeks (35.68%), and pollen stored in hexane at ambient temperature for one week (40.77%; Table 3-13). No or minimal pollen germination was recorded for pollen stored in control conditions at ambient temperature for either three or four weeks; pollen stored in hexane at 4ºC, -20ºC, and -80ºC for 4 weeks; and pollen stored in paraffin oil for two, three, and four weeks at ambient temperature, for three and four weeks 4ºC, and for three and four weeks at -20ºC and -80ºC (Table 3-13).

Table 3-13: Effect of storage medium in combination with storage temperature and duration on pollen germination rate

Storage medium	Storage temperature	Storage (weeks)							
		1		2		3		4	
		TM	BTM	TM	BTM	TM	BTM	TM	BTM
Control[+]	Ambient (25–27ºC)	5.30^{bc}	28.09	0.52^{qrs}	0.27	0.00^{s}	0.00	0.00^{s}	0.00
	4ºC	6.01^{ab}	36.12	$2.84^{fghijkl}$	8.08	2.76^{ghijkl}	7.61	1.68^{lmnopq}	2.82
	-20ºC	5.95^{ab}	35.44	3.99^{def}	15.92	5.97^{ab}	35.68	2.57^{hijkl}	6.58
	-80ºC	6.80^{a}	46.28	$3.43^{defghij}$	11.75	4.22^{cde}	17.79	$3.18^{efghijk}$	10.13
Hexane	Ambient (25–27ºC)	6.38^{ab}	40.77	3.66^{defgh}	13.42	3.89^{defg}	15.13	$2.85^{fghijkl}$	8.09
	4ºC	4.04^{de}	16.28	2.17^{klmno}	4.72	4.53^{cd}	20.50	0.81^{pqrs}	0.66
	-20ºC	3.58^{defghi}	12.84	2.07^{klmno}	4.28	1.29^{mnopqr}	1.66	0.33^{rs}	0.11
	-80ºC	4.30^{cde}	18.52	2.21^{klmn}	4.88	1.12^{nopqrs}	1.25	0.00^{s}	0.00
Paraffin oil	Ambient (25–27ºC)	1.86^{lmnop}	3.45	0.00^{s}	0.00	0.00^{s}	0.00	0.00^{s}	0.00
	4ºC	$3.45^{defghij}$	11.92	1.91^{lmnop}	3.66	1.00^{opqrs}	0.99	0.22^{rs}	0.05
	-20ºC	$3.49^{defghij}$	12.20	2.39^{jklm}	5.70	0.00^{s}	0.00	0.22^{rs}	0.05
	-80ºC	$3.40^{defghij}$	11.58	2.43^{ijklm}	5.88	1.37^{mnopqr}	1.89	0.00^{s}	0.00

[+] = No storage medium; TM = square root transformed means; BTM = back-transformed means; n = 4; Different letters associated with germination rates in the same column indicate statistically significant differences.

3.5 Discussion

In the current study, the pollen germination medium constituents used by Brewbaker and Kwack (1963) facilitated the highest germination rate in mango cv. NMBP-1201 pollen. For this specific germination medium, an increase or decrease in the sucrose concentration (10%) is thought to reduce the pollen germination rate (Brewbaker and Kwack (1963). Brewbaker and Majumder (1961) reported low pollen germination rates when using a sucrose concentration between 2% and 40%, with a noticeable reduction in pollen germination at more than 40% sucrose content and pollen bursting at less than 2% sucrose content. Here, a lack of pollen germination also resulted when using the germination medium described by Chaudhury *et al.* (2010), which may be the result of missing essential nutrients such as calcium, magnesium, and potassium that stimulate and regulate pollen germination and pollen tube growth (Brewbaker and Kwack, 1963; Imani *et al.*, 2011).

This study showed that fresh mango pollen had a significantly higher germination rate (87.22%) than that of mango pollen stored under any of the tested conditions. Similarly, past reports by Dutta *et al.* (2013a) and Martínez-Gómez *et al.* (2002) observed higher pollen germination rates for fresh pollen than that for stored pollen of other fruit crops. However, Chaudhury *et al.* (2010) demonstrated that stored mango pollen exhibiting a pollen germination rate greater than 55%, which is capable of inducing fruit set similar to fresh pollen.

Compared to fresh pollen, significantly lower pollen germination rates in pollen stored in media such as hexane and paraffin oil demonstrates the sensitivity of mango pollen to extended storage media exposure. These results contradict previous studies in other crops that report pollen stored in different organic solvents and mineral oils maintained high pollen viability (40–90%) for short- and long-term periods (Iwanami and Nakamura, 1972; Iwanami, 1973; Jain and Shivanna, 1988b; Jain and Shivanna, 1990; Jain *et al.*, 1990). The data presented here suggest that hexane and paraffin oil are phytotoxic to mango pollen following storage for more than one week. Potential reasons for the phytotoxic effects of this media were not investigated. However, decreased pollen viability from exposure to organic solvents may be the result of leaching of phospholipids, sugars, and amino acids from the stored pollen (Jain and Shivanna, 1988a). The loss of these compounds compromises pollen membrane integrity and consequently affects pollen viability (Jain and Shivanna, 1988a; Jain and Shivanna, 1988b; Jain and Shivanna, 1989).

In addition to having a negative effect on pollen germination, the extraction of pollen from paraffin oil storage is a complex process that is not practical for breeding work in the field. Conversely, pollen extraction from hexane is a rapid, straightforward process. The exposure of hexane-stored pollen to open air on filter paper allows the hexane to evaporate, therefore easily recovering the stored pollen. In addition to maintaining reasonable pollen germination rates (40.77%) following one-week hexane storage at ambient temperature, the ability to rapidly extract pollen from hexane highlights its potential as a suitable mango pollen storage medium. The results of the current study confirm the report of Stanley and Linskens (2012), that organic solvent use is a convenient technique for pollen transport without the need for refrigeration or dry ice.

The data presented here show that pollen germination rates following one-week 4ºC, -20ºC, and -80ºC storage were statistically comparable, which suggests that 4ºC storage is sufficient for routine breeding work performed within a week. These results do not agree with previous studies, which report that a decrease in storage temperature increases mango pollen longevity (Chaudhury *et al.*, 2010; Dutta *et al.*, 2013a; Khan and Perveen, 2009). The decline in mango pollen viability following four-week storage at room temperature may be attributed to a high sensitivity to ambient temperature and low relative humidity (Akond *et al.*, 2012; Dutta *et al.*, 2013a). Common household freezers are sufficient for short-term mango pollen storage (Hanna and Towill, 1995). These results validate existing mango pollen storage practices during hand pollination activities, whereby mango pollen is stored in a refrigerator for one or two days following harvest to accommodate various inconveniences, such as the lack of receptive flowers for crossing. This stored pollen may maintain a germination rate sufficient to induce high-level fruit set in hand-pollinated crosses, similar to results reported by Chaudhury *et al.* (2010) that stored pollen exhibiting a ≥55% pollen germination rate facilitated fruit set comparable to that resulting from the use of fresh mango pollen.

Low pollen germination rates following more than one-week storage indicate the negative effects of extended storage duration on pollen integrity. Differential pollen viability associated with storage at different temperatures may be the result of the temperature-dependent metabolic activity rate of pollen (Dutta *et al.*, 2013a). The loss of pollen viability over time observed here is considerably higher than that reported in prior studies on pollen storage and subsequent germination rates (Akond *et al.*, 2012; Chaudhury *et al.*, 2010; Dutta *et al.*, 2013a). The difference in germination rates measured in this work and those in previous studies may be the

result of genetic effects from different variety in use (Dutta *et al.*, 2013a; Khan and Perveen, 2009). The decrease in stored pollen viability may be associated with the activity of enzymes that reduce respiratory substrates. A decline in respiratory substrates is associated with reduced cellular respiration rates e.g. sugar conversion to organic acids, which is why higher sucrose concentrations are required to obtain optimum germination of stored pollen (Stanley and Linskens, 2012).

In conclusion, the current study shows that, irrespective of storage medium or temperature, mango cv. NMBP-1201 pollen is sensitive to storage that extends for more than one week. Stored pollen exhibited a significant loss in viability despite the use of low temperature and storage media. Hexane as a pollen storage medium maintains up to 40% pollen viability during storage for more than a week at room temperature, and the extraction of mango pollen from hexane is a simple process. In addition to negatively affecting stored pollen germination rates, the extraction of mango pollen from paraffin oil storage is difficult compared to that from hexane, making paraffin oil a less suitable option for pollen storage. Therefore, for routine hybridization activities in mango breeding programs, pollen can be stored in hexane for one week at room temperature without the detrimental loss of pollen viability. The pollen germination test described in this study can also be used to test the germination capability of previously stored pollen in order to avoid using unviable or low-viable pollen. The results of this study have the potential to improve mango breeding efficiency by providing a technique for storing pollen for one week following pollen harvest and its subsequent successful retrieval. This ensures the availability of quality pollen, which may improve the efficiency of routine mango breeding work and result in a higher number of hybridised fruit. However, these results may not be applicable to all mango cultivars as pollen germination differs among cultivars (Ramírez and Davenport, 2016). Therefore, to ensure adequate storage of viable mango pollen, further research is recommended on the effects of other various organic solvents and mineral oils on the pollen of other mango cultivars, as well as the tolerance of this pollen to different storage durations.

Chapter 4: Identification and heritability assessment of the most efficient tool for measuring tree vigour in mango breeding families

4.1 Abstract

Tree vigour refers to the intensity of vegetative growth in fruit trees. In mango, tree vigour is negatively correlated to the bearing of fruit and, therefore, control over vigour is highly desirable for high-density production systems. The assessment of vigour in large fruit tree breeding populations is laborious and time-consuming. A method for rapid vigour assessment is important for improving the efficiency of fruit tree breeding programs. The current study was conducted to develop a method for rapid vigour assessment based on an easily measured trait and estimate its heritability in a mango breeding population. Twelve traits were evaluated to determine their correlation with tree vigour in 40 selected mango trees. A heritability assessment of the morphological tree trait that displayed the highest correlation with tree vigour was carried out. Alongside a number of promising morphological traits such as tree height and branch dry matter, trunk cross-sectional area (TCA) was identified as highly positively correlated with tree vigour (r=0.828). TCA was measured in 1909 progeny from 41 mango breeding families within the Australian Mango Breeding Program. Narrow sense heritability was low (h^2 = 0.23) for TCA, which suggests there is a greater influence of environment on this trait than genotype. However, a number of promising low-vigour families were identified as next-generation parents for future crosses to develop low vigour mango plants. Low TCA heritability encourages the use of a model consisting of other morphological traits exhibiting high correlation with tree vigour, such as tree height and branch dry matter, in conjunction with TCA to assess plant vigour.

4.2 Introduction

Nesme *et al.* (2005) defined tree vigour as the intensity of tree vegetative growth such as long lateral branching. Jerie *et al.* (1989) observed a negative correlation between tree vigour and fruit productivity of fruit trees. Worldwide, a number of traditional mango varieties are characterized as high-vigour and low-yield compared to varieties in other fruit crops (Bally and Ibell, 2015). The increasing popularity of high-density and ultra–high density planting systems is not conducive with the intensive labour requirements needed for both the training and

pruning of high-vigour fruit trees (Byrne, 2012; Horton, 1985). Moreover, due to their large canopies, high-vigour mango trees reduce the efficiency of chemical application as well as their own photosynthetic productivity and that of adjacent trees (Bally and Ibell, 2015). Control of tree vigour improves orchard productivity and labour efficiency, and offers cost-effective management by reducing labour costs and pesticide consumption (Fideghelli *et al.*, 2003; Olmstead *et al.*, 2006). The need for cost-effective orchard management encourages research into mechanisms to alter tree growth and size (Byrne, 2012). The breeding of low-vigour dwarf fruit trees may be a viable option for effective canopy management in high-density planting systems.

A number of methods for measuring tree vigour in woody fruit species have been developed (Barden and Marini, 2001; Barden *et al.*, 2002; Nesme *et al.*, 2005; Saeed *et al.*, 2010; Srivastav *et al.*, 2009). The simplest measurement is based on trunk diameter (Guxiong *et al.*, 1987; Vargas and Romero, 1998). In a historic study, Beakbane and Thompson (1939) highlighted a link between higher phloem to xylem ratios and dwarfing rootstocks in apple. Further studies reported that vegetative growth in apple was associated with the anatomical features of roots (Beakbane, 1953), stems (Beakbane, 1941), and leaves (Beakbane, 1967). Mango tree vigour has been quantified using measurements of stem growth, bark percentage of root, and area of root vessels (Majumdar *et al.*, 1972). A higher phloem to xylem ratio was also associated with dwarf phenotypes in mango trees. Kurian and Iyer (1992) observed that low-vigour mango trees exhibited higher phloem to xylem ratio. However, xylem and phloem ratio is not a rapid enough test for a breeding program that assesses large number of trees each day.

Trunk cross-sectional area (TCA) has also been used to estimate and assess tree vigour in fruit tree crops (Barden *et al.*, 2002; Khatamian and Hilton, 1977). Various leaf traits (Abirami *et al.*, 2011; Guxiong *et al.*, 1987; Liang *et al.*, 2010; Pal *et al.*, 1983; Yanjun *et al.*, 2011; Ying *et al.*, 2010), branch and stem traits (Fen-xue *et al.*, 2008; Ma *et al.*, 2011; Saeed *et al.*, 2010; Shi *et al.*, 2000; Srivastav *et al.*, 2009; Wang and Li, 2006; Yanjun *et al.*, 2011), and root traits (Srivastav *et al.*, 2009) are also reported to correlate with tree vigour in different fruit crops.

The efficiency of mango breeding programs can be improved by more effective parental selection in crosses and assessment of the resulting progeny. Selection of candidate parents for breeding low-vigour/dwarf trees requires rapid and reliable vigour assessment techniques. Traits that are suitable for selection are those that have a moderate to strong correlation with

vegetative vigour and a high level of inheritance in subsequent hybridised generations. Many tree morphological traits reported in peer-reviewed horticultural breeding research are quantitative traits that do not follow Mendelian inheritance (Griffiths *et al.*, 2015). Narrow-sense heritability (h^2) is the ratio of additive variance to the phenotypic variance among individuals in a population (Griffiths *et al.*, 2015). The h^2 value is often employed to predict the phenotypes of breeding progeny because it provides a measure of parental genetic influence on progeny phenotypes. The h^2 value also measures the response of a trait to selective breeding (Griffiths *et al.*, 2015; van Buijtenen, 1992).

Best linear unbiased prediction (BLUP) is a standard technique used in animal breeding for calculating the random effects of a mixed model. This method was devised to estimate breeding values in animals, which form the basis for selecting the most suitable family or individual progeny. Two types of BLUPs are typically employed in plant breeding: the first is based on total genetic variance and the second is based on additive genetic variance. The former is used to estimate the phenotypic performance of a genotype in commercial testing. The latter determines the performance of a genotype as a parent in future crosses, and is also referred to as the breeding value (Piepho *et al.*, 2008).

The current study describes the identification of the most efficient mean to measure tree vigour in mango breeding populations. Once several breeding populations were measured for this vigour-predicting trait, heritability analysis was used to identify families and individuals with the highest trait heritability as candidate parental lines in future breeding programs. This work explores tree morphological traits that assist in parent selection to promote dwarf phenotypes in mango.

4.3 Materials and methods

4.3.1 Overall aim

This study was conducted to investigate the correlation between selected tree morphological traits and tree vigour, with the aim to characterise time-efficiently observable trait that efficiently predicts tree vigour in mango breeding populations.

The experiment was conducted in two stages:

1. Identify the most efficient method of measuring tree vigour by calculating the correlation between 12 morphological traits and tree vigour; and
2. Conduct a heritability analysis for the morphological trait that displays the strongest positive correlation with vegetative vigour, and identify candidate parental lines with low vegetative vigour and high heritability for use in breeding of low-vigour trees.

4.3.2 Identifying the most efficient way of measuring tree vigour

Aim

To develop a rapid but accurate method of measuring tree vigour in mango breeding populations in order to efficiently assess large numbers of progeny. This will be achieved by identifying one or more easily measured tree morphological traits that are correlated with tree vigour (based on canopy volume at a given age).

Experiment site and plant material

Morphological observations were conducted on 40 six-year-old seedlings (non-grafted) from the mango gene pool and mango breeding populations at Southedge Research Station (SERS), Department of Agriculture and Fisheries (DAF), Mareeba (Fig. 4-1). The trees selected for the study had not been pruned (hedging and skirting) for 18 months. Vegetative vigour was represented in these trees by total canopy volume (over the life of the trees; 6 years), which was measured using a modified version of the method described by O'Farrell *et al.* (2010). The canopy shape of experimental trees was cylindrical following an 18-month period of no pruning (hedging and skirting). Canopy height and average canopy radius (n=3) were measured as illustrated in Fig. 4-2, and canopy volume was calculated using the formula described by Frank (2010) for cylindrical shaped canopy, as follows:

$V = \pi r^2 h$

Where V = canopy volume, $\pi = 3.14159$, r = radius of canopy, and h = height of canopy.

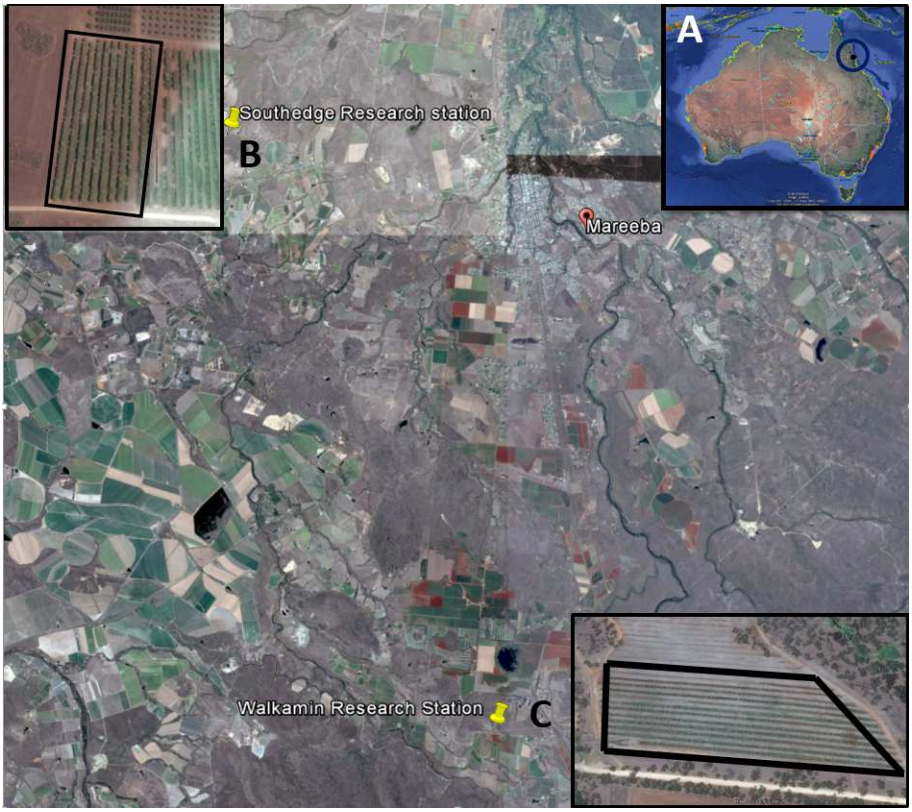

Fig. 4-1: (A) Location of the experimental sites in Queensland, Australia. (B) Aerial view of experimental trees at Southedge Research Station (16°58′44.34″S, 145°20′37.22″E, elevation 457 m). (C) Walkamin Research Station (17°8′14.20″S, 145°24′52.22″E, elevation 576 m).

Tree vigour

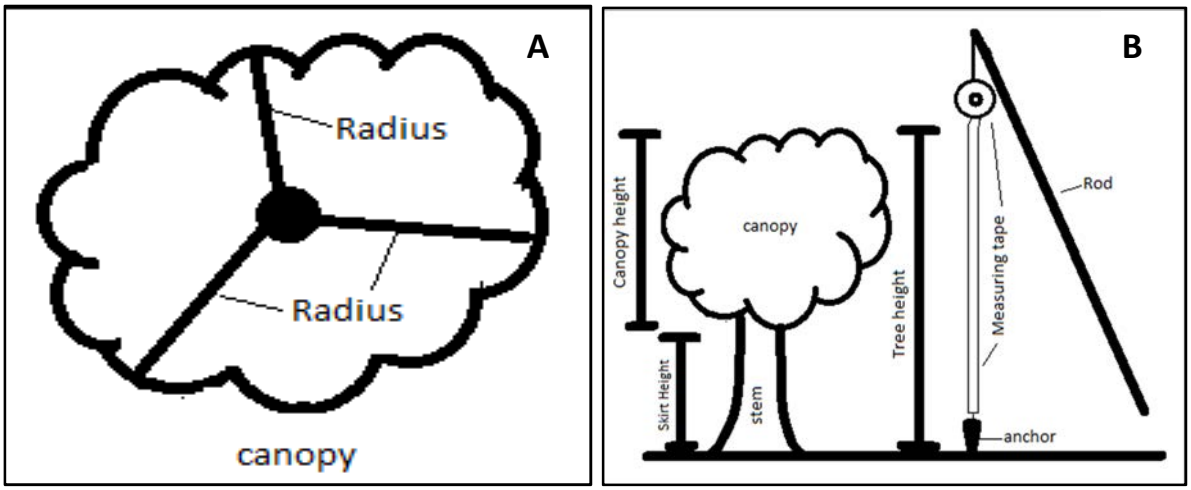

Fig. 4-2: Canopy volume measurement. (A) Measurement of canopy radius at three points under canopy as described by O'Farrell *et al.* (2010). (B) Measurement of tree height and skirt height (distance between ground and canopy bottom). Canopy height was determined as the difference between skirt height and tree height.

Traits measured

A total of 12 morphological characters (leaf- and stem-based traits) were measured, and assessed by correlation analysis with canopy volume. Traits analysed in the experiment were identified from the literature as having a high correlation with tree vigour. Leaf traits were selected because of their relationship with photosynthesis and carbon allocation to stem wood production (Waring *et al.*, 1980). Trunk- and branch-related traits have been associated with hydraulic conductance and stem water potential, which in turn can influence dwarfing (Tombesi *et al.*, 2010; Tombesi *et al.*, 2011).

Leaf traits

Leaf number per growth unit

In general, the growth unit (GU), is a stem axis formed in two stages (an old/well formed section and a current/newly formed section) and develops without interruption (Dambreville, 2012; Hallé and Martin, 1968). The leaf number per GU was recorded for five one-year-old terminal branches and expressed as the average leaf number on the three most recently emerged GUs.

Leaf fresh weight/unit area (1 cm^2)

Five young, fully hardened and expanded mature leaves were removed from the most recently emerged GUs of five terminal branches selected at random. Thus, a total of 25 leaves were measured per tree. A uniform size (1 cm^2) piece of leaf from a defined leaf area (section half way between the leaf border and midrib, and half way between the leaf tip and base) was taken to measure leaf fresh weight. A digital scale (Model FZ3000iWP, A&D Company Ltd, Tokyo, Japan) was used to measure leaf weight.

Leaf dry weight/unit area (1cm^2)

Fresh weight leaf samples were dried in a oven (Model ODW50, Laboratory Equipment Pty Ltd, Marrickville, NSW, Australia) at 60°C for 48 hours. Once dessicated, samples were reweighed using an analytical balance (Model HR-250AZ, A&D Company Ltd, Tokyo, Japan) to determine leaf dry weight (mg).

Leaf thickness

The thickness of the leaf lamina (mm; two decimal places) was measured in a leaf area halfway between the leaf border and the midrib and halfway between the leaf tip and base. Five leaves were measured per tree using a digital vernier caliper (Model TD2082, Jaycar Electronics, Rydalmere, NSW, Australia) and average leaf thickness was calculated.

Branch and stem traits

Diameter of growth unit stem

The diameter of the GU stem (mm) was recorded for five randomly selected terminal branches. On each branch, diameters of the three most recently emerged GUs were measured at the middle of the length of the GU using a digital vernier caliper (Model TD2082, Jaycar Electronics, Rydalmere, NSW, Australia).

Length of growth unit

The length of the GU stem (cm) was recorded for five randomly selected terminal branches. On each branch, the length of the three most recently emerged GUs were measured using a measuring tape.

Trunk cross-sectional area

Trunk cross-sectional area (TCA) was determined using the circumference of the tree at 30 cm from the trunk base, measured with a measuring tape. Circumference was used to calculate TCA (cm^2), as follows:

$$TCA = C^2/4\pi$$

Where C = Tree circumference, and $\pi = 3.14159$.

Wood thickness to bark thickness ratio

Five one-year-old terminal branches were selected to measure their thickness (mm; two decimal places) before and after bark removal. Measurements were made using a digital vernier

caliper (Model TD2082, Jaycar Electronics, Rydalmere, NSW, Australia) and the wood thickness to bark thickness ratio was calculated as follows:

$$\text{wood thickness to bark thickness ratio} = \frac{\text{Wood thickness}}{\text{Bark thickness}}$$

Branch dry matter

The stems, with leaves removed, of five randomly selected terminal branches were weighed (g; two decimal places) using a digital scale (Model FZ3000iWP, A&D Company Ltd, Tokyo, Japan). Both fresh weight and dry weight, the latter following drying in a laboratory oven (Model ODW50, Laboratory Equipment Pty Ltd, Marrickville, NSW, Australia) at 60°C for 48 hours, were measured and branch dry matter was calculated as follows:

$$\text{Branch dry matter (\%)} = \frac{\text{Dry Weight}}{\text{Wet Weight}} \times 100$$

Branching density

Branching density was expressed as the number of terminal branches m^{-2} and measured by determining the average number of terminal branches in the canopy periphery of three randomly selected, equally spaced 1 m^2 canopy subsections.

Statistical analysis

Pearson correlation was selected to identify which of the tree morphological traits described above exhibited the highest linear correlation with tree vigour (canopy volume). Calculations were performed using SAS/STAT software version 9.0. (SAS Institute, Cary, NC, USA) using data from 40 experimental trees in stage 1 (Brand, 1986; De la Rosa *et al.*, 2006; Zorić *et al.*, 2012).

4.3.3 Estimating the heritability of the morphological trait used to assess vigour

Aim

To assess the heritability of tree vigour in mango breeding families from the Australian Mango Breeding Program using the trait identified to possess the highest correlation with tree vigour (TCA), and to identify individual trees suitable as parental lines for mango breeding.

Experiment site and plant material

The heritability of tree vigour, assessed using TCA, was determined in 41 mango breeding families from the Australian Mango Breeding Program located on the Southedge Research Station (SERS) and the Walkamin Research Station (WRS), Department of Agriculture and Fisheries (DAF), Mareeba (Fig. 4-1). The individuals in each breeding family exhibited uneven spatial distribution in rows at both research stations as shown in Fig. 4-3. Eleven families were common across the two research stations.

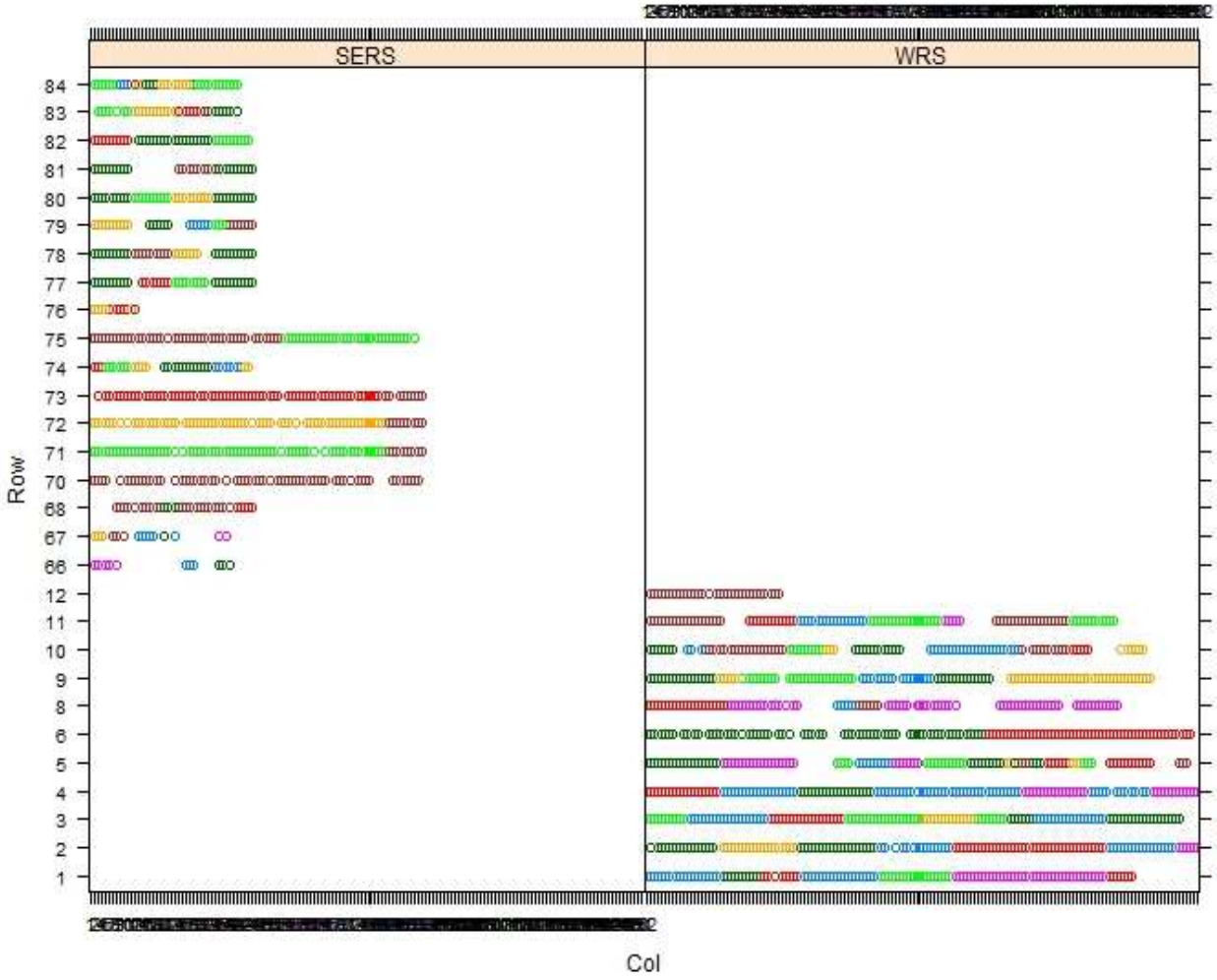

Fig. 4-3: The spatial distribution of individual trees from 41 breeding families measured at the Southedge Research Station (SERS) and the Walkamin Research Station (WRS). X-axis represents family size and Y-axis represents row number as per planting orientation at the respective research stations. Families are depicted in different colours.

The number of individuals within each family for each age category is described in Table 4-1 and 4-2.

Table 4-1: Number of individual trees assessed in each family for each tree age at the Southedge Research Station (SERS).

Tree age	Family									
(years)	**1**	**2**	**5**	**7**	**9**	**12**	**13**	**14**	**18**	**27**
2	0	0	0	0	0	0	0	0	0	0
4	0	0	60	7	21	47	9	0	62	11
5	6	0	3	8	0	6	10	0	4	0
6	4	7	3	0	3	0	3	5	1	4
Tree age	**Family**									
(years)	**29**	**30**	**32**	**33**	**37**	**38**	**201**	**202**	**203**	**204**
2	0	0	0	0	0	0	0	0	0	0
4	17	8	6	16	16	10	0	0	0	0
5	11	0	0	0	0	0	73	38	67	62
6	0	0	0	0	0	0	0	33	34	68

Table 4-2: Number of individual trees assessed in each family for each tree age at the Walkamin Research Station (WRS).

Tree age	Family									
(years)	**1**	**5**	**7**	**12**	**17**	**18**	**21**	**27**	**28**	**29**
2	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	13	6	0	0
5	17	24	0	10	0	9	27	5	5	7
6	0	11	8	44	6	0	35	33	0	1
Tree age	**Family**									
(years)	**30**	**31**	**32**	**37**	**38**	**39**	**41**	**42**	**43**	**47**
2	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	57	0	5	0	0
5	24	0	14	25	18	0	18	0	17	12
6	70	13	18	0	23	0	58	47	80	7
Tree age	**Family**									
(years)	**48**	**50**	**52**	**53**	**54**	**55**	**57**	**58**	**65**	**66**
2	0	0	0	0	46	0	0	0	0	6
4	0	0	0	0	0	0	0	0	31	16
5	6	16	30	10	0	0	11	0	0	0
6	0	0	0	44	37	6	18	16	0	0
Tree age	**Family**									
(years)	**68**	**74**	**200**	**201**						
2	0	17	0	0						
4	17	0	0	3						
5	0	0	64	74						
6	0	0	0	0						

Statistical analysis

TCA was identified in stage one as the trait with the highest correlation with tree vigour. Narrow sense heritabilities were calculated by analyses of TCAs among the measured mango families. The analysis was based on a linear mixed model with parameter estimation using Residual Maximum Likelihood (REML) in the Asreml-R package (Butler *et al.*, 2009). The linear mixed model included terms for additive genetic variance and Family genetic variance. Pedigree information was included in the analysis.

A separate analysis of TCA at each site was performed in ASReml-R incorporating pedigree information. The analysis of each Site was based on the following linear mixed model:

$$y = X\tau + Z_a u_a + Z_f u_f + e$$

Where τ is a vector of fixed effects [Age (year)] with design matrix *X*; u_a is a vector of random additive genetic effects with distribution $u_a \sim N(0, \sigma^2_a A)$, where *A* is the known additive relationship matrix based on the pedigree information; and u_f is a vector of random family effects with distribution $u_f \sim N(0, \sigma^2_f I)$. The vector of random residual effects is given by e, with distribution $e \sim N(0, \sigma^2_e I)$. Analysed trees were not in a completely defined regular grid of rows and columns, so the model does not account for spatial correlation.

The data were also pooled across the two research stations and evaluated in a combined analysis. There were not sufficient common varieties across the two research stations to estimate the genetic correlation between the two sites. The analysis used was based on an approach used for animals (without replication across the research stations) and combined the additive genetic variance across the two sites. A separate mean was fitted for each site by age (year) level and a separate residual variance estimated for each research station.

Hence, the linear mixed model was the same as above, but in the combined analysis, the vector of fixed effects, τ, contained research station by age (year) fixed effects. The model also allowed each trial to have its own residual variance structure and residuals were assumed independent between trials. Hence the residuals were normally distributed with mean zero and variance matrix R [i.e. $e \sim N(0, R)$], where R is a block diagonal matrix [R=diag(R_i), with $R_i = \sigma^2_{ei} I$].

BLUPs were calculated for all breeding families using the combined data from both research stations, as well as data for each breeding family age category from either separate research station. Moreover, for individual progenies, BLUPs for total genetic effect (genetic variance in general, divided into additive genetic variance and dominance variance, important for the selection of individual progeny for commercial trials in breeding programs after qualifying for all desirable traits) and additive genetic effects (breeding values; additive genetic variance and the basis of individual progeny selection as parental lines for future crosses) were calculated to identify suitable candidates for field trials and parent selection, respectively (Griffiths *et al.*, 2015).

4.4 Results and discussion

4.4.1 Identification of the most efficient morphological trait that represents tree vigour

Relationship between tree morphological traits and canopy volume

As described above, tree vigour can be defined as vegetative growth intensity in woody plants (Nesme *et al.*, 2005). Previous work describes the association of a number of morphological tree traits with tree vigour, which are important for evaluating vigour in various woody species (Barden and Marini, 2001; Barden *et al.*, 2002; Nesme *et al.*, 2005; Saeed *et al.*, 2010; Srivastav *et al.*, 2009). However, the methodologies of these studies are not suitable to evaluate vigour of the numerous progeny in mango breeding populations because they are based on cultural practices in commercial production and involve time-intensive assessment. Such methods may be feasible in commercial settings, but are impractical in fruit breeding programs. In this study I evaluated 12 select tree morphological traits in 40 trees, with the trees belonging to different vigour categories at the Southedge Research Station, in order to develop an efficient method of assessing vigour in mango breeding populations.

Relationship between trunk cross-sectional area and canopy volume

TCA is a trait commonly used to estimate tree growth (Khatamian and Hilton, 1977; Nesme *et al.*, 2005; Ro and Park, 2000). The TCA calculated for the analysed trees showed a strong positive correlation ($r = 0.828$, $p<0.0001$) with canopy volume as illustrated by Fig. 4-4A. These results demonstrate that TCA displays the strongest correlation with tree vigour, as measured by canopy volume, compared to that of other morphological traits. The high positive correlation highlights TCA as a powerful indicator of vegetative vigour. This result is

consistent with the conclusions of previous work (Barden *et al.*, 2002; Guxiong *et al.*, 1987; Khatamian and Hilton, 1977; Nesme *et al.*, 2005; Strong and Azarenko, 2000), whereby TCA was identified as an important tool to evaluate tree vigour in various woody fruit species. However, as reported by Nesme *et al.* (2005), the correlation between TCA and vigour is reduced following a certain tree age because of certain orchard management practices such as regular pruning. This idea is also supported by the conclusions of Westwood and Roberts (1970), who observed a linear relationship between TCA and apple tree vigour only while the plants were not heavily pruned. However, TCA is a suitable candidate trait to use as a rapid vigour assessment tool in breeding programs because plants undergo vigour assessment at an early age in these scenarios. Therefore, TCA may be recommended as a reliable measure of vegetative vigour in mango breeding programs. It is more efficient to measure TCA than canopy volume during the assessment of a large number of hybrid seedlings, which is typically performed at an early tree age to identify potential candidates for use in subsequent crosses or commercial adoption.

Relationship between tree height and canopy volume

A positive correlation ($r = 0.780$, $p<0.0001$) observed between tree height and canopy volume in the analysed trees suggests that tree height is a suitable alternate candidate trait to assess vigour (Fig. 4-4B). These results agree with those of Jimenez and Priego (1987), who reported significant differences in tree height associated with avocado of various plant vigour categories. However, this correlation between tree height and vigour may be less in avocado, where orchard management systems include annual pruning.

Relationship between branching density and canopy volume

A positive correlation ($r = 0.466$, $p<0.001$) between branching density and canopy volume was also observed in the analysed trees as shown in Fig. 4-4C. However, branching density was only moderately correlated with tree canopy volume. Furthermore, the practicality of using branching density to measure vigour may be limited because branching density can be affected by regular pruning.

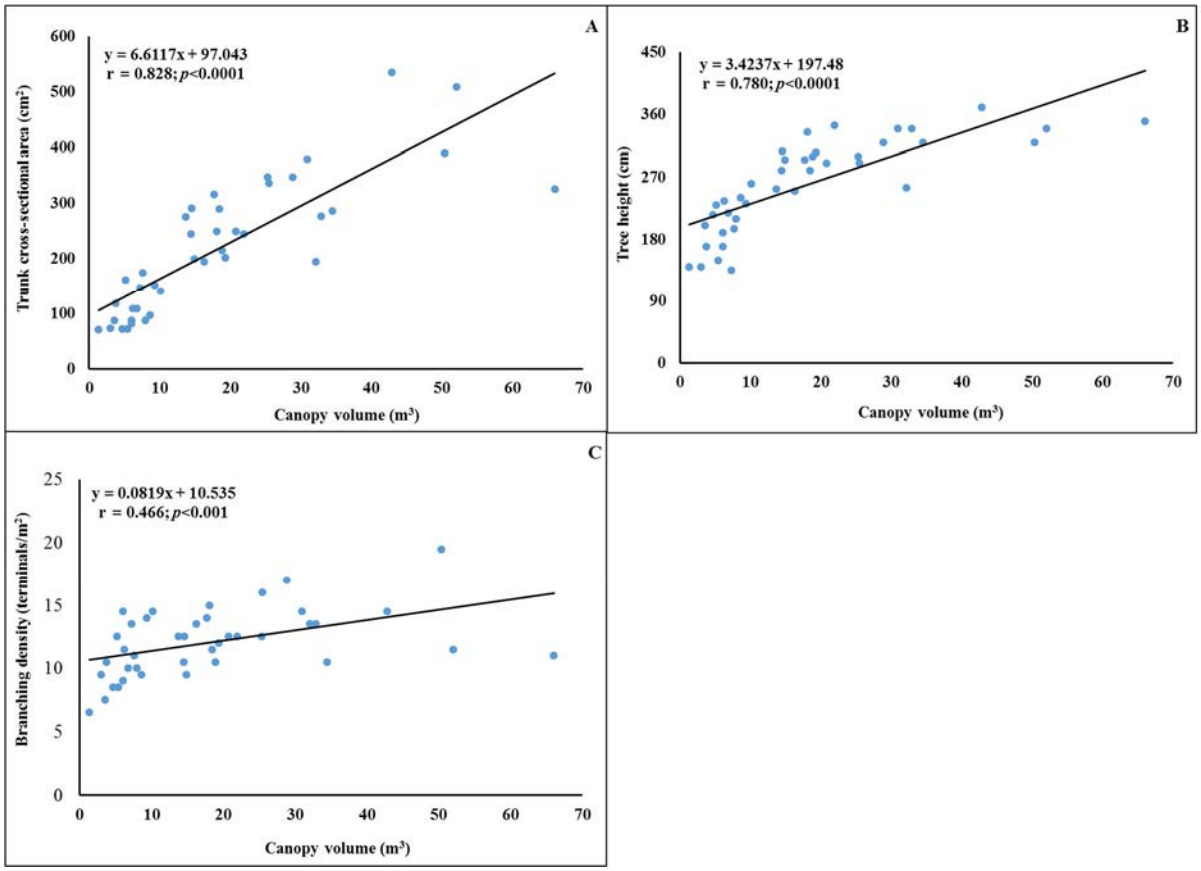

Fig. 4-4: Linear correlation of (A) trunk cross-sectional area (cm^2), (B) tree height (cm), and (C) branching density (terminals/m^2) with canopy volume (m^3). r = Pearson correlation coefficient, n = 40 trees.

Relationship between leaf number per growth unit and canopy volume

The leaf number per GU in the analysed trees showed a weak positive correlation (r = 0.361; $p<0.05$) with canopy volume (Fig. 4-5A), hence there was a weak positive relationship between leaf number per GU and vigour. These results differ from those reported by Yanjun *et al.* (2011) who found that leaf number per GU does not significantly differ among mango varieties that display different vigour.

Relationship between leaf fresh weight/unit area and canopy volume

Leaf fresh weight/unit area calculated for the analysed trees was weakly negatively correlated with canopy volume (r = -0.387, $p<0.05$; Fig. 4-5B), which makes leaf fresh weight/unit area a poor vigour assessment tool.

Relationship between leaf dry weight and canopy volume

Leaf dry weight, calculated as the percentage of leaf fresh weight, showed a positive correlation ($r = 0.427$, $p<0.001$) with canopy volume in the analysed trees (Fig. 4-5C). Although this correlation was statistically significant ($p<0.001$), the relatively lower r value (0.427) suggests that leaf dry weight may not be a useful predictor of tree vigour. This observation does not agree with those of Yanjun *et al.* (2011) and Caruso *et al.* (1997), who reported that leaf dry weight did not significantly vary among mango and peach varieties that exhibit different vigour. This irregular behaviour can be caused by various factors such as tree age (Chalmers and van den Ende, 1975), seasonal vegetative, reproductive growth pattern (De Jong *et al*., 1987) and tree genotype (De Jong and Doyle, 1984).

Relationship between leaf thickness and canopy volume

The correlation observed between leaf thickness and canopy volume in the analysed trees was not significant ($r = 0.091$, $p<0.05$; Fig. 4-5D) and therefore, because of the absence of a relationship with canopy volume, leaf thickness is a poor candidate trait for vigour assessment. In pear, however, Ying *et al.* (2010) found higher leaf thickness associated with dwarf phenotypes and proposed leaf thickness as a pre-selection index for growth potential. Earlier work performed by Chong and Andrews (2002) also reported leaf thickness as a good indicator of vigour in cherry rootstock. However, Beakbane (1967) obtained variable results regarding leaf thickness in apple rootstocks of different vigour categories. Here, the leaves of dwarf rootstocks were thinner than those of high-vigour rootstocks in 8- and 16-year-old unpruned trees (Beakbane (1967); however, another study reported no differences in leaf thickness among rootstocks in 2- and 4-year-old pruned trees of varying vigour (Chong and Andrews, 2002).

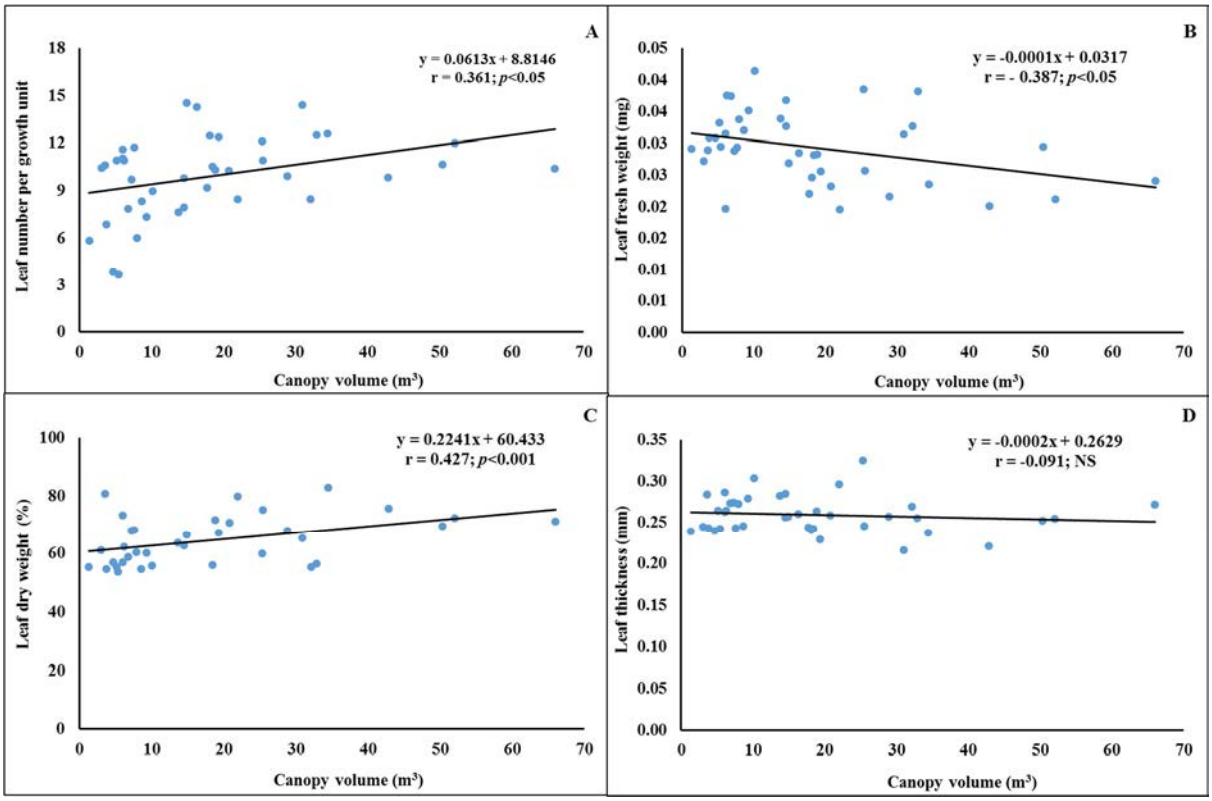

Fig. 4-5: Linear correlation of (A) leaf number per growth unit, (B) leaf fresh weight (mg), (C) leaf dry weight (% fresh weight), and (D) leaf thickness (mm) with canopy volume (m^3). r = Pearson correlation coefficient, n = 40 trees.

Relationship between growth unit length and canopy volume

A strong positive correlation (r = 0.505, p<0.0001) was found between GU length and canopy volume in the analysed trees (Fig. 4-6A), meaning that GU length can be considered a viable alternative vigour predictor. Previous work also supports these results, such as Yanjun *et al.* (2011) who reported that GU length is significantly different among mango varieties that differ in vigour. There is also evidence that dwarf rootstocks decrease the length of the scion shoot length. Cao *et al*. (2008) found that scion shoot length was significantly shorter in dwarf rootstock compared to semi-dwarf and high-vigour apple rootstocks.

Relationship between growth unit stem diameter and canopy volume

There was no correlation (r = 0.002, p<0.05) observed between GU stem diameter and canopy volume in the analysed trees (Fig. 4-6B), and thus GU stem diameter possesses a lower degree of association with tree vigour and is not an ideal candidate trait for vigour assessment. These results are not supported by an earlier work performed by Yanjun *et al.* (2011), who reported that GU stem diameter is significantly different among mango varieties of variable vigour.

Srivastav *et al.* (2009) reported similar findings that shoot diameter had a lower degree of association with vigour.

Relationship between branch dry matter and canopy volume

A positive correlation (r = 0.468, $p<0.001$) was observed between branch dry matter and canopy volume calculated in the analysed trees (Fig. 4-6C), however this association was only moderate. Therefore, branch dry matter is not an ideal trait for predicting tree vigour. Past reports propose that fresh and dry branch weight is a reliable prediction of vigour in mango during early growth stages (Srivastav *et al.*, 2009).

Relationship between bark thickness and canopy volume

Bark thickness had a significant weak negative correlation with canopy volume calculated in the analysed trees (r = -0.319, $p<0.05$; Fig. 4-6D) implying that bark thickness is negatively associated with vigour. These results are supported by Jimenez and Priego (1987), who reported that thicker bark is associated with dwarf trees. Thicker bark in dwarf trees is related to the higher degradation of auxins by lAA-oxidase, peroxidase, and phenolic compounds present in the bark. This reduction of auxin supply leads to reduced production of cytokinins in the roots, which alters the normal growth pattern of the tree (Lochard and Schneider, 1981).

Relationship between the wood thickness to bark thickness ratio and canopy volume

Wood thickness to bark thickness ratio displayed a non-significant (r = 0.163, $p<0.05$) correlation with canopy volume calculated in the analysed trees (Fig. 4-6E). Bark to wood ratio is a poor indicator of rootstock vigour (Chong and Andrews, 2002); however, another study showed that bark percentage is useful in predicting mango rootstock vigour at the nursery growth stage (Abirami *et al.*, 2011).

Determination of the most efficient tree morphological trait to assess vigour

Based on the above results, TCA was selected as a rapid vigour assessment tool because this trait exhibited the highest association (r = 0.828, $p<0.0001$) with canopy volume, which reflected vegetative vigour. Furthermore, TCA is the simplest and most time-efficient measurement out of the analysed traits.

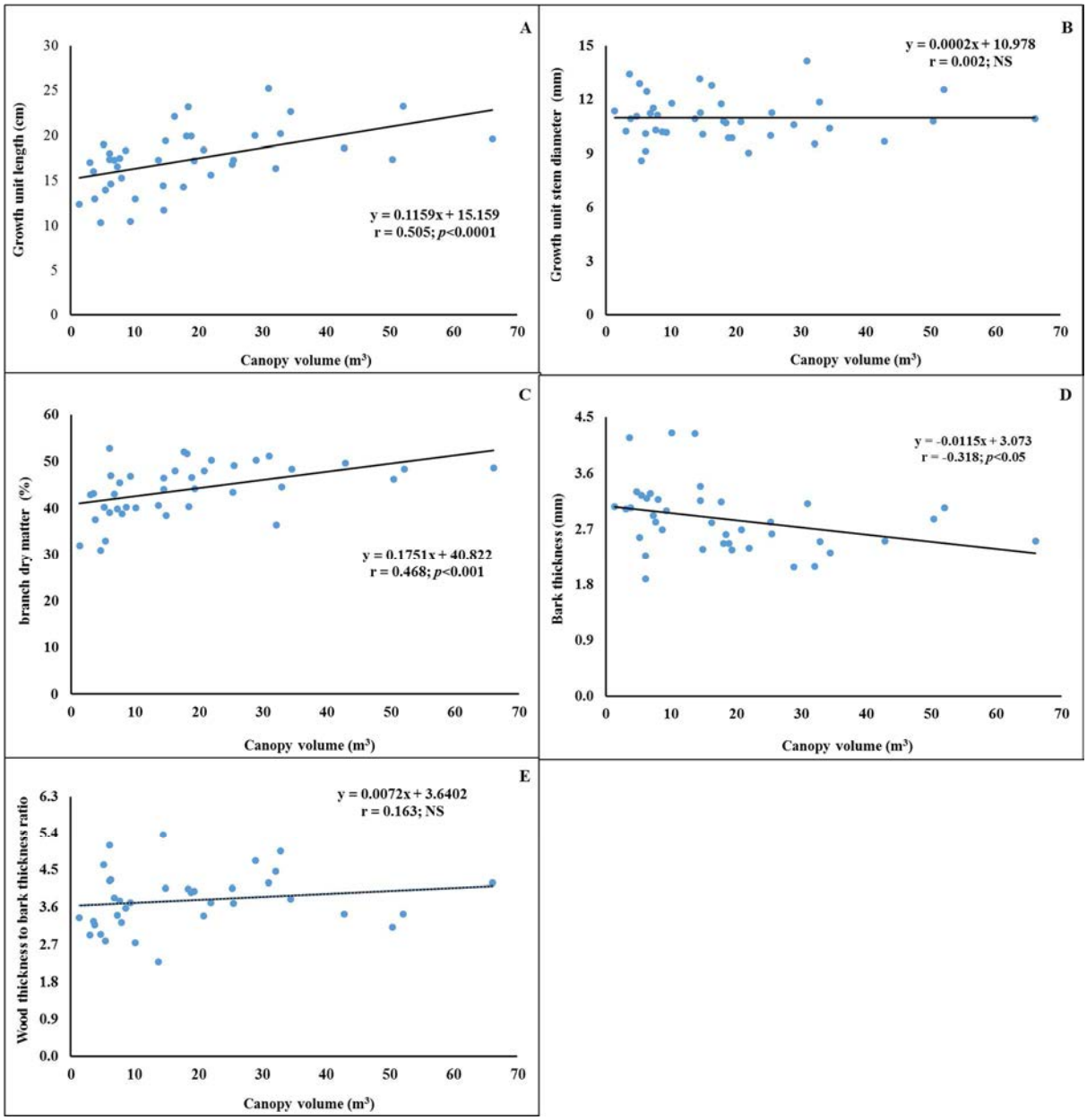

Fig. 4-6: Linear correlation of (A) growth unit length (cm), (B) growth unit stem diameter (mm), (C) branch dry matter (%), (D) bark thickness (mm), and (E) wood thickness to bark thickness ratio with canopy volume (m^3). r = Pearson correlation coefficient, n = 40 trees.

4.4.2 Estimating the heritability of the efficient vigour assessment tool identified in the first stage

TCA was identified as the optimal efficient vigour assessment measurement out of twelve potential tree morphological traits, because it displayed the highest correlation ($r = 0.828$, $p<0.0001$) with canopy volume out of all the analysed traits and it is rapid to measure.

Mean trunk cross-sectional area (TCA) measurement across mango breeding families

Characteristics of the test population

The test population was spread across two research stations and included 41 mango breeding families consisting of 1909 progeny. All families were subdivided based on age into 2, 4, 5, and 6 year old categories. Only 69 trees from 3 families, all located at WRS, were placed in the 2 year old category. These individuals were much younger than the other analysed trees, and so were excluded from the analysis. Hence the analysis was based on 4-, 5-, and 6-year-old trees from the two research stations WRS and SERS. Family 72 was excluded entirely as it was made up of only 2-year-old trees. For all mango breeding families, the mean TCA for each age category at either research station is given in Table 4-3. The experimental population possesses some non-ideal characteristics, such as that the size and location of the breeding families is not uniform across the two research stations, and only eleven breeding families are common to both research stations.

Table 4-3: Mean TCA (cm^2) of mango breeding family progeny across four age categories located at Southedge and Walkamin Research Stations (SERS and WRS, respectively).

Family	SERS				WRS			
	Age categories (years)				Age categories (years)			
	2	4	5	6	2	4	5	6
1	-	-	107.23	169.07	-	-	104.27	-
2	-	-	-	177.57	-	-	-	-
5	-	97.54	103.49	166.96	-	-	123.53	143.80
7	-	121.76	130.92	-	-	-	-	158.04
9	-	156.10	-	199.00	-	-	-	-
12	-	113.10	121.16	-	-	-	187.78	134.03
13	-	141.15	178.01	174.24	-	-	-	-
14	-	-	-	187.38	-	-	-	-
17	-	-	-	-	-	-	-	84.44
18	-	106.62	155.86	258.55	-	-	146.61	-
21	-	-	-	-	-	81.41	127.83	140.43
27	-	122.95	-	159.54	-	90.65	177.49	175.6

28	-	-	-	-	-	-	147.77	-
29	-	168.75	178.37	-	-	-	150.73	169.12
30	-	150.00	-	-	-	-	140.75	186.42
31	-	-	-	-	-	-	-	151.89
32	-	104.56	-	-	-	-	115.68	149.64
33	-	139.04	-	-	-	-	-	-
37	-	158.21	-	-	-	-	141.27	-
38	-	156.42	-	-	-	-	154.72	193.76
39	-	-	-	-	-	82.23	-	-
41	-	-	-	-	-	-	169.58	202.79
42	-	-	-	-	-	114.34	-	214.09
43	-	-	-	-	-	-	151.40	170.77
47	-	-	-	-	-	-	143.78	173.49
48	-	-	-	-	-	-	118.08	-
50	-	-	-	-	-	-	156.05	-
52	-	-	-	-	-	-	120.2	-
53	-	-	-	-	-	-	79.72	140.76
54	-	-	-	-	36.13	-	-	174.72
55	-	-	-	-	-	-	-	129.89
57	-	-	-	-	-	-	123.06	154.33
58	-	-	-	-	-	-	-	102.36
65	-	-	-	-	-	91.65	-	-
66	-	-	-	-	-	82.61	-	-
68	-	-	-	-	-	93.61	-	-
74	-	-	-	-	48.44	-	-	-
200	-	-	-	-	-	-	91.31	-
201	-	-	120.89	-	-	50.04	124.35	-
202	-	-	95.21	117.07	-	-	-	-
203	-	-	114.08	122.10	-	-	-	-
204	-	-	159.55	150.24	-	-	-	-

SERS = Southedge Research Station, WRS = Walkamin Research Station

Estimation of TCA heritability

TCA narrow-sense heritability was estimated for either research station separately as well as for both research stations combined.

Estimation of TCA narrow-sense heritability across mango breeding families

Plant breeders usually develop large numbers of breeding populations every year. Parent selection is important for breeding program success when selecting from the abundant progeny that result from these crosses. Parent selection ensures that individuals with desirable features contribute to the genepool of the next generation (Bauer *et al.*, 2006). Predicting offspring phenotype from parental phenotype is important to efficiently improve a crop through breeding.

Knowledge of narrow-sense heritability (h^2) is used to make such predictions. The h^2 value estimates the extent to which an individual's genotype governs its offspring's phenotype, and specifies which plant traits can be improved through selection (Falconer, 1989; Griffiths *et al.*, 2015).

The h^2 estimates of TCA in the mango breeding population at WRS (0.26), SERS (0.09), and both research stations combined (0.23) were low (Table 4-4). This indicates substantial heterogeneity in estimates of family genetic components and additive genetic components in breeding families at both research stations.

This study shows variable h^2 for mango populations at the two different experimental sites. The TCA h^2 value in mango at WRS and SERS was 0.26 and 0.09, respectively. Possible reasons for the lower h^2 observed at SERS are lower additive variance, different environmental conditions, and the presence of different mango breeding families, in spite of the 11 families that are common to both research stations. Due to an insufficient number of common breeding families, genetic correlation was not estimated between the two research stations. The h^2 value is a measurement based on specific environment effects and breeding population. An estimate made using one population and environment may not be useful for another population or environment (Griffiths *et al.*, 2015). The low TCA h^2 value of combined mango populations at both experimental sites (0.23) suggests that this trait is under weak additive genetic control and is poorly heritable. This is the first assessment of TCA heritability in mango, and contrasts with the results reported for cacao that showed moderate TCA heritability in that species ($h^2 = 0.5$; Padi *et al.* (2016). Therefore, for TCA in mango breeding populations, the genotype weakly determines the phenotype due to higher non-additive variance. Thus, it is recommended that additional morphological traits are included as a model to assess plant vigour in mango. However, the applied statistical analysis shows that statistically significant difference is still displayed among the different breeding families. Therefore, this significant difference can be exploited in mango breeding by selecting optimal individuals from the most appropriate breeding families.

Table 4-4: TCA variance and heritability components in 41 mango breeding families from the two research stations.

Research station	Family number	Family genetic variance	Additive genetic variance	Residual variance	Narrow-sense heritability
WRS	33	250.30	659.92	1590.11	0.26
SERS	20	577.04	248.00	2008.79	0.09
Combined	41	412.94	529.66	1658.47^{WRS} 1843.57^{SERS}	0.23

WRS = Walkamin Research Station; SERS = Southedge Research Station

Best linear unbiased prediction (BLUP) estimation for TCA in mango breeding families

Breeding values were predicted for 41 breeding families located at both SERS and WRS.

BLUP estimation for TCA in mango breeding population

Family BLUP estimation

BLUP is a standard technique used for calculating random effects of mixed models and is widely applied in animal breeding. However, BLUP is confined to the estimation of genetic and non-genetic components of variance (Piepho *et al.*, 2008). This technique is also used to calculate breeding values and select the most suitable family or individual progeny based on their breeding value. The 10 breeding families with the lowest TCA were the families 17, 58, 200, 202, 203, and 53, 1, 55, 5, and 201 (Fig. 4-7 and Table 4-4). Family 17 had the lowest BLUP for TCA compared to other families that displayed low TCA, but this family was not significantly different to families 58, 200, and 202 due to a relatively higher standard error. This uncertainty in prediction may be because there are only 6 highly variable trees for family 17. The 10 highest predicted breeding families for TCA bearing are 42, 41, 29, 9, 38, 13, 30, 50, 14, and 37 (Fig. 4-7 and Table 4-5).

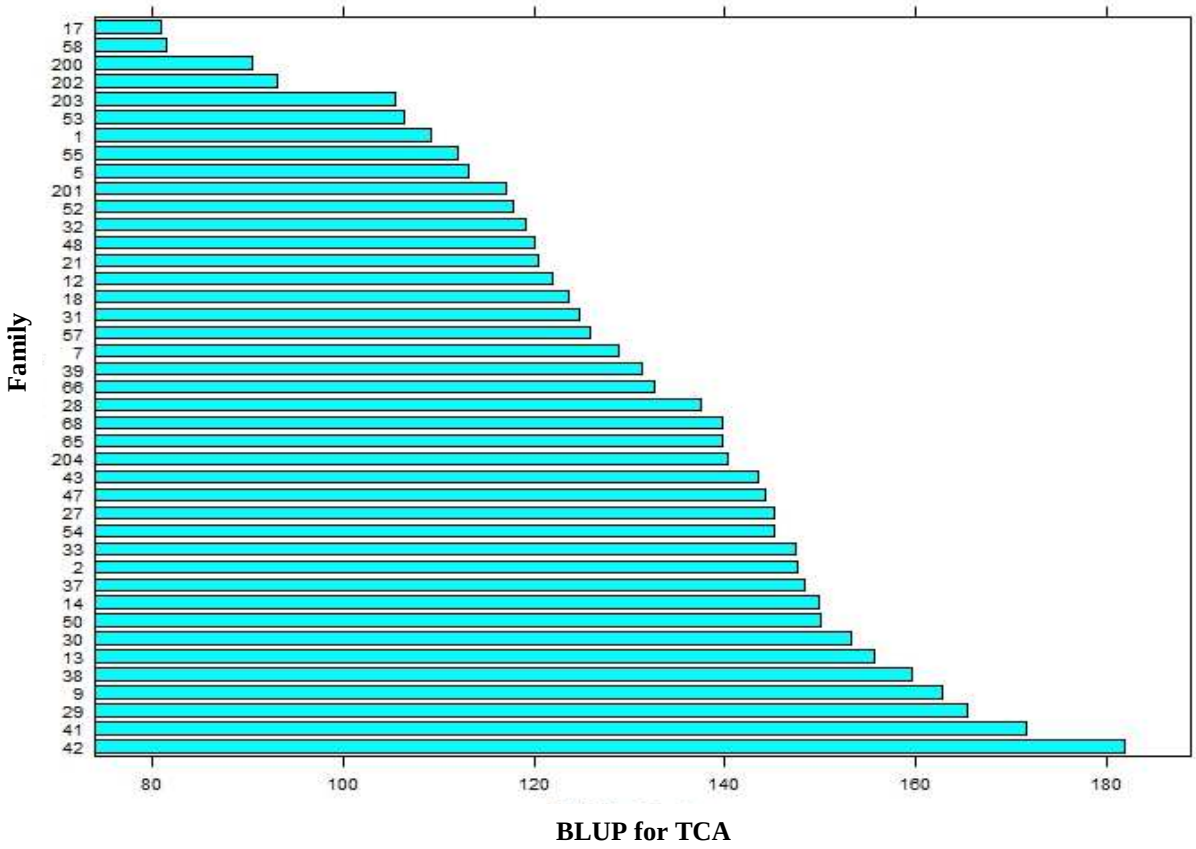

Fig. 4-7: The predicted BLUPs for trunk cross-sectional area (TCA; cm^2) for the 41 families from both research stations.

Table 4-5: The BLUPs for trunk cross-sectional area (TCA; cm^2), with standard error and ranking for the 41 mango breeding families from both research stations.

Family	Predicted Value	Standard Error	Ranking
17	80.99	13.94	1
58	81.53	9.77	2
200	90.38	5.69	3
202	93.00	5.94	4
203	105.40	5.27	5
53	106.49	6.02	6
1	109.15	7.75	7
55	112.07	13.97	8
5	113.22	4.69	9
201	117.05	3.89	10
52	117.83	7.58	11
32	119.20	6.75	12
48	120.04	13.90	13
21	120.48	4.99	14
12	121.92	4.49	15
18	123.69	5.54	16
31	124.79	10.63	17
57	125.86	7.55	18
7	128.94	8.34	19
39	131.25	8.03	20
66	132.69	10.77	21
28	137.54	14.67	22
68	139.74	10.62	23
65	139.77	9.09	24
204	140.36	5.01	25
43	143.47	4.89	26
47	144.26	8.92	27
27	145.16	5.51	28
54	145.18	7.11	29
33	147.42	10.13	30
2	147.63	13.82	31
37	148.32	6.65	32
14	149.83	15.43	33
50	150.14	9.69	34
30	153.22	4.68	35
13	155.71	8.70	36
38	159.71	5.98	37
9	162.84	8.63	38
29	165.54	6.96	39
41	171.60	5.25	40
42	181.87	6.11	41

Overall standard error of difference = 12.25

BLUP estimation for individuals

Two types of individual predictions (BLUPs) were calculated.

Total genetic effect

The prediction of total genetic effect for each individual is important for germplasm selection because it calculates the phenotypic performance of a given genotype. The 20 individuals with the smallest TCA out of 1909 individuals in the entire mango population are described in Fig. 4-8 and Table 4-6. Most individuals in this subgroup belong to open-pollinated families (200 and 202), and the remainder are from families 17 and 58. BLUP estimation for total genetic effect concluded that there was no significant difference in TCA BLUPs among these 20 individuals. These individuals, based on low TCA BLUP in addition to a number of other traits, are suitable candidates for the commercial adoption stage, whereby selected genotypes are tested in a limited commercial setting before their release as novel cultivars to farmers in mango breeding programs.

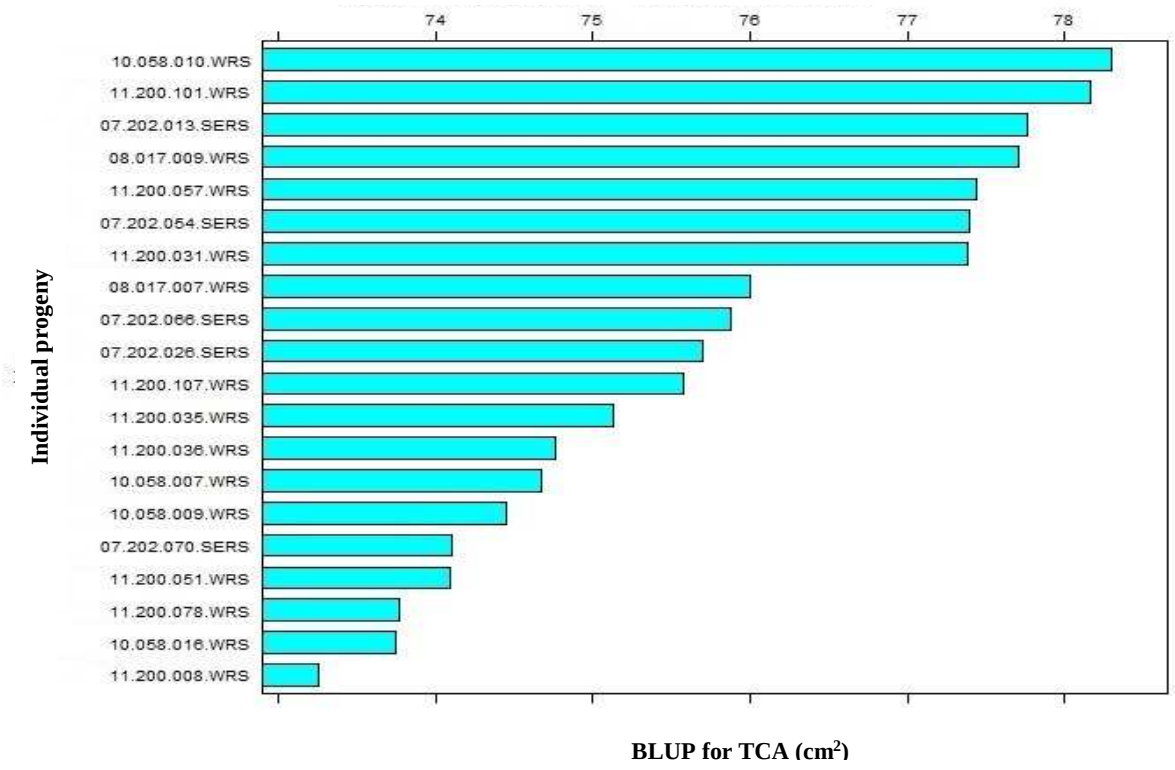

Fig. 4-8: Twenty individuals with the smallest predicted TCA based on BLUP for TCA total genetic effect.

Table 4-6: The 20 individuals with the smallest predicted TCA based on BLUP for TCA total genetic effect, along with standard error.

Individual ID	Predicted Value	Standard Error
11.200.008.WRS	73.25	18.65
10.058.016.WRS	73.74	17.59
11.200.078.WRS	73.77	18.65
11.200.051.WRS	74.09	18.65
07.202.070.SERS	74.10	18.93
10.058.009.WRS	74.45	17.59
10.058.007.WRS	74.67	17.59
11.200.036.WRS	74.77	18.65
11.200.035.WRS	75.14	18.65
11.200.107.WRS	75.58	18.65
07.202.026.SERS	75.70	18.90
07.202.066.SERS	75.88	18.93
08.017.007.WRS	76.00	19.61
11.200.031.WRS	77.39	18.65
07.202.054.SERS	77.40	18.90
11.200.057.WRS	77.44	18.65
08.017.009.WRS	77.71	19.61
07.202.013.SERS	77.76	18.90
11.200.101.WRS	78.17	18.65
10.058.010.WRS	78.30	17.59

Additive genetic effects

Additive genetic effects (or breeding values) for the TCA trait are more useful for breeders as they predict the likelihood of an individual, when used as a parent, to produce progeny with low TCAs. All parents in the pedigree of mango breeding families were included in these predictions, ranked according to the lowest predicted additive genetic effects for TCA, and the lowest ranking 50 individuals are presented in Fig. 4-9. A couple of parents (e.g. *M. laurina* Lombok) are in these lowest ranking 50, but a number of individuals are below them (from families 53 and 202) that are likely to promote the small TCA trait in future crosses. The other parents in the pedigree have a higher TCA than the lowest ranking 50 individuals. The individuals with the largest negative additive genetic effect are likely to be the optimal parents for promoting small TCA in future generations. Most individuals from families 53 and 58 had the largest negative BLUP for TCA additive genetic effects. These individuals are best to use as parents in future crosses.

There are many other highly ranked traits such as yield and fruit colour to consider when selecting optimal parents for future crosses. It may be prudent to select the parents of the best families, e.g. family 17, 58, or 53, alongside the optimal individuals in these families, as these parents are compatible and can be crossed to produce progeny with low TCA.

The open-pollinated families 200 and 202 also displayed low TCA. The greater performance of the open-pollinated population is because most agriculturally important traits have large non-additive variance, and therefore open-pollinated crosses are more successful than controlled crosses (Lavi *et al.*, 1993a; Lavi *et al.*, 2004). The difference in h^2 between the research stations clearly explains the substantial difference observed for additive variance in mango populations at both research stations. The higher non-additive variance at WRS is due to the large proportion of open-pollinated individuals in the breeding population present at that research station (Table 4-2).

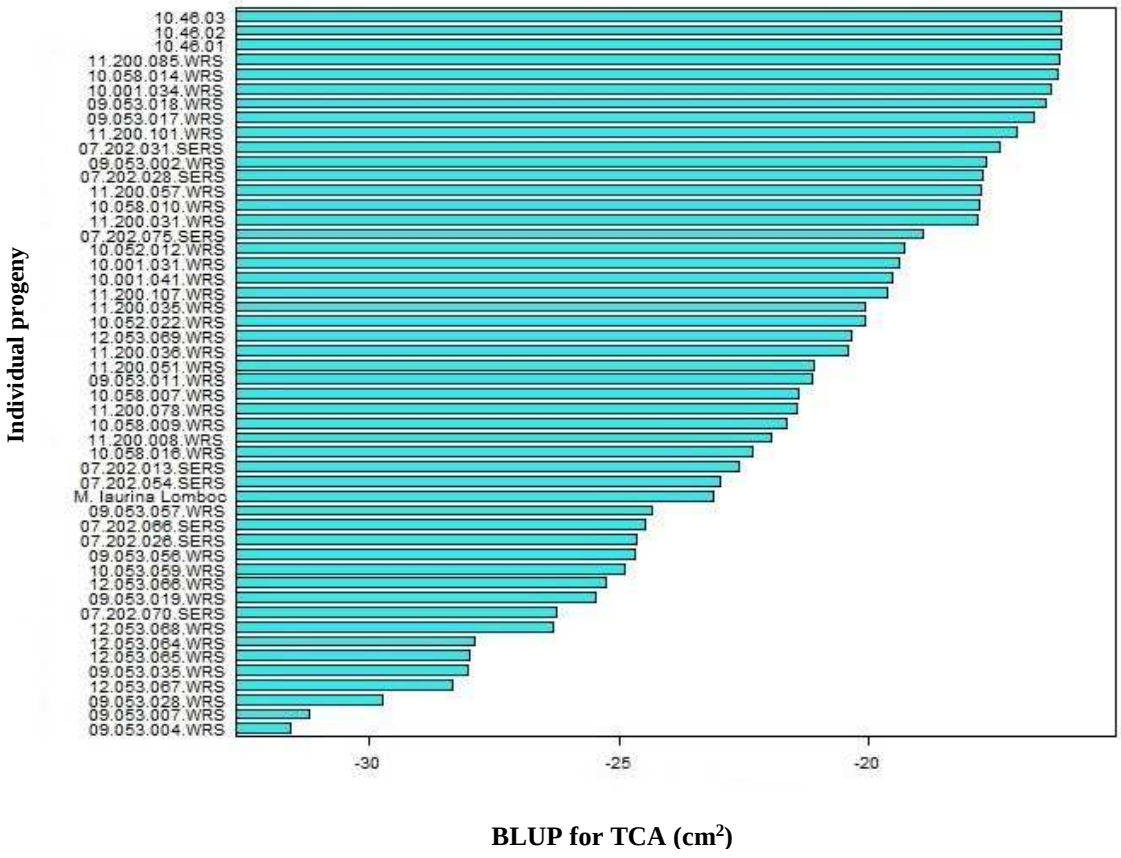

Fig. 4-9: Predicted BLUPs for 50 individual from 41 families with the largest negative TCA additive genetic effects.

BLUP prediction for different mango breeding family age categories located at both research stations

The predicted average TCA for each age category at each research station is detailed in Table 4-7. No significant differences were observed in TCA BLUPs of 5- and 6-year-old families located at both research stations. At WRS, significantly lower BLUP for TCA resulted for 4-year-old families. However, the predicted values need to be considered with caution as different families were measured at each research station and for each age category (Table 4-7).

Table 4-7: The predicted average TCA (cm^2) for each age category at each research station.

Age	Research Station	Predicted Value	Standard Error
4	SERS	114.13	9.82
	WRS	75.66	11.43
5	SERS	133.50	9.46
	WRS	127.31	9.27
6	SERS	144.12	9.83
	WRS	152.52	9.31

WRS = Walkamin Research Station, SERS = Southedge Research Station, Overall standard error of difference = 6.56.

4.4.3 Statistical method and study design improvement

The study employed a mixed linear model, incorporating pedigree information to predict TCA breeding value from unbalanced data collected from the two research stations. This technique has been previously employed in mango to predict breeding value (Hardner *et al.*, 2012). Incorporation of pedigree in the model allows individuals from irregular mating systems to be used to predict the breeding value (Hardner *et al.*, 2012; Henderson, 1975) and manipulates genetic correlation among relatives included in the pedigree (Piepho *et al.*, 2008)

The design of the study was not ideal, and was potentially affected by unbalanced numbers and age categories of individuals in breeding families, insufficient breeding families common to the two experimental sites, and unbalanced positions of individuals in rows and columns, which is not suitable for the spatial analysis approach of Gilmour *et al.* (1997). Elimination of these constraints can improve the study design and possible the outcomes.

4.4.4 Implication for selection (breeding & deployment)

The low TCA heritability in mango, as demonstrated in this study, suggests that only modest gains in mango breeding are possible using selection based on this phenotype. The breeding values indicated by the study can be used to select suitable parents with low BLUP for additive genetic effect. However, parental selection will depend on the presence of other desirable fruit quality traits in addition to low BLUP for additive genetic effect. Individuals with high BLUP for total genetic effect can be deployed in commercial trials with the anticipation that they also generate quality fruit and possess other desirable traits such as regular fruit bearing.

Low TCA heritability also suggests that the inclusion of one or two additional morphological traits in the model may improve vigour heritability assessment in mango. A model including TCA, tree height, and branch dry matter may be more robust in predicting mango vigour than that possible using TCA alone, which is a poorly heritable trait. The same strategy was employed by Nesme *et al.* (2005), whereby a set of morphological traits was used to model plant vigour in apple orchards.

4.5 Conclusion

Among the morphological traits measured in this study, TCA is poorly heritable, indicating that environment has a stronger influence on this trait than genotype. A number of promising low-vigour breeding families were identified in this experiment. This chapter contributes to the understanding of morphological traits that correlate with mango tree vigour and provides tools for parent selection in mango breeding programs focused on reducing plant vigour.

Chapter 5: Effect of foliar application of plant growth regulators on fruit set and fruit retention in the two mango cultivars NMBP-1243 and Keitt

5.1 Abstract

Mango is one of the most important tropical/subtropical fruit crops, with an annual global production of 45.225 million tons (FAOSTAT, 2014b). Mango is a particularly important fruit in Asia (Chapman, 2000). Mango yields are affected by the tendency for irregular or erratic bearing of fruit, which, in addition to influencing regular crop production, can also lower the efficiency of mango breeding programs (Bally *et al.*, 2009a). A key issue in mango cultivation worldwide is low fruit retention due to premature fruit drop. Low fruit set and high fruit drop significantly affects breeding efficiency and plays a role in low yields of commercial mango orchards (Singh *et al.*, 2005). Foliar application of plant growth regulators (PGRs) such as auxins and gibberellins is an established fruit drop management strategy in fruit tree crops including litchi (Singh and Lal, 1980), grapefruit (El-Zeftawi, 1980), and mango (Singh, 2009). This experiment was designed to study the effects of PGRs on fruit set, retention, and quality in the two mango cultivars NMBP-1243 and Keitt during the 2014 and 2015 growing seasons at Walkamin Research Station, Department of Agriculture and Fisheries, Mareeba. Three PGRs, namely naphthylacetic acid (25 and 50 ppm), 2,4-D (25 and 40 ppm) and gibberellic acid (5 and 10 ppm) were sprayed on the inflorescences of selected mango trees at full bloom stage alongside control material (no spray). Following treatment, twenty-five panicles per tree were selected and tagged to observe fruit set and retention until harvest. 2,4-D (40 ppm) spray treatment either significantly reduced or did not affect fruit set in both mango varieties in both the 2014 and 2015 seasons. However, inflorescences of both cv. NMBP-1243 and cv. Keitt trees sprayed with 2,4-D had significantly higher fruit retention compared to that in control trees. Foliar 2,4-D spray reduced fruit size and weight in cv. NMBP-1243, whereas no impact on the size and weight of cv. Keitt mangoes was observed with this treatment. The results of this study demonstrate that foliar PGR application at flowering stage may not increase fruit set, but may result in fruit retention increased up to two fold that of untreated trees. If such a nutritional management strategy is applied in commercial orchards, the resulting increase in

fruit retention can improve mango breeding efficiency as well as tree productivity and farm profitability.

5.2 Introduction

Mango (*Mangifera indica* L.) is an important tropical/subtropical fruit crop and is produced commercially in more than 90 countries (Crane, 2008). In spite of profuse mango flowering in various mango-producing countries, considerable monetary losses are caused by fruit drop (Hagemann *et al.*, 2014; Malik *et al.*, 2003; Singh *et al.*, 2005). Mango tree productivity is the product of fruit set at flowering and subsequent rates of fruit drop throughout fruit maturation (Chadha, 1993; Hagemann *et al.*, 2014; Singh *et al.*, 2005). Regular crop production is important to avoid an interrupted fruit supply, which can cause considerable disparities between farm yields and affect productivity of the commercial fruit industry. In addition to the influence of fruit drop on crop production regularity and farm profitability (Bains *et al.*, 1997a; Chadha, 1993; Monselise and Goldschmidt, 1982), fruit drop reduces the efficiency of mango breeding programs (Bally *et al.*, 2009a; Singh *et al.*, 2005). Mango fruit drop occurs primarily at early stages of fruit growth as the result of either reduced pollen viability (Issarakraisila and Considine, 1994), lack or failure of fertilization (Ram, 1992), embryo abortion (Lakshminarayana and Aguilar, 1975), or hormonal and nutrient imbalances (Nuñez-Elisea, 1986; Ram, 1992; Singh and Singh, 1995). Hormonal imbalances lead to the formation of an abscission layer between the fruit and pedicel that results in fruit drop. Auxins, gibberellins, and ethylene are the plant hormones that are directly involved in the signalling and formation of the abscission layer (Singh *et al.*, 2005).

Mango trees produce an abundance of flowers, in panicle inflorescences (Hagemann *et al.*, 2014). Each panicle contains two types of flowers, namely male and perfect/hermaphrodite. There are 500–1000 flowers per panicle and 200–3000 panicles per mango tree (Guzman-Estrada, 1997; Kurup, 1967; Nagao and Nishina, 1993; Ram, 1992). Fruit drop occurs at all developmental stages during the mango season; however, fruit drop is extensive in the four weeks following fruit set (Davenport and Nunez-Elisea, 1983; Naqvi *et al.*, 1998; Nuñez-Elisea, 1986; Prakash and Ram, 1984; Singh *et al.*, 2005). Despite profuse flowering, fruit yield can be a considerably low proportion of perfect flower number (0.1%), and this relationship between flower number and final fruit number depends upon cultivar identity, flower sex ratio, pollen viability, and environmental conditions during the pollination process (Bally *et al.*,

2009a; Guzman-Estrada, 1997; Prakash and Ram, 1984; Ram, 1992). Factors that contribute towards fruit drop can be categorised as either biotic or abiotic. An increase in fruit set and retention can improve the crossing success rate following hand pollination (≤18.6%) (Iyer and Dinesh, 1997; Lavi *et al.*, 1993b) and hence the number of generated hybrids, which would greatly enhance the efficiency of mango breeding programs as well as commercial farm yields and profitability.

Plant growth regulators (PGRs), both naturally occurring and synthetic, have been an important element in agricultural production. However, most PGR applications are limited to high-value horticultural crops (El-Otmani *et al.*, 2000; Gianfagna, 1995; Thomas, 1982). Endogenous PGRs regulate fruit attachment to the peduncle. Heavy fruit drop during early fruit development is the result of a lower concentration of auxins and gibberellins and a higher concentration of growth inhibitors in the fruit. This hormonal imbalance reduces fruit growth and leads to fruit drop (Prakash and Ram, 1984; Ram, 1992). The importance of PGRs for preventing fruit drop is well documented (Davenport and Nunez-Elisea, 1983; Hagemann *et al.*, 2014; Malik *et al.*, 2003; Sakhidin *et al.*, 2011; Singh *et al.*, 2005; Singh, 2009). Exogenous application of synthetic PGRs at different growth stages ameliorates PGR deficiency and increases fruit retention in mango (Prakash and Ram, 1984; Ram, 1992; Singh *et al.*, 2005).

Synthetic PGRs such as naphthaleneacetic acid (NAA), 2,4-Dichlorophenoxyacetic acid (2,4-D), and gibberellic acid (GA_3) have been successfully used to control pre-harvest fruit drop in various fruit crops (Ward, 2004). NAA is a synthetic auxin documented to effectively manage fruit drop in many fruit crops such as apple (Yuan and Carbaugh, 2007; Yuan and Li, 2008), citrus (Anthony and Coggins, 2001), and mango (Prakash and Ram, 1984). GA_3 effectively reduces fruit drop and increases fruit retention in fruit crops including litchi (Singh and Lal, 1980), grapefruit (El-Zeftawi, 1980), and mango (Singh, 2009). A further synthetic auxin, 2,4-D, also efficiently suppresses fruit drop in crops such as citrus (Anthony and Coggins, 1999; Stewart *et al.*, 1951). In mango, research on PGR effects has shown that their use reduces natural fruit drop. Mango fruit drop management has been successfully practiced using NAA (Ram, 1992), 2,4-D (Ram, 1983), and GA_3 (Ahmed *et al.*, 2012; Singh, 2009). However, little research effort has been devoted to investigating the effect of PGRs on Australian mango varieties in the tropical environmental conditions of Queensland.

This chapter deals with fruit set following one month of full bloom and fruit retention until harvest in two Australian mango varieties in response to the foliar application of three PGRs, namely NAA, and 2,4-D, and GA_3. This study tests the hypothesis that foliar PGR application improves fruit set and retention in these two mango cultivars in the tropical environmental conditions of Queensland. The results of this study will improve current understanding of PGR spray impact on fruit set and retention in both mango breeding projects and commercial fruit production.

5.3 Materials and methods

5.3.1 Study site and sampling of trees

The experiment was conducted over the 2014 and 2015 consecutive growing seasons at Walkamin Research Station (17°8′17″S 145°25′41″E; Elevation: 599.23 m), Department of Agriculture and Fisheries, Mareeba (Fig. 5-1). Four-year-old cv. NMBP-1243 and cv. Keitt mango trees grafted on 'Kensington Pride' rootstock, were used in the experiment. Trees were spaced in a 7 × 6 arrangement and managed according to the practices described in Table 5-1.

Table 5-1: Horticultural management practices applied to experimental trees.

Operation	2014		2015	
	Frequency	**Month**	**Frequency**	**Month**
Irrigation	Per 4 days	June-Dec	Per 4 days	June-Dec
Mowing	Per month	Jan; Feb; March; May	Per month	July; Sep; Nov
Weedicide spray	Per month	Jan; April; May	Per month	Jan

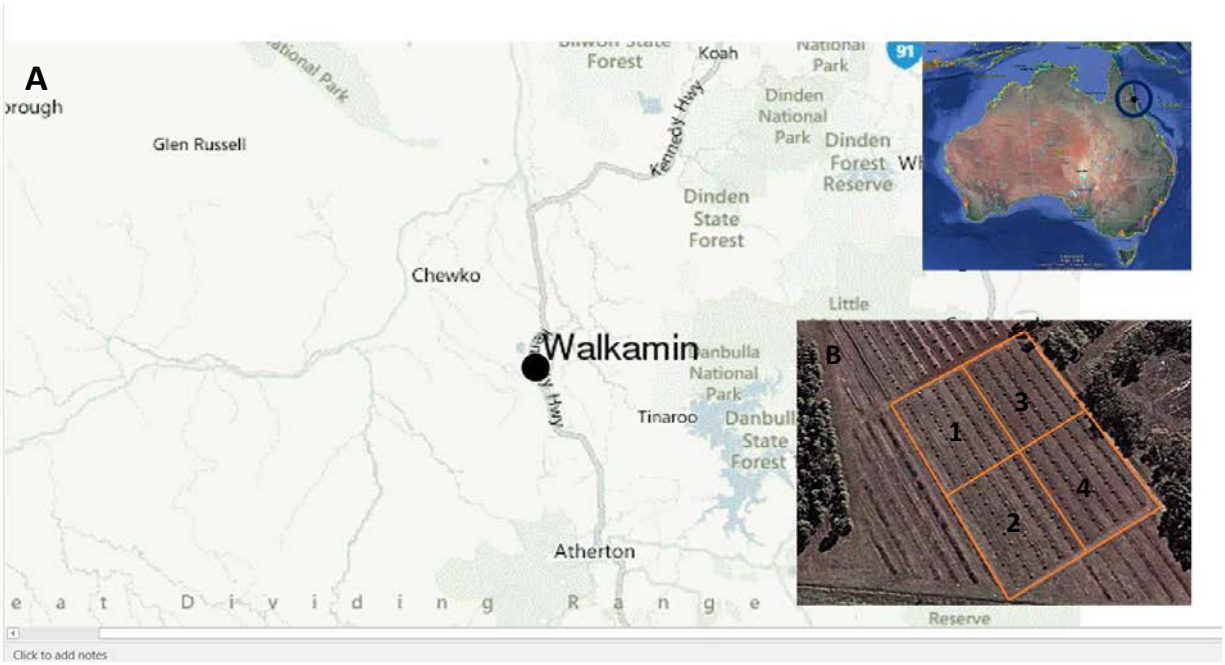

Fig 5-1: (A) Location of the experimental site in Queensland, Australia. (B) Aerial view of the experimental mango block including (1) NMBP-1243, (2) Keitt, (3) NMBP-1201, and (4) R2E2 cultivars.

5.3.2 Plant growth regulator treatments

Three PGRs (NAA, 2,4-D, and GA_3) were selected as experimental treatments based previous reports of their efficacy in reducing fruit drop in different mango cultivars (Ram, 1983; Roemer, 2011; Singh, 2009). Each PGR was applied at two concentrations as described in Table 5-2.

Table 5-2: PGR treatments.

Treatment	**Spray concentration**	**Details**
Control		No spray
Naphthaleneacetic acid (NAA)	25 ppm	NAA Stop Drop®, Kendon Chemical & MNFG. Co. Pty Ltd, Fairfield, Victoria, Australia; a.i. 20g/L 1-Naphthylacetic acid
	50 ppm	
2,4-Dichlorophenoxyacetic acid (2,4-D)	25 ppm	Amicide® 625, Nufarm Australia Ltd., Laverton North, Victoria, Australia; a.i. 625 g/L 2,4-D
	40 ppm	
Gibberellic acid (GA_3)	5 ppm	ProGibb® G.A., Valent BioSciences Corporation, Libertyville, Illinois, USA; a.i. 100 g/L Gibberellic acid
	10 ppm	

All PGR treatments were applied at full bloom (flowering in more than 90% of all panicles; BBCH scale 615; (Delgado *et al.*, 2011; Rajan *et al.*, 2011). In 2014, PGRs were applied on 11 September for both mango varieties, and in the 2015 season, PGRs were applied to cv. NMBP-1243 on 26 September and to cv. Keitt on 12 October. PGR applications were carried out using a knapsack spray (Model CP3, Cooper Pegler & Co. Limited, Burgess Hill, Sussex, England). PGRs were diluted in water to create the desired application concentration (Table 5-2) and sprayed over the entire tree until runoff. Following treatment, a maximum of twenty-five panicles were selected and tagged for the monitoring of fruit set and retention.

5.3.3 Data collection

Fruit set, retention, and quality data were collected as outlined below.

Assessment of fruit set and retention

Measurement of fruit retention (fruit count) on tagged panicles began at 28 days after full bloom (DAFB) and continued at fortnightly intervals. Fruit count at 28 DAFB was considered as the fruit set per panicle because fruit set occurs approximately 14 DAFB in mango (Notodimedjo, 2000) and early fruit drop occurs during the first three to four weeks of fruit development due to self-incompatibility, failure of pollination or fertilization, embryo abortion, or competition among developing fruit (Davenport and Nunez-Elisea, 1983; Guzman-Estrada, 1997; Singh *et al.*, 2005). Fruit counts continued until harvest, which was at 98 DAFB for cv. NMBP-1243 and 126 DAFB for cv. Keitt. The fruit set data was expressed as mean fruit number per panicle. The fruit retention data of all tagged panicles in a tree were converted to a single fruit retention value per panicle before further statistical analysis, and hence these data are expressed as mean percentage fruit retention per panicle.

Fruit quality measurement

Fruit sampling and post-harvest handling

Four mature, hard green, uniform-sized, healthy, and blemish-free mango fruit were harvested with long stems from each experimental tree, with fruit selection performed at random. Fruit were transported in plastic field bins to the horticulture laboratory, Department of Agriculture and Fisheries, Mareeba. Fruit were de-stemmed and de-sapped before they were placed in 400 g 100 L^{-1} Septone Mango Wash® solution (ITW AAMTech, Wetherill Park, NSW, Australia)

for 1 minute to neutralise sap residues. Following de-sapping, mangoes were removed from the wash solution and rinsed with clean water. Fruit were then dipped in a hot (52°C) 120 mL 100 L^{-1} water Scholar® fungicide solution (Syngenta Australia Pty Ltd, Macquarie Park, NSW, Australia; a.i. 230 g/L fludioxonil) for 5 minutes. Fruit were then air-dried and maintained at 21°C and 90% relative humidity to ripen until they reached the stage suitable for consumption (Hofman *et al.*, 2010).

Fruit weight

Fruit were weighed using a digital balance (Model FZ3000iWP, A&D Company Ltd, Tokyo, Japan) at ripe stage (n=4 fruit).

Fruit size

The length, width, and depth of fruit were measured to determine fruit size (Fig. 5-2). Fruit length was measured along the axis from the stalk attachment site to the furthest opposite point. Fruit width was measured at the broadest fruit section perpendicular to fruit length. Fruit measurements were performed using digital vernier caliper (Model TD2082, Jaycar Electronics, Rydalmere, NSW, Australia) at ripe stage (n=4 fruit).

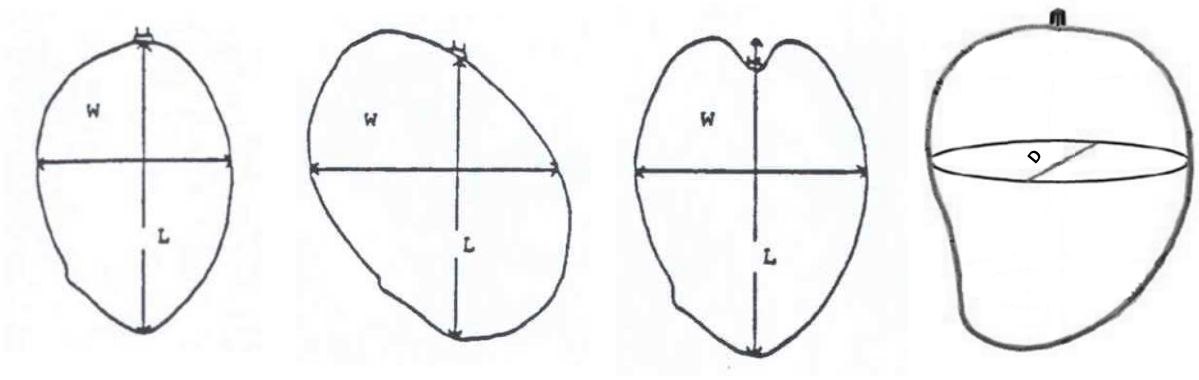

Fig 5-2: Mango fruit length (L), width (W), and depth (D) measurement, performed as previously described (Dhameliya *et al.*, 2016; UPOV, 2006).

Stone weight

Mango seeds, referred to as stones, were removed from fruit and weighed using a digital balance (Model FZ3000iWP, A&D Company Ltd, Tokyo, Japan) at ripe stage (n=4 fruit).

Stone weight percentage

Stone weight percentage was calculated as follows:

$$\text{Stone weight (\%)} = \frac{\text{Stone weight}}{\text{Fruit weight}} \times 100$$

Fruit dry matter contents

Fruit dry matter contents were measured at ripe stage (Hofman *et al.*, 2010). Fruit flesh was weighed before (wet weight) and after (dry weight) drying at 60ºC for 48 h in an oven (Model ODW50, Laboratory Equipment Pty Ltd, Marrickville, NSW, Australia) (n=4 fruit). Fruit dry matter was calculated as follows:

$$\text{Fruit dry matter (\%)} = \frac{\text{Dry weight}}{\text{Wet weight}} \times 100$$

Total Soluble Solids

A digital refractometer (Model DBR-1, Starr Instruments, Dandenong South, Victoria, Australia) was used to estimate total soluble solids (TSS). For this, 2–3 drops of mesocarp juice were placed on the prism of the refractometer and TSS was recorded as °Brix (n=4 fruit).

5.3.4 Statistical design and analysis

Field layout

The experiment was created following a Completely Randomised Design (RCD) with 7 treatments and 5 replications. Each treatment unit consisted of one tree. A maximum of twenty-five panicles were selected per tree for evaluation of fruit retention.

Statistical Design

The effect of treatments on fruit set and retention were statistically analysed using two methods: (1) residual maximum likelihood (REML) under factorial arrangement with two factors (product and spray concentration) using Genstat version 18.0 (VSN International Ltd., Hemel Hempstead, UK), and (2) generalised linear model (GLM) using SAS/STAT software version 9.0. (SAS Institute, Cary, NC, USA). Results are presented as treatment means.

Analysis of variance (ANOVA) was used to test the significance of PGR treatment on the various fruit quality parameters measured. Least significant difference (LSD) at $p \leq 0.05$ was used to establish significant differences between the treatment means (Singh and Janes, 2000).

5.4 Results

The results obtained for either mango variety are described separately.

5.4.1 Effect of PGR treatments on mango cv. NMBP-1243

Effect of PGR treatments on fruit set and retention in mango cv. NMBP-1243

Effect of PGR treatments on fruit set in cv. NMBP-1243

Fruit set is the retention and growth of the ovary following pollination. Fruit set is an important event in tree phenology (Srivastava, 2002). In mango cv. NMBP-1243, fruit set at 28 DAFB was not significantly affected by foliar application with any of the various individual PGR treatments over the 2014 and 2015 seasons (Table 5-3 and 5-8). Data shown in Table 5-13 reveals that the interactive effects of PGR treatments and seasons significantly altered fruit set in this cultivar.

Combined data for individual PGRs (Table 5-4 and 5-9) and combined data for all PGRs (Table 5-5 and 5-10) also revealed no significant difference regarding fruit set between control and PGR-treated trees in both seasons. Furthermore, there was no significant effect of PGR spray concentration on fruit set in either season (Table 5-6 and 5-11). The interactive effects of PGRs and spray concentrations on fruit set were significant, although the difference in fruit set was slight in the 2014 season (Table 5-7) and not observed in the 2015 season (Table 5-12).

Effect of PGR treatments on fruit retention at 42 DAFB in cv. NMBP-1243

Foliar application of various individual PGR treatments had no significant effect on fruit retention in cv. NMBP-1243 at 42 DAFB in either the 2014 or 2015 season (Table 5-3 and 5-8). Interactive effects of PGRs and season on fruit retention were also non-significant (Table 5-13).

Combined data for all PGRs did not reveal significant difference in fruit retention at 42 DAFB between control and PGR-treated trees in either growing season (Table 5-4 and 5-9). However,

combined data for individual PGRs in the 2014 season showed significantly higher fruit retention at 42 DAFB in 2,4-D-treated trees compared to that in control and other PGR treated trees, whereas this combined data showed no difference in fruit retention at 42 DAFB resulting from treatment with other PGRs (Table 5-5). In the 2015 season, no significant difference was observed in fruit retention at 42 DAFB among PGR-treated and control trees (Table 5-10). Also, neither PGR spray concentration (Table 5-6 and 5-11) nor the interactive effects of PGRs and PGR spray concentration (Table 5-7 and 5-12) significantly affected fruit retention at 42 DAFB in either season.

Effect of PGR treatments on fruit retention at 56 DAFB in cv. NMBP-1243

In the 2014 season, data for various individual PGR treatments showed that fruit retention at 56 DAFB was significantly higher on trees sprayed with 40 ppm 2,4-D (Table 5.3), resulting in fruit retention (36.00% fruit per panicle) which was 2.21 fold higher than that in control trees and significantly higher ($p \leq 0.0001$) than that in all other PGR-treated trees. Trees sprayed with 40 ppm 2,4D (36% fruit per panicle) has significantly higher fruit retention at 56 DAFB than that caused by other PGR treatments, whereas fruit retention resulting from all other PGR treatments were not significantly different from each other (Table 5-3).

In the 2015 season, data for various individual PGR treatments showed significant differences ($p \leq 0.05$) between fruit retention in experimental trees (Table 5-8). Trees sprayed with 25 ppm 2,4-D had the highest level of fruit retention (12.50% fruit per panicle), which was 1.76 fold higher than fruit retention in control trees. Statistically comparable to fruit retention following 25 ppm 2,4-D treatment, treatment with either 50 ppm NAA, 40 ppm 2,4-D, 5 ppm GA_3, or 10 ppm GA_3 also resulted in higher fruit retention at 56 DAFB compared to that in control trees. The lowest level of fruit retention was observed in control trees, which were comparable to that in trees sprayed with 25 ppm NAA and 40 ppm 2,4-D (Table 5-8). Furthermore, the interactive effects of season and PGR treatments on fruit retention at 56 DAFB were significant ($p \leq 0.001$; Table 5-13).

In the 2014 season, combined data for all PGRs showed that fruit retention at 56 DAFB was not affected by foliar application of PGRs, as no significant difference was observed between control and treated trees (Table 5-4). However in the 2015 season, combined data for all PGRs showed that PGR-treated trees had significantly higher fruit retention at 56 DAFB compared to that in control trees (Table 5-9). Combined data for individual PGRs revealed that foliar

application of 2,4-D resulted in significantly higher fruit retention at 56 DAFB compared to that following treatment with other PGRs in the 2014 season (Table 5-5); whereas comparable data for the 2015 season highlighted no significant difference among control and PGR-treated trees (Table 5-10). PGR spray concentrations were seen to significantly affect fruit retention at 56 DAFB, in that high PGR spray concentrations were conducive to higher fruit retention than low PGR spray concentrations in the 2014 season (Table 5-6); however, this result was not apparent in comparable data from the 2015 season (Table 5-11). The interactive effects of PGRs and treatment spray concentrations did not reveal changes in fruit retention at 56 DAFB in either season (Table 5-7 and 5-12).

Effect of PGR treatments on fruit retention at 70 DAFB in cv. NMBP-1243

The average fruit retention at 70 DAFB in cv. NMBP-1243 was significantly affected by various individual PGR treatments in the 2014 season (Table 5-3), but not in the 2015 season (Table 5-8). Trees treated with 40 ppm 2,4-D in the 2014 season displayed the highest level of fruit retention (20.99% fruit per panicle), which was 2.4 fold higher than that in control trees (Table 5-3). The remaining PGR treatments in the 2014 season led to fruit retentions that were statistically comparable to each other and to that in control trees (Table 5-3). However, interactive effects of seasons and individual PGR treatments indicated were significant for fruit retention at 70 DAFB (Table 5-13).

Combined data for all PGRs revealed that fruit retention at 70 DAFB was not significantly altered by PGR treatment in either season (Table 5-4 and 5-9). Combined data for individual PGRs showed that foliar application of 2,4-D resulted in the highest fruit retention, which was 1.83 fold higher than that recorded following NAA treatment, the PGR treatment yielding the lowest fruit retention rate at 70 DAFB in the 2014 season (Table 5-5). This same result was not observed in the 2015 season, where PGR treatments were not seen to significantly change fruit retention (Table 5-10). Also, PGR spray concentration and the interactive effects of PGRs and treatment spray concentrations were not associated with significant changes in fruit retention at 70 DAFB in either season (Table 5-6 and Table 5-11, and Table 5-7 and 5-12, respectively).

Effect of PGR treatments on fruit retention at 84 DAFB in cv. NMBP-1243

In the 2014 season, only 40 ppm 2,4-D treatment caused a significantly higher level of fruit retention in cv. NMBP-1243 at 84 DAFB (18.53% fruit per panicle), which was 2.42 fold higher than the lowest fruit retention level observed in control trees (7.65% fruit per panicle; Table 5-3). In contrast, in the 2015 season, various individual PGR treatments were seen to significantly affect fruit retention at 84 DAFB (Table 5-8). In this season, the highest fruit retention was recorded in trees sprayed with 50 ppm NAA (8.26% fruit per panicle), which was 2.32 fold higher than that in control trees (Table 5-8). All other PGR treatments aside from 10 ppm GA_3 resulted in fruit retention at 84 DAFB that was statistically comparable to that in control trees (Table 5-8). Data presented in Table 5-13 reveals that the interactive effects of season and PGR treatments were significant for fruit retention at 84 DAFB.

For both the 2014 and 2015 season, combined data for all PGRs revealed no significant difference between control and treated cv. NMBP-1243 trees for fruit retention at 84 DAFB (Table 5-4 and 5-9). Using combined data for individual PGRs from the 2014 season, it was apparent that foliar application of 2,4-D led to the highest fruit retention at 84 DAFB, which was 1.90 fold higher than the lowest fruit retention rate observed for PGR-treated trees (NAA treatment; Table 5-5). However, in the 2015 season, combined data for individual PGRs revealed no significant effect of PGR treatment on fruit retention 84 DAFB (Table 5-10). Furthermore, PGR spray concentration in either season (Table 5-6 and 5-11) and the interactive effects of PGR treatment and treatment spray concentrations in the 2014 season (Table 5-7) had no significant impact on fruit retention at 84 DAFB. However, in the 2015 season, the combined effect of NAA treatment at a high spray concentration impacted fruit retention at 84 DAFB, resulting in a level 2.32 fold higher than fruit retention following high-spray concentration 2,4-D treatment (lowest fruit retention out of any PGR treatment/spray concentration and control; Table 5-12).

Effect of PGR treatments on fruit retention at 98 DAFB in cv. NMBP-1243

Fruit retention at 98 DAFB in cv. NMBP-1243 was significantly affected by various individual PGR treatments in the 2014 season. The highest fruit retention rate (13.62% fruit per panicle) was recorded in trees treated with 40 ppm 2,4-D, which was 1.87 fold higher than that in control trees (Table 5-3). The remaining PGR treatments did not significantly affect fruit retention at 98 DAFB (Table 5-3). Similar to the 2014 season, PGR treatments had a significant effect on

fruit retention at 98 DAFB in the 2015 season. However, in this season, trees sprayed with 50 ppm NAA displayed the highest fruit retention at 98 DAFB (8.26% fruit per panicle), which was 2 fold higher than that in control trees (Table 5-8). Trees sprayed with 25 ppm 2,4-D, 5 ppm GA_3, and 10 ppm GA_3 exhibited similar fruit retention at 98 DAFB as that observed in 50 ppm NAA–treated trees (Table 5-8). In contrast with the 2014 season data, trees in the 2015 season treated with 40 ppm 2,4-D had the lowest fruit retention at 98 DAFB, which was statistically comparable to that in control trees (Table 5-8). The interactive effects of seasons and PGR treatments had a significant impact on fruit retention at 98 DAFB (Table 5-13).

Combined data for all PGRs revealed no significant difference between control and PGR-treated cv. NMBP-1243 trees for fruit retention at 98 DAFB in either season (Table 5-4 and 5-9). However, combined data for individual PGRs revealed that foliar application of 2,4-D in the 2014 season caused higher fruit retention compared to that for all other treatments, which was 1.65 fold higher than that observed for NAA (lowest fruit retention out of any treatment; Table 5-5). Similar data for the 2015 season showed no significant effect of PGRs on fruit retention at 98 DAFB (Table 5-10).

Fruit retention at 98 DAFB was not significantly affected by PGR spray concentrations in either season (Table 5-6 and 5-11) or the interactive effects of PGRs and PGR spray concentrations in the 2014 season (Table 5-7); however high-spray concentration NAA treatment imparted high-level fruit retention at 98 DAFB in the 2015 season, which was 2.32 fold higher than that observed following high-spray concentration 2,4-D treatment (Table 5-12).

Table 5-3: The effect of six foliar-applied PGR treatments on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2014 season.

	Fruit set	**Fruit retention**				
Treatment	**28**	**42**	**56**	**70**	**84**	**98**
Control	8.90	41.61^{b}	16.29^{bc}	8.87^{b}	7.65^{b}	7.24^{b}
25 ppm NAA	8.27	42.06^{b}	15.05^{c}	9.74^{b}	8.40^{b}	6.90^{b}
50 ppm NAA	8.89	39.59^{b}	17.22^{bc}	9.36^{b}	7.92^{b}	6.82^{b}
25 ppm 2,4-D	8.40	49.60^{ab}	23.82^{b}	14.00^{b}	12.63^{b}	9.09^{b}
40 ppm 2,4-D	5.11	58.97^{a}	36.00^{a}	20.99^{a}	18.53^{a}	13.62^{a}
5 ppm GA_3	7.42	40.68^{b}	15.18^{c}	10.61^{b}	9.74^{b}	8.39^{b}
10 ppm GA_3	8.32	43.03^{b}	18.99^{bc}	10.67^{b}	8.19^{b}	6.87^{b}
Level of significance	NS	NS	$p\leq0.0001$	$p\leq0.001$	$p\leq0.001$	$p\leq0.05$
l.s.d.	-	-	7.78	5.84	5.38	4.03

Numbers in bold indicate days after full bloom. NS=non-significant at $p\leq0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=5

Table 5-4: The combined effect of three PGRs on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2014 season.

Treatment	**Fruit set**	**Fruit retention**				
	28	**42**	**56**	**70**	**84**	**98**
Control	8.90	41.61	16.29	8.87	7.65	7.24
Treated	7.74	45.66	21.04	12.56	10.90	8.62
Level of significance	NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p\leq0.05$. n=5

Table 5-5: The effect of three PGRs on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2014 season. Data are the average of two PGR spray concentrations.

Treatment	**Fruit set**	**Fruit retention**				
	28	**42**	**56**	**70**	**84**	**98**
Control	8.90	41.61^{b}	16.29^{b}	8.87^{b}	7.65^{b}	7.24^{b}
NAA	8.58	40.83^{b}	16.14^{b}	9.55^{b}	8.16^{b}	6.86^{b}
2,4-D	6.76	54.28^{a}	29.91^{a}	17.49^{a}	15.57^{a}	11.35^{a}
GA_3	7.87	41.86^{b}	17.09^{b}	10.64^{b}	8.96^{b}	7.63^{b}
Level of significance	NS	$p\leq0.05$	$p\leq0.05$	$p\leq0.05$	$p\leq0.05$	$p\leq0.05$
l.s.d	-	10.21	5.50	4.13	3.80	2.85

Numbers in bold indicate days after full bloom. NS=non-significant at $p\leq0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=5

Table 5-6: The effect of PGR spray concentrations on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2014 season.

Spray concentrations	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	8.90	41.61	16.29[b]	8.87	7.65	7.24
Low	8.04	44.11	18.02[b]	11.45	10.25	8.13
High	7.44	47.2	24.07[a]	13.67	11.54	9.10
Level of significance	NS	NS	$p \leq 0.05$	NS	NS	NS
l.s.d.	-	-	4.49	-	-	-

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-7: The interactive effects of six foliar-applied PGR treatments and treatment spray concentrations on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2014 season.

Treatment	Spray concentration	Fruit set	Fruit retention				
		28	**42**	**56**	**70**	**84**	**98**
Control		8.90[a]	41.61	16.29	8.87	7.65	7.24
NAA	Low	8.27[a]	42.06	15.05	9.74	8.40	6.90
	High	8.89[a]	39.59	17.22	9.36	7.92	6.83
2,4-D	Low	8.41[a]	49.6	23.82	14.00	12.63	9.09
	High	5.11[b]	58.97	36.00	20.98	18.52	13.62
GA_3	Low	7.43[ab]	40.68	15.18	10.62	9.74	8.39
	High	8.32[a]	43.03	18.99	10.67	8.19	6.87
Level of significance		$p \leq 0.05$	NS	NS	NS	NS	NS
l.s.d		2.56	-	-	-	-	-

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=5. Spray concentrations for each treatment are presented in Table 5-2

Table 5-8: The effect of six foliar-applied PGR treatments on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2015 season.

Treatment	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	11.88	13.22	7.07^{c}	4.86	4.14^{bc}	4.14^{bc}
25 ppm NAA	9.49	14.13	8.27^{bc}	5.86	5.12^{bc}	5.12^{bc}
50 ppm NAA	8.64	17.66	12.13^{a}	8.99	8.26^{a}	8.26^{a}
25 ppm 2,4-D	10.59	17.32	12.50^{a}	9.90	6.23^{abc}	6.23^{abc}
40 ppm 2,4-D	11.82	14.82	10.10^{abc}	6.77	3.56^{c}	3.56^{c}
5 ppm GA_3	10.77	16.65	11.82^{ab}	8.72	5.81^{abc}	5.81^{abc}
10 ppm GA_3	10.11	16.86	11.40^{ab}	7.53	6.52^{ab}	6.52^{ab}
Level of significance	NS	NS	$p \leq 0.05$	NS	$p \leq 0.05$	$p \leq 0.05$
l.s.d	-	-	3.68	-	2.96	2.96

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=5.

Table 5-9: The combined effects of three PGRs on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2015 season.

Treatment	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	11.88	13.23	7.07^{b}	4.86^{b}	4.14	4.14
Treated	10.24	16.24	11.04^{a}	7.96^{a}	5.92	5.92
Level of significance	NS	NS	$p \leq 0.05$	$p \leq 0.05$	NS	NS
l.s.d.	-	-	2.81	2.86	-	-

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=5.

Table 5-10: The effect of three PGRs on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2015 season. Data are the average of two PGR spray concentrations.

Treatment	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	11.88	13.23	7.07	4.86	4.14	4.14
NAA	9.07	15.90	10.2	7.42	6.69	6.69
2,4-D	11.21	16.07	11.3	8.34	4.90	4.90
GA_3	10.44	16.76	11.6	8.13	6.17	6.17
Level of significance	NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. n=5.

Table 5-11: The effect of PGR spray concentration on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2015 season.

Spray concentrations	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	11.88	13.23	7.07	4.86	4.14	4.14
Low	10.29	16.03	10.86	8.16	5.72	5.72
High	10.19	16.45	11.21	7.76	6.11	6.11
Level of significance	NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-12: The interactive effects of foliar-applied PGR treatments and spray concentrations on fruit set and retention rates per panicle in mango cv. NMBP-1243 in the 2015 season.

Treatment	Spray concentration	Fruit set	Fruit retention				
		28	**42**	**56**	**70**	**84**	**98**
Control		11.88	13.23	7.07	4.86	4.14^{bc}	4.14^{bc}
NAA	Low	9.49	14.13	8.27	5.85	5.12^{bc}	5.12^{bc}
	High	8.64	17.66	12.14	8.99	8.26^{a}	8.26^{a}
2,4-D	Low	10.59	17.32	12.5	9.90	6.24^{abc}	6.24^{abc}
	High	11.82	14.82	10.10	6.77	3.56^{c}	3.56^{c}
GA_3	Low	10.77	16.65	11.82	8.73	5.81^{abc}	5.81^{abc}
	High	10.12	16.86	11.4	7.53	6.52^{ab}	6.52^{ab}
Level of significance		NS	NS	NS	NS	$p \leq 0.05$	$p \leq 0.05$
l.s.d.		-	-	-	-	2.96	2.96

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-13: The interactive effects of seasons and foliar-applied PGRs on fruit set and retention rates per panicle in mango cv. NMBP-1243.

Season	Treatment	Fruit set	Fruit retention				
		28	**42**	**56**	**70**	**84**	**98**
2014	Control	8.90	41.61	16.29	8.87	7.65	7.24
	25 ppm NAA	8.27	42.06	15.05	9.74	8.40	6.90
	50 ppm NAA	8.89	39.59	17.22	9.36	7.92	6.82
	25 ppm 2,4-D	8.41	49.60	23.82	14.00	12.63	9.09
	40 ppm 2,4-D	5.11	58.97	36.00	20.99	18.53	13.62
	5 ppm GA_3	7.43	40.68	15.18	10.61	9.74	8.39
	10 ppm GA_3	8.32	43.03	18.99	10.67	8.19	6.87
2015	Control	11.88	13.22	7.07	4.86	4.14	4.14
	25 ppm NAA	9.49	14.13	8.27	5.86	5.12	5.12
	50 ppm NAA	8.64	17.66	12.13	8.99	8.26	8.26
	25 ppm 2,4-D	10.59	17.32	12.50	9.90	6.23	6.23
	40 ppm 2,4-D	11.82	14.82	10.10	6.77	3.56	3.56
	5 ppm GA_3	10.77	16.65	11.82	8.72	5.81	5.81
	10 ppm GA_3	10.12	16.86	11.40	7.53	6.52	6.52
Level of significance		$p \leq 0.0001$	NS	$p \leq 0.0001$	$p \leq 0.001$	$p \leq 0.0001$	$p \leq 0.001$
C.V.		21.16	26.00	31.09	39.66	42.91	39.53

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. C.V.=co-efficient of variation. n=5.

Effect of PGR treatments on fruit quality in cv. NMBP-1243

Effect of PGR treatments on fruit weight in cv. NMBP-1243

Fruit weight was significantly influenced by various individual PGR treatments in both the 2014 and 2015 seasons (Table 5-14 and 5-20). In the 2014 season, only 25 ppm 2,4-D and 40 ppm 2,4-D treatments had a significant effect by reducing fruit weight compared to that in the control (Table 5-14). Highest fruit weight (511.74 g) was recorded in trees sprayed with 25 ppm NAA, but this measurement was statistically comparable with data for control trees and those sprayed with 50 ppm NAA, 5 ppm GA_3, and 10 ppm GA_3. Meanwhile, lowest fruit weight (365.59 g) was observed in trees treated with 40 ppm 2,4-D (Table 5-14). In the 2015 season, only trees sprayed with 40 ppm 2,4-D possessed fruit with significantly reduced weight compared to that resulting from other PGR treatments and in the control (Table 5-20).

Combined data for all PGRs showed that PGR treatment had no significant impact on fruit weight in the 2014 season (Table 5-15), but a significant reduction in fruit weight resulted from PGR treatment in the 2015 season (Table 5-21). Combined data for individual PGRs revealed

that foliar application of 2,4-D resulted in the lowest fruit weight, which was significantly lower than that for the control and other PGR treatments in both the 2014and 2015 seasons (Table 5-16 and 5-22). PGR spray concentrations in either season (Table 5-17 and 5-23) and the interactive effects of PGRs and spray concentrations in the 2014 season (Table 5-18) had no significant impact on fruit weight; however, in the 2015 season, foliar application of high-spray concentration 2,4-D caused reduced fruit weight, which was significantly lower than that resulting from high-spray concentration NAA treatment (highest fruit weight) and all other PGR treatments and the control (Table 5-24).

Effect of PGR treatments on fruit length in cv. NMBP-1243

Data detailed in Table 5-14 and 5-20 summarise the significant influence of various individual PGR treatments on fruit length in the 2014 and 2015 seasons (Table 5-14 and 5-20). Foliar application of 40 ppm 2,4-D resulted in a fruit length of 100.06 mm, which was significantly decreased compared to that in the control and caused by other PGR treatments (Table 5-14). Similar minimum fruit length was observed in trees sprayed with 40 ppm 2,4-D in the subsequent 2015 season, whereas all other PGR treatments had non-significant significant effects on fruit length compared with one another and fruit length in control trees (Table 5-20).

Combined data for all PGRs highlighted no significant difference in fruit length between control and treated trees in both the 2014 and 2015 seasons (Table 5-15 and 5-21); however, combined data for individual PGRs showed trees sprayed with 2,4-D had fruit with significantly less length compared to other PGR-treated trees and control trees in either season (Table 5-16 and 5-22). PGR spray concentrations (Table 5-17 and 5-23) and the interactive effects between PGRs and PGR spray concentrations (Table 5-18 and 5-24) did not significantly affect fruit length in both the 2014 and 2015 seasons.

Effect of PGR treatments on fruit width in cv. NMBP-1243

Statistical analysis showed that various individual PGR treatments had a significant effect on fruit width in the 2014 and 2015 seasons (Table 5-14 and 5-20). In the 2014 season, foliar application of 40 ppm 2,4-D reduced fruit width to a level comparable with that observed following 25 ppm 2,4-D treatment, but significantly less than that observed for the control and all other treatments (Table 5-14). The greatest measurement of fruit width (97.46 mm) was made in fruit harvested from trees sprayed with 25 ppm NAA, which was significantly

comparable to that in control trees and trees sprayed with either 50 ppm NAA, 5 ppm GA_3, or 10 ppm GA_3 (Table 5-14). Similar results for fruit width following PGR treatment were recorded for the 2015 season, whereby foliar spray of 40 ppm 2,4-D significantly reduced fruit width, and fruit width in control trees and those sprayed with all other PGR treatments were statistically comparable (Table 5-20).

Combined data for all PGRs showed no significant difference in fruit width between control and PGR-treated trees in the 2014 season (Table 5-15); however, control trees had greater fruit width compared to that of PGR-treated trees in the 2015 season (Table 5-21). Combined data for individual PGRs showed that 2,4-D treatment resulted in fruit with the least width (Table 5-16 and 5-22), which was significantly reduced compared to that for the control and other PGR treatments in the 2014 season. PGR spray concentrations in both the 2014 and 2015 seasons (Table 5-17 and 5-23) and the interactive effects of PGRs and PGR spray concentrations in the 2014 season (Table 5-18) had no significant impact on fruit width. However, in the 2015 season, high-spray concentration foliar application of 2,4-D resulted in significantly reduced fruit width (Table 5-24).

Effect of PGR treatments on fruit depth in cv. NMBP-1243

Fruit depth significantly differed between trees treated with various individual PGR treatments in the 2014 and 2015 seasons (Table 5-14 and 5-20). The maximum fruit depth in the 2014 season (85.82 mm) was measured in fruit harvested from trees sprayed with 25 ppm NAA, which was statistically comparable to fruit depth in control trees and trees sprayed with all other PGR treatments except for 25 ppm and 40 ppm 2,4-D (Table 5-14). 25 ppm and 40 ppm 2,4-D treatment resulted in the minimum fruit depths (79.17 mm and 77.38 mm, respectively) that were statistically comparable with each other (Table 5-14). In the 2015 season, fruit harvested from trees sprayed with 40 ppm 2,4-D again exhibited the minimum fruit depth (73.00 mm), which was significantly reduced compared to that in the control and that resulting from the remaining PGR treatments (Table 5-20).

Combined data for all PGRs showed that fruit harvested from control and PGR-treated trees had statistically comparable depth in both the 2014 and 2015 seasons (Table 5-15 and 5-21). Combined data for individual PGRs revealed that foliar application of 2,4-D yielded the lowest fruit depth in either season, which was significantly reduced compared to that resulting in the control and from other PGRs (Table 5-16 and 5-22). Fruit depth did not differ significantly as

a result of either different PGR spray concentrations in both seasons (Table 5-17 and 5-23) or the interactive effects of PGRs and PGR spray concentrations in the 2014 season (Table 5-18). High-spray concentration foliar application of 2,4-D caused significant reduction in fruit depth compared to that in control trees (greatest fruit depth) and trees treated with all other spray concentrations of other PGRs (Table 5-24).

Effect of PGR treatments on fruit TSS in cv. NMBP-1243

Foliar application of various individual PGR treatments had a significant effect on fruit TSS in the 2014 season (Table 5-14), with the highest level of TSS recorded for fruit harvested from 10 ppm GA_3–treated trees (14.90%), which was statistically comparable with the TSS in fruit obtained from 5 ppm GA_3– and 25 ppm 2,4-D–treated trees. The least amount of TSS in the 2014 season was measured in fruit from 40 ppm 2,4-D–treated trees (13.17; Table 5-14). In contrast, in the 2015 season, PGR treatments had no significant influence on fruit TSS (Table 5-20).

Combined data for all PGRs showed that fruit TSS did not differ significantly between control and PGR-treated trees in both the 2014 and 2015 seasons (Table 5-15 and 5-21). Combined data for individual PGRs revealed that foliar application of GA_3 significantly increased fruit TSS compared to the resulting from other PGRs in the 2014 season (Table 5-16). However, the same data for the 2015 season showed no significant difference among the PGR treatments and the control (Table 5-22). PGR spray concentrations did not alter fruit TSS in both seasons (Table 5-17 and 5-23). The interactive effects of PGRs and PGR spray concentration showed that, in the 2014 season, high-level 2,4-D treatment resulted in the lowest fruit TSS, which was significantly reduced compared to that in fruit from all other experimental trees, including high-level GA_3–treated trees (the highest fruit TSS; Table 5-18). In the 2015 season, high-level GA_3 treatment again produced fruit with the highest TSS, which was statistically comparable to all other treatments except for low-spray concentration GA_3 and high-spray concentration NAA, which both produced fruit with significantly less TSS at statistically comparable levels (Table 5-24).

Effect of PGR treatments on fruit dry matter contents in cv. NMBP-1243

Fruit dry matter contents (DM) did not differ significantly as result of foliar application of various individual PGR treatments in both the 2014 and 2015 seasons (Table 5-14 and 5-20).

Furthermore, combined data for all PGRs (Table 5-15 and 5-21) and combined data for individual PGRs (Table 5-16 and 5-22) showed that DM were not significantly affected by PGR treatment in either season. PGR spray concentrations did not affect DM in the 2014 season (Table 5-17); however, in the 2015 season, significantly lower DM were recorded in fruit harvested from trees sprayed with low PGR spray concentrations (Table 5-23). The interactive effects of PGRs and PGR spray concentrations did not significantly affect DM in both seasons (Table 5-18 and 5-24).

Table 5-14: The effect of PGR treatments at two spray concentrations on mango cv. NMBP-1243 fruit quality in the 2014 season.

Treatment	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit depth (mm)	TSS (%)	DM (%)
Control	489.92[a]	112.65[a]	95.61[a]	82.76[a]	13.95[b]	14.09
25 ppm NAA	511.74[a]	113.57[a]	97.46[a]	85.82[a]	13.92[b]	14.09
50 ppm NAA	479.67[a]	110.84[ab]	94.51[ab]	83.29[a]	13.99[b]	14.22
25 ppm 2,4-D	413.35[b]	106.34[b]	91.02[bc]	79.17[b]	14.20[ab]	14.03
40 ppm 2,4-D	365.59[b]	100.06[c]	87.97[c]	77.38[b]	13.17[c]	13.58
5 ppm GA_3	493.12[a]	113.11[a]	96.26[a]	83.82[a]	14.41[a]	14.14
10 ppm GA_3	497.42[a]	113.84[a]	96.58[a]	85.29[a]	14.90[a]	14.77
Level of significance	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.001$	$p \leq 0.0001$	$p \leq 0.001$	NS
C.V.	8.25	3.47	3.16	3.33	4.11	4.25

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. C.V.=coefficient of variation. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-15: The combined effect of PGR treatments on mango cv. NMBP-1243 fruit quality in the 2014 season.

Treatment	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit depth (mm)	TSS (%)	DM (%)
Control	489.9	112.65	95.61	82.76	13.945	14.09
Treated	460.1	109.62	93.96	82.46	14.094	14.14
Level of significance	NS	NS	NS	NS	NS	NS

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-16: The effect of three PGR treatments on mango cv. NMBP-1243 fruit quality in the 2014 season. Data are the average of two PGR spray concentrations.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	489.9[a]	112.65[a]	95.61[a]	82.76[a]	13.95[b]	14.09
NAA	495.7[a]	112.20[a]	95.98[a]	84.55[a]	13.95[b]	14.15
2,4-D	389.5[b]	103.20[b]	89.49[b]	78.27[b]	13.68[b]	13.80
GA_3	495.3[a]	113.47[a]	96.42[a]	84.55[a]	14.65[a]	14.45
Level of significance	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	NS
l.s.d.	35.11	3.51	2.73	2.52	0.53	-

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-17: The effect of PGR spray concentration on of mango cv. NMBP-1243 fruit quality in the 2014 season.

Spray concentrations	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	489.9	112.65	95.61	82.76	13.945	14.09
Low	44.11	111.00	94.91	82.93	14.173	14.09
High	47.2	108.24	93.02	81.99	14.01	14.19
Level of significance	NS	NS	NS	NS	NS	NS

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-18: The interactive effects of PGRs and PGR spray concentration on mango cv. NMBP-1243 fruit quality in the 2014 season.

Treatment	Spray concentrations	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit depth (mm)	TSS (%)	DM (%)
Control		489.9	112.65	95.61	82.76	13.95^{b}	14.09
NAA	Low	511.7	113.57	97.45	85.81	13.92^{b}	14.09
	High	479.7	110.84	94.51	83.29	13.99^{b}	14.22
2,4-D	Low	413.3	106.33	91.02	79.17	14.20^{ab}	14.03
	High	365.6	100.06	87.97	77.38	13.17^{c}	13.58
GA_3	Low	493.1	113.11	96.26	83.81	14.41^{ab}	14.14
	High	497.4	113.84	96.58	85.29	14.89^{a}	14.77
Level of significance		NS	NS	NS	NS	$p \leq 0.05$	NS
l.s.d.		-	-	-	-	0.75	-

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. TSS=total soluble solids. DM=dry matter contents. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-19: Variation in mango cv. NMBP-1243 fruit quality within and between tree levels in the 2014 season.

Variation level	Estimator	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit depth (mm)	TSS (%)	DM (%)
Within Trees	Mean SE	26.67	2.555	2.041	1.65	0.35	0.30
	Mean SD	53.35	5.11	4.082	3.29	0.69	0.60
Between Trees	Mean	464.4	110.1	94.2	82.50	14.07	14.13
	SD	61.4	5.931	4.221	3.85	0.72	0.64
	SEM	10.38	1.002	0.714	0.65	0.12	0.11

SE=standard error. SD=standard deviation. SEM=standard error of means. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-20: The effect of PGR treatments at two spray concentrations on mango cv. NMBP-1243 fruit quality in the 2015 season.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	457.34[a]	107.09[a]	93.89[a]	82.36[a]	10.50	16.17
25 ppm NAA	406.67[a]	101.19[a]	88.54[b]	80.11[a]	10.86	16.81
50 ppm NAA	446.25[a]	106.37[a]	92.10[ab]	83.30[a]	10.23	15.95
25 ppm 2,4-D	433.62[a]	105.60[a]	92.72[ab]	80.61[a]	10.64	16.86
40 ppm 2,4-D	310.58[b]	93.82[b]	81.41[c]	73.00[b]	10.53	16.22
5 ppm GA_3	410.50[a]	101.87[a]	90.45[ab]	80.29[a]	10.29	16.13
10 ppm GA_3	430.40[a]	104.57[a]	92.21[ab]	82.09[a]	11.03	16.09
Level of significance	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.0001$	$p \leq 0.0001$	NS	NS
C.V.	11.61	4.95	4.25	4.11	5.19	3.70

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-21: The combined effect of PGR treatments on mango cv. NMBP-1243 fruit quality in the 2015 season.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	457.3[a]	112.65	93.89[a]	82.36	10.495	16.17
Treated	406.3[b]	109.62	89.57[b]	79.90	10.595	16.34
Level of significance	$p \leq 0.05$	NS	$p \leq 0.05$	NS	NS	NS
l.s.d.	47.50	-	3.80	-	-	-

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-22: The effect of PGR treatments on mango cv. NMBP-1243 fruit quality in the 2015 season. Data are the average of two PGR spray concentrations.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	457.3[a]	112.65[a]	93.89[a]	82.36[a]	10.495	16.17
NAA	426.5[a]	112.20[a]	90.32[ab]	81.71[a]	10.543	16.38
2,4-D	372.1[b]	103.20[b]	87.07[b]	76.80[b]	10.583	16.54
GA_3	420.5[a]	113.47[a]	91.33[a]	81.19[a]	10.66	16.11
Level of significance	$p \leq 0.05$	$p \leq 0.001$	$p \leq 0.05$	$p \leq 0.001$	NS	NS
l.s.d.	43.98	3.51	3.51	3.02	-	-

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-23: The effect of PGR spray concentration on mango cv. NMBP-1243 fruit quality in the 2015 season.

Spray concentrations	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	457.3	112.65	93.89	82.36	10.50	16.17[ab]
Low	416.9	111.00	90.57	80.34	10.59	16.60[a]
High	395.7	108.24	88.57	79.46	10.60	16.09[b]
Level of significance	NS	NS	NS	NS	NS	$p \leq 0.05$
l.s.d.	-	-	-	-	-	0.64

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. TSS=total soluble solids. DM=dry matter contents. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-24: The interactive effects of PGRs and their spray concentration on mango cv. NMBP-1243 fruit quality in the 2015 season.

Treatment	Spray concentrations	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control		457.3^{a}	112.65	93.89^{a}	82.36^{a}	10.50^{ab}	16.17
NAA	Low	406.7^{a}	113.57	88.54^{b}	80.11^{a}	10.86^{ab}	16.81
	High	446.2^{a}	110.84	92.10^{ab}	83.30^{a}	10.23^{b}	15.95
2,4-D	Low	433.6^{a}	106.33	92.72^{ab}	80.61^{a}	10.64^{ab}	16.85
	High	310.6^{b}	100.06	81.41^{c}	73.00^{b}	10.53^{ab}	16.22
GA_3	Low	410.5^{a}	113.11	90.45^{ab}	80.29^{a}	10.29^{b}	16.13
	High	430.4^{a}	113.84	92.21^{ab}	82.08^{a}	11.03^{a}	16.1
Level of significance		$p\leq0.001$	NS	$p\leq0.001$	$p\leq0.001$	$p\leq0.05$	NS
l.s.d.		62.19	-	4.97	4.27	0.71	-

NS=non-significant at $p\leq0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. TSS=total soluble solids. DM=dry matter contents. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-25: Variation in mango cv. NMBP-1243 fruit quality within and between tree levels in the 2015 season.

Variation level	Estimator	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Within Trees	Mean SE	25.99	3.04	2.25	1.77	0.49	0.47
	Mean SD	51.99	6.09	4.50	3.54	0.98	0.95
Between Trees	Mean	413.60	102.90	90.19	80.25	10.58	16.32
	SD	63.29	6.32	5.29	4.38	0.57	0.64
	SEM	10.70	1.07	0.89	0.74	0.09	0.11

SE=standard error. SD=standard deviation. SEM=standard error of means. TSS=total soluble solids. DM=dry matter contents. n=5.

5.4.2 Effect of PGR treatments on mango cv. Keitt

Effect of PGR treatments on fruit set and retention of mango cv. Keitt

Effect of PGR treatments on fruit set in cv. Keitt

Following various individual foliar applied PGR treatments, statistical analysis revealed no significant effect on fruit set in cv. Keitt in both the 2014 and 2015 seasons (Table 5-26 and 5-31). Combined data for all PGRs showed that fruit set differed between the control and PGR-sprayed trees in both seasons, with control trees displaying significantly higher fruit set (Table 5-27 and 5-32). However, combined data for individual PGRs did not reflect significant differences in fruit set between control and PGR-treated trees in either season (Table 5-28 and 5-33). PGR spray concentrations (Table 5-29 and 5-34) and the interactive effects of PGRs and PGR spray concentrations (Table 5-30 and 5-35) did not significantly impact fruit set in both seasons. Fruit set differed non-significantly by interaction of PGRs and spray concentrations.

Effect of PGR treatments on fruit retention at 42 DAFB in cv. Keitt

Fruit retention did not differ significantly at 42 DAFB in experimental cv. Keitt trees as a result of foliar application of various individual PGR treatments in both the 2014 and 2015 seasons (Table 5-26 and 5-31). Combined data for all PGRs (Table 5-27 and 5-32) as well as combined data for individual PGRs (Table 5-28 and 5-33) revealed no significant difference in fruit retention at 42 DAFB in control and PGR-treated trees in either season. Both PGR spray concentrations (Table 5-29 and 5-34) and the interactive effects of PGR treatment and PGR spray concentrations (Table 5-30 and 5-35) had no significantly impact on fruit retention at 42 DAFB in both seasons.

Effect of PGR treatments on fruit retention at 56 DAFB in cv. Keitt

Data for cv. Keitt at 56 DAFB indicates a significant effect of various individual PGR treatments on fruit retention in experimental trees in the 2014 season (Table 5-26). Trees sprayed with 40 ppm 2,4-D exhibited the highest rate of fruit retention (36.14% fruit per panicle), which was 1.5 fold higher than that in control trees, but statistically comparable to that resulting from 25 ppm 2,4-D and 5 ppm GA_3 treatment (Table 5-26). Control trees displayed the lowest fruit retention (24.25% fruit per panicle), which was statistically comparable to that resulting from 25 ppm NAA, 50 ppm NAA, and 10 ppm GA_3 treatment

(Table 5-26). In contrast to the 2014 season, no significant difference was observed in fruit retention at 56 DAFB following various PGR treatments in the 2015 season (Table 5-31).

Combined data for all PGRs showed that PGR-treated trees had significantly higher fruit retention at 56 DAFB compared to that in control trees in the 2014 season (Table 5-27) but this same effect was not observed in comparable data from the 2015 season (Table 5-32). Combined data for individual PGRs showed that, at 56 DAFB, fruit retention was increased by 2,4-D treatment in the 2014 season, and that this increase was significantly higher that fruit retention rates in control trees and trees treated with other PGRs (Table 5-28); however, comparable data from the 2015 season showed no significant effect of PGR treatment (Table 5-33). Fruit retention at 56 DAFB in both seasons was not significantly affected by either PGR spray concentrations (Table 5-29 and 5-34) or the interactive effects of PGR treatment and PGR spray concentrations (Table 5-30 and 5-35).

Effect of PGR treatments on fruit retention at 70 DAFB in cv. Keitt

In the 2014 season, average cv. Keitt fruit retention at 70 DAFB was significantly affected by various individual PGR treatments, with trees treated with 40 ppm 2,4-D exhibiting the highest fruit retention (32.01% per panicle), which was 1.8 fold higher than that in control trees (18.40% fruit per panicle; lowest fruit retention; Table 5-26). However, comparable data from the 2015 season showed no significant effect of any of the PGR treatments on fruit retention at 70 DAFB (Table 5-31).

Combined data for all PGRs showed significantly higher fruit retention at 70 DAFB in PGR-treated trees compared to that in control trees in the 2014 season (Table 5-27); however, in the 2015 season, comparable data showed no significant difference between control and PGR-treated trees (Table 5-32). Combined data for individual PGRs showed that PGR application did not influence fruit retention at 70 DAFB in both seasons (Table 5-28 and 5-33). Both PGR spray concentrations (Table 5-29 and 5-34) and the interactive effects of PGRs and PGR spray concentrations (Table 5-30 and 5-35) had no significant impact on fruit retention at 70 DAFB in both seasons.

Effect of PGR treatments on fruit retention at 84 DAFB in cv. Keitt

In cv. Keitt at 84 DAFB, fruit retention differed significantly in response to various individual PGR treatments in the 2014 season, with trees treated with 40 ppm 2,4-D exhibiting the highest fruit retention (30.45% fruit per panicle), which was 1.76 fold higher than that in control trees (Table 5-26). Control trees had the lowest fruit retention at 84 DAFG (17.21% fruit per panicle), which was statistically comparable to that in trees treated with 25 ppm NAA and 10 ppm GA_3 (Table 5-26). However, comparable data from the 2015 season showed no significant effect of any of the PGR treatments on fruit retention at 84 DAFB (Table 5-31).

Combined data for all PGRs showed that fruit retention at 84 DAFB differed between control and PGR-treated trees in the 2014 season, with significantly higher fruit retention observed following PGR treatment (Table 5-27), but this same result was not observed in comparable data from the 2015 season (Table 5-32). Combined data for individual PGRs showed that application of each PGR significantly increased fruit retention at 84 DAFB compared to that in control trees (Table 5-28). 2,4-D-treated trees displayed the highest fruit retention at 84 DAFB among all PGR-treated trees in the 2014 season (Table 5-28); however, in the 2015 season, the foliar application of PGRs did not significantly affect fruit retention at 84 DAFB (Table 5-33). PGR spray concentrations also had no significant impact on fruit retention at 84 DAFB in both seasons (Table 5-29 and 5-34). The interactive effects of PGRs and PGR spray concentrations significantly affected fruit retention at 84 DAFB in the 2014 season, with the highest level of fruit retention recorded in trees treated with high-spray concentration 2,4-D and control trees exhibiting the lowest fruit retention, which was statistically comparable to that in high-spray concentration GA_3–treated trees (Table 5-30). In the 2015 season, fruit retention at 84 DAFB was not significantly impacted by the interactive effects of PGRs and PGR spray concentrations (Table 5-35).

Effect of PGR treatments on fruit retention at 98 DAFB in cv. Keitt

In the 2014 season, fruit retention at 98 DAFB in cv. Keitt was significantly affected by foliar application of various individual PGR treatments, with the highest fruit retention observed in trees sprayed with 40 ppm 2,4-D (28.49% fruit per panicle), which was 1.78 fold higher than that in control trees (Table 5-26). This high fruit retention at 98 DAFB following 40 ppm 2,4-D treatment was statistically comparable to that resulting from 50 ppm NAA, 25 ppm 2,4-D,

and 5 ppm GA_3 treatment (Table 5-26). Comparable data from the 2015 season showed no significant effect of any of the PGR treatments on fruit retention at 98 DAFB (Table 5-31).

Combined data for all PGRs showed that, in the 2014 season, foliar application of PGRs significantly increased fruit retention at 98 DAFB compared to fruit retention in control trees (Table 5-27); however, no significant effect of PGR treatment on fruit retention at 98 DAFB was observed in the 2015 season (Table 5-32). In the 2014 season, combined data for individual PGRs showed that fruit retention at 98 DAFB differed significantly as a result of PGR treatment, with the highest fruit retention exhibited by trees treated with 2,4-D, which was significantly higher than that in NAA-treated trees but statistically comparable to that in GA_3-treated trees (Table 5-28). Comparable data for the 2015 season revealed no significant impact of any PGR treatment on fruit retention at 98 DAFB (Table 5-33). PGR spray concentrations also did not significantly impact fruit retention at 98 DAFB in both seasons (Table 5-29 and 5-34). The interactive effects of PGRs and PGR spray concentrations significantly affected fruit retention at 98 DAFB, with high-spray concentration 2,4-D treatment resulting in the highest fruit retention, which was significantly higher than that in control trees and that resulting from low-spray concentration NAA treatment, but was statistically comparable to that resulting from all other treatments (Table 5-30). In the 2015 season, the interactive effects of PGRs and PGR spray concentrations did not significantly impact fruit retention at 98 DAFB (Table 5-35).

Effect of PGR treatments on fruit retention at 112 DAFB in cv. Keitt

Data in Table 5-26 shows that there was significant difference in cv. Keitt fruit retention at 112 DAFB in experimental trees treated with various individual PGRs at different spray concentrations in the 2014 season. Trees treated with 40 ppm 2,4-D displayed the highest fruit retention at 112 DAFB (28.49% fruit per panicle), which was 1.83 fold higher than that in control trees and statistically similar to that in 50 ppm NAA–, 25 ppm 2,4-D–, and 5 ppm GA_3–treated trees. The lowest fruit retention at 112 DAFB was recorded in control trees (15.49% fruit per panicle), which was statistically comparable with fruit retention in 25 ppm NAA– and 10 ppm GA_3–treated trees (Table 5-26).

Combined data for all PGRs in the 2014 season showed significant difference in fruit retention at 112 DAFB between control and PGR-treated trees, with PGR-treated displaying significantly higher fruit retention than that in control trees (Table 5-27). Combined data for individual PGRs in the 2014 season showed that PGR treatment significantly improved fruit

retention at 112 DAFB, with 2,4-D treatment resulting in the highest fruit retention, which was significantly higher than that in control and NAA-treated, but statistically comparable with GA_3-treated trees (Table 5-28). Fruit retention at 112 DAFB was not significantly affected by different PGR spray concentrations in the 2014 season (Table 5-29). The interactive effects of PGRs and PGR spray concentrations significantly affected fruit retention at 112 DAFB in the 2014 season, with the highest fruit retention recorded in trees treated with high-spray concentration 2,4-D. This high-level fruit retention was significantly higher than that in control trees and trees treated with high-spray concentration GA_3, but statistically comparable to that resulting from the remaining PGR treatments at either spray concentration (Table 5-30).

Effect of PGR treatments on fruit retention at 126 DAFB in cv. Keitt

In the 2014 season at 126 DAFB, average fruit retention was significantly influenced by various individual PGR treatments, with trees treated with 40 ppm 2,4-D exhibiting the highest fruit retention (26.86% fruit per panicle), which was 1.92 fold higher than that in control trees and statistically comparable to that in trees treated with 50 ppm NAA, 25 ppm 2,4-D, and 5 ppm GA_3. Control trees exhibited the lowest fruit retention at 126 DAFB (13.98% fruit per panicle), which was not significantly different from fruit retention in 25 ppm NAA– and 10 ppm GA_3–treated trees (Table 5-26).

Combined data for all PGRs showed that fruit retention differed significantly between control and PGR treated trees at 126 DAFB in the 2014 season, with higher fruit retention observed in PGR-treated trees compared to that in control trees (Table 5-27). However, combined data for individual PGRs (Table 5-28), data for PGR spray concentrations (Table 5-29), and data for the interactive effects of PGRs and PGR spray concentrations (Table 5-30) revealed no significant change in fruit retention at 126 DAFB for any treatment in the 2014 season.

Table 5-26: The effect of six foliar-applied PGR treatments on fruit set and retention rates per panicle in mango cv. Keitt in the 2014 season.

Treatment	Fruit set	Fruit retention						
	28	**42**	**56**	**70**	**84**	**98**	**112**	**126**
Control	7.48	56.58	24.25^{c}	18.40^{c}	17.21^{d}	16.00^{d}	15.49^{d}	13.98^{c}
25 ppm NAA	6.63	54.57	29.15^{bc}	22.72^{bc}	20.44^{cd}	18.89^{cd}	18.76^{cd}	16.91^{bc}
50 ppm NAA	6.06	62.74	29.26^{bc}	26.56^{ab}	24.21^{bc}	23.18^{abc}	23.06^{abc}	22.57^{ab}
25 ppm 2,4-D	6.21	59.91	33.85^{ab}	26.87^{ab}	24.70^{bc}	23.70^{abc}	23.70^{abc}	22.20^{ab}
40 ppm 2,4-D	6.09	58.31	36.14^{a}	32.01^{a}	30.45^{a}	28.49^{a}	28.49^{a}	26.86^{a}
5 ppm GA_3	6.08	62.31	33.26^{ab}	27.95^{ab}	26.17^{ab}	24.99^{ab}	24.99^{ab}	22.35^{ab}
10 ppm GA_3	6.92	55.13	27.54^{bc}	21.93^{bc}	21.05^{bcd}	19.92^{bcd}	19.92^{bcd}	19.01^{bc}
Level of significance	NS	NS	$p\leq0.05$	$p\leq0.05$	$p\leq0.001$	$p\leq0.001$	$p\leq0.001$	$p\leq0.001$
l.s.d	-	-	6.38	6.94	5.63	5.47	5.56	6.02

Numbers in bold indicate days after full bloom. NS=non-significant at $p\leq0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d=least significant difference. n=5.

Table 5-27: The combined effect of three PGRs on fruit set and retention rates per panicle in mango cv. Keitt in the 2014 season.

Treatment	Fruit set	Fruit retention						
	28	**42**	**56**	**70**	**84**	**98**	**112**	**126**
Control	7.48^{a}	56.58	24.25^{b}	18.41^{b}	17.21^{b}	16.00^{b}	15.49^{b}	13.98^{b}
Treated	6.34^{b}	58.83	31.53^{a}	26.34^{a}	24.50^{a}	23.20^{a}	23.15^{a}	22.24^{a}
Level of significance	$p\leq0.05$	NS	$p\leq0.001$	$p\leq0.001$	$p\leq0.001$	$p\leq0.001$	$p\leq0.001$	$p\leq0.001$
l.s.d.	1.12	-	4.87	5.30	4.30	4.17	4.24	2.25

Numbers in bold indicate days after full bloom. NS=non-significant at $p\leq0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d=least significant difference. n=5.

Table 5-28: The effect of three PGRs on fruit set and retention rates in panicles of mango cv. Keitt in the 2014 season. Data are the average of two PGR spray concentrations.

Treatment	Fruit set	Fruit retention						
	28	**42**	**56**	**70**	**84**	**98**	**112**	**126**
Control	7.48	56.58	24.25^{c}	18.41	17.21^{c}	16.00^{c}	15.49^{c}	13.98
NAA	6.35	58.65	29.20^{bc}	24.64	22.32^{b}	21.04^{b}	20.91^{b}	21.52
2,4-D	6.16	59.11	34.99^{a}	29.44	27.58^{a}	26.09^{a}	26.09^{a}	24.53
GA_3	6.51	58.72	30.40^{b}	24.94	23.61^{ab}	22.46^{ab}	22.46^{ab}	20.68
Level of significance	NS	NS	$p\leq0.05$	NS	$p\leq0.05$	$p\leq0.05$	$p\leq0.05$	NS
l.s.d.	-	-	4.51	-	3.98	3.86	3.93	-

Numbers in bold indicate days after full bloom. NS=non-significant at $p\leq0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d=least significant difference. n=5.

Table 5-29: The effect of PGR spray concentration on fruit set and retention rates per panicle in mango cv. Keitt in the 2014 season.

Spray concentrations	Fruit set	Fruit retention						
	28	**42**	**56**	**70**	**84**	**98**	**112**	**126**
Control	7.48	56.58	24.25	18.41	17.21	16.00	15.49	13.98
Low	6.31	58.93	32.09	25.85	23.77	22.53	22.48	21.67
High	6.36	58.73	30.98	26.83	25.23	23.86	23.82	22.81
Level of significance	NS	NS	NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p\leq0.05$. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-30: The interactive effects of foliar-applied PGR treatments and PGR spray concentrations on fruit set and retention rates per panicle in mango cv. Keitt in the 2014 season.

Treatment	Spray concentration	Fruit set	Fruit retention						
		28	**42**	**56**	**70**	**84**	**98**	**112**	**126**
Control		7.48	56.58	24.25	18.41	17.21^{d}	16.00^{d}	15.49^{d}	13.98
NAA	Low	6.63	54.57	29.15	22.72	20.44^{cd}	18.89^{cd}	18.76^{cd}	20.47
	High	6.06	62.74	29.25	26.56	24.21^{bc}	23.18^{abc}	23.06^{abc}	22.57
2,4-D	Low	6.22	59.91	33.84	26.87	24.70^{bc}	23.70^{abc}	23.70^{abc}	22.20
	High	6.10	58.31	36.14	32.01	30.45^{a}	28.49^{a}	28.49^{a}	26.86
GA_3	Low	6.09	62.31	33.26	27.95	26.17^{ab}	24.99^{ab}	24.99^{ab}	22.35
	High	6.93	55.13	27.55	21.93	21.05^{bcd}	19.92^{bcd}	19.92^{bcd}	19.01
Level of significance		NS	NS	NS	NS	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	NS
l.s.d.		-	-	-	-	5.63	5.47	5.56	-

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-31: The effect of six foliar-applied PGR treatments on fruit set and retention rates per panicle in mango cv. Keitt in the 2015 season.

Treatment	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	12.83	15.50	9.02	7.17	7.17	7.17
25 ppm NAA	8.29	17.00	10.82	10.48	10.48	10.48
50 ppm NAA	10.23	17.38	12.79	8.62	8.62	8.62
25 ppm 2,4-D	9.56	17.10	11.69	10.64	10.64	10.64
40 ppm 2,4-D	9.89	23.60	18.66	14.36	14.36	14.36
5 ppm GA_3	11.34	25.18	22.24	18.07	18.07	18.07
10 ppm GA_3	8.10	22.32	15.71	13.45	13.45	13.45
Level of significance	NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. n=3.

Table 5-32: The combined effect of three PGRs on fruit set and retention rates per panicle in mango cv. Keitt in the 2015 season.

Treatment	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	12.83^a	15.49	9.02	7.17	7.17	7.17
Treated	9.29^b	20.43	15.32	20.43	12.60	12.60
Level of significance	$p \leq 0.05$	NS	NS	NS	NS	NS
l.s.d	3.51	-	-	-	-	-

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. l.s.d.=least significant difference. n=3.

Table 5-33: The effect of three PGRs on fruit set and retention rates per panicle in mango cv. Keitt in the 2015 season. Data are the average of two PGR spray concentrations.

Treatment	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	12.83	15.49	9.02	7.17	7.17	7.17
NAA	9.30	17.19	11.81	9.55	9.55	9.55
2,4-D	9.73	20.35	15.18	12.50	12.50	12.50
GA_3	8.84	23.75	18.97	15.76	15.76	15.76
Level of significance	NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. n=3.

Table 5-34: The effect of PGR spray concentration on fruit set and retention rates per panicle in mango cv. Keitt in the 2015 season.

Spray concentrations	Fruit set	Fruit retention				
	28	**42**	**56**	**70**	**84**	**98**
Control	12.83	15.49	9.02	7.17	7.17	7.17
Low	10.02	19.76	14.91	13.06	13.06	13.06
High	8.55	21.1	15.72	12.14	12.14	12.14
Level of significance	NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. n=3. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-35: The interaction of foliar-applied PGR treatments and PGR spray concentration on fruit set and retention rates per panicle in mango cv. Keitt in the 2015 season.

Treatment	Spray concentration	Fruit set	Fruit retention				
		28	**42**	**56**	**70**	**84**	**98**
Control		12.83	15.49	9.02	7.17	7.17	7.17
NAA	Low	9.16	17.00	10.82	10.48	10.48	10.48
	High	9.43	17.38	12.80	8.62	8.62	8.62
2,4-D	Low	9.57	17.10	11.68	10.64	10.64	10.64
	High	9.89	23.60	18.67	14.36	14.36	14.36
GA_3	Low	11.34	25.18	22.24	18.07	18.07	18.07
	High	6.33	22.31	15.70	13.45	13.45	13.45
Level of significance		NS	NS	NS	NS	NS	NS

Numbers in bold indicate days after full bloom. NS=non-significant at $p \leq 0.05$. n=3. Spray concentrations for each treatment are presented in Table 5-2.

Effect of PGR treatment on fruit quality in mango cv. Keitt

Effect of PGR treatments on fruit weight in cv. Keitt

Fruit weight in cv. Keitt was significantly influenced by various individual PGR treatments in the 2014 season (Table 5-36). Except for 25 ppm NAA and 5 ppm GA_3, fruit weight following all PGR treatments was comparable to that of control fruit. The highest fruit weight was recorded in control trees (729.85 g), which was statistically comparable to fruit weights resulting from 50 ppm NAA, 25 ppm 2,4-D, 40 ppm 2,4-D and 10 ppm GA_3 treatment. Lowest fruit weight (648.36 g) was observed in trees treated with 5 ppm GA_3, which was statistically similar to fruit weight following 25 ppm NAA and 50 ppm NAA treatment (Table 5-36). However, in the 2015 season, fruit weight was not significantly affected by any PGR treatment (Table 5-42).

Combined data for all PGRs showed that fruit weight did not significantly differ between control and PGR-treated trees in both seasons (Table 5-37 and 5-43). Combined data for individual PGRs showed that PGR foliar application significantly affected fruit weight in the 2014 season, with the highest fruit weight recorded in trees treated with NAA, which was significantly higher than that in 2,4-D-treated trees and statistically comparable to that in GA_3-treated trees (Table 5-38). However, combined data for individual PGRs showed that PGR treatment did not significantly affect fruit weight in the 2015 season (Table 5-44). Fruit weight was not significantly affected by both PGR spray concentrations (Table 5-40 and 5-45) and the interactive effects of PGRs and PGR spray concentrations (Table 5-40 and 5-46) in both seasons.

Effect of PGR treatments on fruit length in cv. Keitt

Fruit length was not significantly affected by various individual PGR treatments in both seasons (Table 5-36 and 5-42). Combined data for all PGRs revealed no significant difference for fruit length between control and PGR-treated trees in both seasons (Table 5-37 and 5-43). Furthermore, combined data for individual PGRs showed that PGR treatment (Table 5-38 and 5-44), in addition to PGR spray concentrations (Table 5-39 and 5-45) and the interactive effects of PGRs and PGR spray concentrations (Table 5-40 and 5-46) did not significantly affect fruit length in both seasons.

Effect of PGR treatments on fruit width in cv. Keitt

Statistical analysis revealed no significant effect of PGR treatments on fruit width in the 2014 and 2015 seasons (Table 5-36 and 5-42). Furthermore, combined data for all PGRs (Table 5-37 and 5-43) and combined data for individual PGRs (Table 5-38 and 5-44) showed no significant impact of PGR treatment on fruit width in both seasons. Similarly, variation in PGR spray concentrations (Table 5-39 and 5-45) and the interactive effects of PGRs and PGR spray concentrations (Table 5-40 and 5-46) did not significantly alter fruit width in either season.

Effect of PGR treatments on fruit depth in cv. Keitt

Data in Table 5-36 and 5-42 indicates that various individual foliar PGR applications had no significant impact on fruit depth in both seasons. Combined data for all PGRs (Table 5-37 and 5-43) and combined data for individual PGRs (Table 5-38 and 5-44) showed that control and

PGR-treated trees produced fruit with statistically similar depth in both seasons. In addition, different PGR spray concentrations (Table 5-39 and 5-45) and the interactive effects of PGRs and PGR spray concentrations (Table 5-40 and 5-46) did not significantly change fruit depth in both seasons.

Effect of PGR treatments on fruit TSS in cv. Keitt

Foliar application of various individual PGR treatments had no significant effect on fruit TSS in both seasons (Table 5-36 and 5-42). Combined data for all PGRs showed that control and PGR-treated trees exhibited statistically comparable fruit TSS in both seasons (Table 5-37 and 5-43). Combined data for individual PGRs showed that PGR treatment significantly changed fruit TSS in the 2014 season, with the highest fruit TSS recorded in trees treated with 2,4-D, which was significantly higher than fruit TSS following NAA treatment and statistically comparable to fruit TSS in control trees and GA_3-treated trees (Table 5-38). Comparable data for the 2015 season revealed no significant impact of PGR treatment on fruit TSS (Table 5-44). Both PGR spray concentrations (Table 5-39 and 5-45) and the interactive effects of PGRs and PGR spray concentrations (Table 5-40 and 5-46) had no significant impact on fruit TSS in both seasons.

Effect of PGR treatments on fruit dry matter contents in cv. Keitt

Fruit dry matter contents (DM) did not vary significantly in response to various individual PGR treatments in both seasons (Table 5-36 and 3-42). Combined data for all PGRs revealed no significant difference in DM between PGF-treated and control trees in both seasons (Table 5-37 and 5-43). Statistical analysis revealed no significant difference in DM in both seasons using combined data for individual PGRs (Table 5-38 and 5-44), data for PGR spray concentrations (Table 5-39 and 5-45), and data for the interactive effects of PGRs and PGR spray concentrations (Table 5-40 and 5-46).

Effect of PGR treatments on stone weight in cv. Keitt

Fruit stone weight was recorded in the 2015 season, which revealed that it was not affected by various individual PGR treatments (Table 5-42). Statistical analysis revealed non-significant difference in stone weight between treated and control trees using combined data for all PGRs (Table 5-43), data for different PGR spray concentrations (Table 5-44), and data for the

interactive effects of PGRs and PGR spray concentrations (Table 5-45). However, combined data for individual PGRs showed significantly lower stone weight resulting from 2,4-D treatment (Table 5-44). The highest stone weight was recorded in control trees, which was statistically comparable to that in NAA- and GA_3-treated trees (Table 5-44).

Effect of PGR treatments on fruit stone weight percentage in cv. Keitt

Fruit stone weight percentage was analysed in the 2015 season. Statistical analysis showed no significant impact of various individual PGR treatments on stone weight percentage (Table 5-42). Combined data for all PGRs (Table 5-43) and combined data for individual PGRs (Table 5-44) showed statistically similar fruit stone weight percentages for control and PGR-treated trees. Also, different PGR spray concentrations (Table 5-45) and the interactive effects of PGRs and PGR spray concentrations (Table 5-46) did not significantly affect fruit stone weight percentage.

Table 5-36: The effect of PGR treatments at two spray concentrations on mango cv. Keitt fruit quality in the 2014 season.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	729.85[a]	135.40	106.88	90.25	13.43	15.66
25 ppm NAA	664.83[bc]	131.09	103.84	86.67	13.17	15.24
50 ppm NAA	672.51[abc]	131.04	104.71	86.56	13.07	15.56
25 ppm 2,4-D	710.29[ab]	133.88	106.14	88.32	13.88	16.16
40 ppm 2,4-D	728.09[a]	134.04	107.02	89.98	13.89	15.84
5 ppm GA_3	648.36[c]	131.14	104.13	87.24	13.85	15.98
10 ppm GA_3	708.01[ab]	135.30	107.13	88.13	13.60	15.86
Level of significance	$p \leq 0.05$	NS	NS	NS	NS	NS
C.V.	6.59	2.77	2.81	2.96	4.31	3.53

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-37: The combined effect of PGR treatments on mango cv. Keitt fruit quality in the 2014 season.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	729.8	135.40	106.88	90.25	13.425	15.664
Treated	688.7	132.75	105.49	87.82	13.573	15.772
Level of significance	NS	NS	NS	NS	NS	NS

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-38: The effect of PGR treatments on mango cv. Keitt fruit quality in the 2014 season. Data are the average of two PGR spray concentrations.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	729.8[a]	135.40	106.88	90.25	13.42[ab]	15.66[ab]
NAA	668.7[c]	131.06	104.27	86.62	13.12[b]	15.40[b]
2,4-D	719.2[ab]	133.96	106.58	89.15	13.88[a]	16.00[a]
GA_3	678.2[bc]	133.22	105.63	87.68	13.72[a]	15.92[a]
Level of significance	$p \leq 0.05$	NS	NS	NS	$p \leq 0.05$	$p \leq 0.05$
l.s.d.	41.94	-	-	-	0.54	0.51

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. TSS=total soluble solids. DM=dry matter contents. l.s.d.=least significant difference. n=5.

Table 5-39: The effect of PGR spray concentrations on mango cv. Keitt fruit quality in the 2014 season.

Spray concentrations	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	729.8	135.40	106.88	90.25	13.425	15.664
Low	674.5	132.03	104.70	87.41	13.63	15.794
High	702.9	133.46	106.29	88.22	13.52	15.75
Level of significance	NS	NS	NS	NS	NS	NS

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-40: The interactive effects of PGRs and PGR spray concentrations on mango cv. Keitt fruit quality in the 2014 season.

Treatment	Spray concentrations	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control		729.8	135.4	106.88	90.25	13.425	15.664
NAA	Low	664.8	131.09	103.84	86.67	13.17	15.24
	High	672.5	131.04	104.71	86.56	13.07	15.56
2,4-D	Low	710.3	133.87	106.14	88.32	13.88	16.16
	High	728.1	134.04	107.02	89.97	13.89	15.84
GA_3	Low	648.4	131.13	104.13	87.24	13.85	15.98
	High	708.0	135.3	107.13	88.13	13.595	15.85
Level of significance		NS	NS	NS	NS	NS	NS

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. n=5. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-41: Variation in mango cv. Keitt fruit quality within and between tree levels in the 2014 season.

Variation level	Estimator	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Within Trees	Mean SE	31.83	2.58	1.94	1.66	0.39	0.43
	Mean SD	63.66	5.15	3.87	3.32	0.79	0.86
Between Trees	Mean	694.6	133.10	105.70	88.16	13.55	15.76
	SD	51.51	3.84	3.01	2.75	0.62	0.58
	SEM	8.71	0.65	0.51	0.47	0.11	0.10

SE=standard error. SD=standard deviation. SEM=standard error of means. TSS=total soluble solids. DM=dry matter contents. n=5.

Table 5-42: The effect of PGR treatments at two spray concentrations on mango cv. Keitt fruit quality in the 2015 season.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)	Stone Weight (g)	SWP (%)
Control	583.34	135.39	100.85	81.21	8.65	16.32	51.40	8.91
25 ppm NAA	481.07	117.50	95.37	80.44	8.98	16.34	43.66	8.77
50 ppm NAA	543.46	130.03	99.67	78.64	8.16	15.49	51.98	9.68
25 ppm 2,4-D	421.10	114.21	89.85	75.18	8.34	15.60	37.50	7.95
40 ppm 2,4-D	490.13	115.61	98.12	80.94	9.30	16.76	37.46	7.83
5 ppm GA_3	478.94	122.06	97.55	77.31	9.02	16.95	49.59	10.32
10 ppm GA_3	490.88	125.27	95.99	78.72	8.75	16.46	45.05	9.20
Level of significance	NS	NS	NS	NS	NS	NS	NS	NS
C.V.	15.30	7.82	5.27	4.84	11.60	5.54	14.48	11.27

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. SWP=stone weight percentage. n=3.

Table 5-43: The combined effect of PGR treatments on mango cv. Keitt fruit quality in the 2015 season.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)	Stone Weight (g)	SWP (%)
Control	583.1	135.4^{a}	100.9	81.21	8.64	16.32	51.4	8.90
Treated	484.3	120.8^{b}	96.1	78.54	8.76	16.27	44.2	9.16
Level of significance	NS	$p \leq 0.05$	NS	NS	NS	NS	NS	NS
l.s.d.	-	12.85	-	-	-	-	-	-

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. TSS=total soluble solids. DM=dry matter contents. SWP=stone weight percentage. n=3.

Table 5-44: The effect of PGR treatments on mango cv. Keitt fruit quality in the 2015 season. Data are the average of two PGR spray concentrations.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)	Stone Weight (g)	SWP (%)
Control	583.1	135.4	100.9	81.21	8.64	16.32	51.40[a]	8.90
NAA	512.3	123.8	97.5	79.54	8.57	15.92	47.82[a]	9.40
2,4-D	456.1	114.9	94.0	78.06	8.82	16.18	37.50[b]	8.32
GA_3	485.4	123.7	96.8	78.01	8.88	16.71	47.33[a]	9.76
Level of significance	NS	NS	NS	NS	NS	NS	$p \leq 0.05$	NS
l.s.d.	-	-	-	-	-	-	8.11	-

NS=non-significant at $p \leq 0.05$. Different letters following treatment means indicate a statistically significant difference. TSS=total soluble solids. DM=dry matter contents. SWP=stone weight percentage. n=3.

Table 5-45: The effect of PGR spray concentration on mango cv. Keitt fruit quality in the 2015 season.

Spray concentrations	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)	Stone Weight (g)	SWP (%)
Control	583.1	135.4	100.9	81.21	8.64	16.32	51.40	8.90
Low	460.1	117.9	94.3	77.64	8.78	16.30	43.60	9.42
High	508.3	123.6	97.9	79.43	8.73	16.24	44.80	8.90
Level of significance	NS	NS	NS	NS	NS	NS	NS	NS

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. SWP=stone weight percentage. n=3. Spray concentrations for each treatment are presented in Table 5-2.

Table 5-46: The interactive effects of PGRs and PGR spray concentration on mango cv. Keitt fruit quality in the 2015 season.

Treatment	Spray concentrations	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)	Stone Weight (g)	SWP (%)
Control		583.1	135.4	100.9	81.21	8.64	16.32	51.4	8.90
NAA	Low	481.4	117.5	95.40	80.44	8.97	16.34	43.7	9.12
	High	543.6	130.0	99.70	78.64	8.16	15.49	52.0	9.68
2,4-D	Low	421.3	114.2	89.80	75.17	8.34	15.60	37.5	8.81
	High	490.2	115.6	98.10	80.94	9.29	16.76	37.5	7.83
GA_3	Low	479.3	122.1	97.50	77.31	9.02	16.95	49.6	10.32
	High	491.5	125.3	96.00	78.72	8.75	16.46	45.1	9.20
Level of significance		NS	NS	NS	NS	NS	NS	NS	NS

NS=non-significant at $p \leq 0.05$. TSS=total soluble solids. DM=dry matter contents. SWP=stone weight percentage. n=3. Spray concentrations for each treatment are presented in Table 5-2

Table 5-47: Variation in mango cv. Keitt fruit quality within and between tree levels in the 2015 season.

Variation level	Estimator	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)	Stone Weight (g)	SWP (%)
Within Trees	SE Mean	38.95	4.40	2.66	2.21	0.44	0.38	4.61	0.58
	SD Mean	77.90	8.79	5.31	4.41	0.87	0.77	9.22	1.16
Between Trees	Mean	498.40	122.90	96.77	78.92	8.74	16.28	45.23	9.12
	SD	80.46	10.97	5.47	3.80	0.93	0.92	7.99	1.11
	SEM	17.56	2.39	1.19	0.83	0.20	0.20	1.74	0.24

SE=standard error. SD=standard deviation. SEM=standard error of means. TSS=total soluble solids. DM=dry matter contents. SWP=stone weight percentage. n=3.

5.5 Discussion

5.5.1 Fruit set

Fruit set refers to the transformation of an ovary into developing fruit following successful pollination and fertilisation. In mango, higher fruit set contributes towards successful conventional breeding and commercial fruit production by increasing fruit number at harvest. Fruit set is regulated by endogenous phytohormones (De Jong *et al.*, 2015), including auxins, gibberellins, and cytokinins in fruit tree crops (Kumar *et al.*, 2014). Foliar application of synthetic PGRs has been used to improve fruit set in several fruit tree crops (Anthony and Coggins, 1999; Anthony and Coggins, 2001; El-Otmani *et al.*, 2000; Singh and Lal, 1980). The current study found that the response to PGR applications varied between cultivars and crop seasons. The variation observed between cultivars might be the result of different plant carbohydrate reserves during each experimental season, which may occur because of alternate bearing and insufficient plant nutrients resulting from poor soil nutrition management. Genotypic variation and variation in bearing habits may also contribute towards the variable response of experimental varieties, such as the difference in bearing habits of mango cv. Keitt (bunch-bearing; multiple fruit per panicle) and mango cv. NMBP-1243 (terminal bearing; single fruit per panicle). In alternate bearing varieties, during the "on-crop" year, a higher concentration of plant carbohydrates is available for fruit growth and development compared to that during the "off-crop" year (Lakso *et al.*, 2006).

A significant difference in fruit set was observed following PGR treatment in cv. NMBP-1243 trees in the 2014 season, with the lowest fruit set measured in trees treated with high-spray concentration 2,4-D (40 ppm). This result is in agreement with a previous study that reported lower fruit set in Dusehri mango trees sprayed with 2,4-D compared to fruit set in trees sprayed with NAA and GA_3 (Ahmed *et al.*, 2012). However, in the current study, fruit set in 2,4-D-treated trees was lower than that in control trees. A previous study by Aliyu *et al.* (2011) also reports that following PGR spray of cashew trees, the lowest fruit set was observed in trees treated with 2,4-D. The results of the current study are contradictory to many previous reports in various fruit crops that report higher fruit set in response to foliar application of auxins including 2,4-D (García-Martínez and García-Papí, 1979; Tuan and Chung-Ruey, 2013b; Tuan and Chung-Ruey, 2013a). Despite the absence of supporting data, the lower fruit set in 2,4-D-treated trees may be the result of poor soil management for those particular experimental trees

that withholds sufficient nutrition required in the plant for accelerated growth and development of the ovary into fruit, which underlies the fruit setting process. This study also showed PGR identity (product/active ingredient) is more important than spray concentration, because spray concentration did not impact fruit set in the experimental mango trees.

Poor fruit set (0–18.6%) after crossing is one of the major constraints in mango breeding (Iyer and Dinesh, 1997; Lavi *et al.*, 1993b). Low hybrid numbers per crossed panicle result from the hand pollination techniques used in mango breeding (Bally *et al.*, 2009a). In this study, fruit set was not significantly improved by foliar-applied PGRs in general. Apart from poor tree management, the naturally low crossing success rate, low pollen viability, and the receptivity of the stigma in mango may contribute towards the poor fruit set response to PGR treatments observed in the experimental trees. However, in a commercial setting with adequate nutrients available to mango trees, fruit set may ultimately be improved by foliar-applied PGRs. To draw a comprehensive conclusion regarding the role of plant/soil nutrient status in mango fruit set response to foliar-applied PGRs, further research is needed that includes PGR treatment on different varieties at multiple locations, encompassing sites with either poor or adequate soil nutrition management. Mango breeding efficiency and commercial yields may then be improved by implementing the outcomes of such extended research.

5.5.2 Fruit retention

Foliar application of synthetic PGRs corrects hormonal imbalances, and their favourable impacts on fruit retention underlie their widespread use to reduce pre-harvest fruit drop in various fruit crops (Anthony and Coggins, 1999; Anthony and Coggins, 2001; Singh and Lal, 1980; Tuan and Chung-Ruey, 2013b). In this study, the patterns of fruit retention in cv. NMBP-1243 and cv. Keitt are comparable to the observed patterns of fruit set following foliar application of PGRs. Fruit retention differed in the experimental varieties in both seasons. In the "on-crop" 2014 season, 2,4-D-sprayed trees displayed higher fruit retention during fruit maturation until harvest in both varieties. These results are consistent with the findings of Chattha *et al.* (1999), who reported higher fruit retention six weeks following fruit set in mango cv. Samar Behisht Chaunsa in response to 40 ppm 2,4-D foliar application. Similar results were also reported by El-Otmani *et al.* (1990), who observed significantly reduced pre-harvest fruit drop in four citrus varieties following foliar application of 16 mg l^{-1} 2,4-D at the fruit colour break stage. These results are also supported by Nawaz *et al.* (2008), who described

significantly higher fruit retention in Kinnow mandarin trees sprayed with 10 ppm 2,4-D during the last week of November compared to that in control trees. The auxins are reported to prevent fruit drop during early fruitlet development in citrus (Guardiola and García-Luis, 2000).

As opposed to in the 2014 season, higher fruit retention was exhibited by cv. NMBP-1243 trees sprayed with 50 ppm NAA, 25 ppm 2,4-D, 5 ppm, and 10 ppm GA_3 during the "off-crop" 2015 season. These results are supported by the findings of Ahmed *et al.* (2012), who showed that trees sprayed with 35 ppm NAA displayed the highest fruit retention. Arteca (1996) also reported that foliar auxin sprays were an effective strategy to increase fruit retention by maintaining the hormonal balance that avoids the development of an abscission zone, thereby mitigating fruit drop in fruit crops. During the 2015 season, cv. Keitt mango trees exhibited a poor response to foliar PGR spraying in terms of fruit retention, which may be attributed to lower competition between the fruitlets from low flowering and subsequent fruit set in this variety. Data also revealed that PGR spray concentration did not impact fruit retention rates, which highlights the importance of suitable PGR selection. This finding agrees with Anthony and Coggins (1999), who emphasised that better PGR selection as opposed to varying PGR spray concentration contributes towards fruit retention in citrus. In this previous work, different PGRs were applied at three different spray concentrations (4, 8, and 16 mg L^{-1}) at the commercially recommended growth stage, which revealed a significant difference in fruit drop in response to PGR variation and no impact on fruit drop by varying spray concentrations.

The average effects of PGR application during this experiment did not significantly improve tree productivity compared to that in control trees, which demonstrates the potential positive as well as negative impacts of PGRs on fruit set and retention in experimental trees. Poor management of the experimental trees in this study may have contributed towards the neutral average performance of PGR-treated trees. This hypothesis is supported by the work of Anthony and Coggins (1999), who concluded that fruit drop is potentially affected by cultural practices.

In fruit breeding programs, nutrient management is not necessarily optimal because high yields in the trees used for crossing is not required for breeding. Poor nutrient management may cause alternate bearing in the trees used in breeding programs. Despite the inefficient and slow nature of the classical breeding system in mango, significantly higher fruit retention may improve breeding efficiency by maximising the number of fruit that yield from crosses. Fruit crops such

as mango have a large number of flowers and a considerably low number of mature fruit at harvest (Lavi *et al.*, 2004). Before performing hand pollination, foliar application of 2,4-D or NAA on the panicles may enhance final fruit retention up to two fold more than fruit retention in panicles that receive no PGR treatment. In a commercial setting, improved soil management translates to adequate nutrient availability for rapidly growing fruitlets in mango trees. Higher tree productivity and farm yield are expected with the use of foliar-applied PGRs in commercial orchards.

5.5.3 Fruit quality

Foliar spraying of different PGRs including auxins and gibberellic acid has increased fruit size and quality in cashew and citrus fruit crops (Aliyu *et al.*, 2011; Guardiola and García-Luis, 2000). In mango cv. NMBP-1243, high-spray concentration 2,4-D application significantly reduced weight as well as the size of the resulting fruit, which is not in agreement with the results of Singh *et al.* (1959), who reported significantly higher fruit size in mango cv. Fajri following 10 ppm 2,4-D and 20 ppm NAA application at six months after bloom. However, as observed here, fruit weight and size in mango cv. Keitt were not significantly changed by 2,4-D treatment. Previous studies on citrus fruit crops report either increased fruit size in trees treated with foliar-applied 2,4-D (Modise *et al.*, 2009) or no change in fruit size following comparable treatments (Agustí *et al.*, 2006). In citrus crops, the dual effect of increasing or decreasing the fruit weight and size has been reported for synthetic auxins application under different conditions. Foliar application of 2,4-D at flowering stage may reduce fruitlet growth rate, delay fruit abscission, and decrease final fruit size and weight (Guardiola and García-Luis, 2000). Poor nutrient management may also play a role in decreased fruit weight and size. Poor nutrient management for trees treated with PGRs may lead to insufficient nutrient supply for growing fruitlets and therefore higher competition between them (Guardiola *et al.*, 1993).

During the 2014 season, fruit weight and size in cv. Keitt mangoes were not significantly affected by 2,4-D treatment as opposed to cv. NMBP-1243 mangoes. The impacts of 2,4-D treatment may be genotype-specific. However, in cv. Keitt, foliar application of NAA resulted in significantly smaller fruit compared to that in control trees, which may be attributed to a reduced growth rate of fruitlets caused by NAA treatment. This reduced fruitlet growth rate may lead to the development of significantly smaller fruit (Guardiola and García-Luis, 2000). Variation in fruit quality within and between tree levels suggests that fruit are relatively

uniform and there is not outstanding variation between or within trees sprayed with PGR (Table 5-19, 5-25, 5-41, and 5-47). The fruit weight and size are of no concern in mango breeding unless the fruit does not produce a healthy stone. However, fruit weight and size is a major concern in commercial mango production. Improved soil management can overcome the problem of small fruit size resulting from foliar-applied PGRs.

5.6 Implication for mango breeding and commercial production

The study suggests that foliar application of NAA (8.26 percent fruit retention per panicle in NMBP-1243) and 2,4-D (13.62 and 26.86 percent fruit retention per panicle in cv. NMBP-1243 and cv. Keitt respectively) at full bloom results in higher fruit retention in mango, and therefore can be employed as an effective remedy for fruit drop. 2,4-D treatment reduced the fruit size of cv. NMBP-1243 mangoes in both the 2014 and 2015 season, whereas comparable treatment did not affect fruit size of cv. Keitt mangoes. A smaller fruit size does not affect breeding program efficiency. However, smaller fruit size may not be suitable for commercial mango production. Better nutrient and soil management in conjunction with foliar PGR application may improve final fruit size and weight.

From the results of this study, it is concluded that foliar application of 2,4-D at full bloom may be used as pre-harvest fruit drop management tool to improve mango breeding efficiency by ensuring the availability of a maximum number of fruit following crossing. However, in commercial orchards, adequate nutritional management should be practiced to preserve suitable fruit size in trees treated with 2,4-D.

Chapter 6: Effect of foliar application of micronutrients on the fruit set and fruit retention of mango cvs. NMBP-1201 and R2E2

6.1 Abstract

Fruit set and retention are important phenological events in fruit production that directly contribute towards yields in commercial mango orchards and breeding efficiency. Fruit set and retention are multidimensional processes that are directly or indirectly governed by nutritional factors. This study aimed to improve fruit set, retention, and quality in the two mango varieties NMBP-1201 and R2E2 by foliar application of micronutrients, namely zinc and boron at two spray concentrations, at the start of bloom. Results showed that these treatments did not improve either fruit set, retention, or quality in either mango variety.

6.2 Introduction

Low fruit set and retention significantly affect mango breeding efficiency and contribute towards low yields in commercial mango orchards (Bally *et al.*, 2009a; Chadha, 1993; Khemira, 1991; Singh *et al.*, 2005). Fruit setting and retention are multidimensional processes that are directly or indirectly governed by nutritional factors (Chaplin and Westwood, 1980; Motesharezade *et al.*, 2001). The fruit set percentage in mango is approximately 0.1% of perfect flower number (Bally *et al.*, 2009a; Guzman-Estrada, 1997; Prakash and Ram, 1984). The majority of early-set fruit (10–50%) is dropped during the first three to four weeks of fruit growth and development. Low fruit set and high fruit drop significantly reduce mango breeding efficiency (Singh *et al.*, 2005) and cause low yields in mango orchards (Malik and Singh, 2003; Prakash and Ram, 1984). Foliar application of nutrients is an efficient process and is 6–20 times more effective in providing nutrients to the plant compared to soil nutrient application (Fageria *et al.*, 2009; Sankar *et al.*, 2013; Swietlik and Faust, 1984). Furthermore, compared to soil nutrient application, foliar application of nutrients require lower application rates, provide uniform fertiliser distribution, and elicit a rapid response in plants (Keshavarz *et al.*, 2011; Umar *et al.*, 1999). Micronutrients, including boron, chlorine, copper, iron, manganese, nickel, and zinc, are essential plant nutrients that are required in concentrations less than 100 µg/g of plant dry weight (Welch, 1995). Among these micronutrients, zinc (Zn) and boron (B) play

important roles in pollination, fruit set, and total yield in higher plants (Motesharezade *et al.*, 2001).

Higher plants require the essential micronutrient B for normal growth (Marschner, 2012). B plays an important role in pollen germination and pollen tube growth, and is consequently involved in successful fruit set (Nyomora *et al.*, 1997). B affects cell wall structure and cell elongation, which are important for pollen tube development, and root growth (Barker and Pilbeam, 2015). B foliar application increases yield, particularly in crops grown in sandy soil where B availability to the plant is low (Nyomora *et al.*, 1997; Yogaratnam and Greenham, 1982). Foliar application of B significantly affects fruit set in many tree crops because this treatment overcomes typical limited B mobility to opening flowers despite adequate B supply for vegetative plant growth (Hanson, 1991a; Nyomora *et al.*, 1997). In plant species where B is transported via the phloem, such as celery and peach, foliar B application enriches bud B concentration during photosynthetic active periods; however, in species where B is not transported via the phloem, such as mango and walnut, foliar B application should be carried out directly on buds in the spring (Brown and Shelp, 1997). Foliar B application is an effective way to enhance bud and flower B concentrations, which results in increased fruit set and yield in *Prunus*, *Malus*, and *Pyrus* species (Batjer and Thompson, 1949; Callan *et al.*, 1978; Chaplin *et al.*, 1977; Hanson, 1991a). B is also required for fruitlet retention because it influences sink strength of the developing embryo through auxin-mediated events (Sarlikioti *et al*., 2011).

Boron application improves fruit set in several fruit tree crops even when adequate leaf B concentrations are observed, suggesting that normal plant B content may not be sufficient for optimum fruit set due to B phloem immobility. Foliar B application increased fruit set in 'Italian' prune (*Prunus domestica* L.) trees, which had 27–38 µg g^{-1} DW foliar B concentrations and no observable B deficiency symptoms (Callan *et al.*, 1978; Chaplin *et al.*, 1977; Hanson and Breen, 1985). Similar effects were observed in 'Barcelona' hazelnut (*Corylus avellana* L.) trees, which displayed no B deficiency symptoms and had 26–86 µg g^{-1} DW foliar B concentrations (Shrestha *et al.*, 1987). In hazelnut, the normal range of foliar B concentration is 11–40 µg g^{-1} DW (Shear and Faust, 1980). B deficiency results in symptoms such as poorly developed stamens, lower fruit set, and low fruit quality (Swietlik and Faust, 1984). In mango, B application has been shown to increase fruit set (Rajput *et al.*, 1976) and fruit retention (Singh and Dhillon, 1987). Foliar application of boric acid on the Langra cultivar improved flushes,

inflorescence, fruit setting percentage, and fruit biochemical characteristics (Rajput *et al.*, 1976). B deficiency in mango leads to low fruit set (de Wet *et al.*, 1989)

Zinc is also an important plant micronutrient because it plays crucial roles as both the metal component of enzymes and as a structural or functional part of different enzyme systems (Bernhard, 1961). Zn is important for the synthesis of phytochemicals and phytohormones, including auxins (Bally, 2009; Marschner, 2012). Zn encourages pollen tube growth via its effect on tryptophan biosynthesis, which is an auxin precursor in higher plants (Chaplin and Westwood, 1980). Comparable with B, Zn deficiency also causes lower fruit set in fruit crops (Swietlik and Faust, 1984). Zn soil application is not particularly effective because Zn does not have high soil mobility. Foliar application is an effective means of providing Zn to plants, although repeated sprays are required to alleviate Zn deficiency because foliar-absorbed Zn is poorly translocated within the plant (Swietlik, 2002). In another study, foliar application of Zn + potassium (K) + salicylic acid [Zn (0.25% $ZnSO_4$), K (0.25% K_2SO_4), in combination with salicylic acid (10μM)] proved to be an effective treatment that decreased fruit drop in Kinnow mandarin (Ashraf *et al.*, 2012). Also, foliar application of Zn was shown to increase the fruit yield and quality of Valencia orange (Rodríguez *et al.*, 2005) and can also improve fruit set in mango (Daulta *et al.*, 1981).

There are few reports describing the effects of foliar application of micronutrients such as B and Zn on fruit set and retention in mango cultivars in the tropical climate of North Queensland. Hence, this chapter explores the effect of foliar B and Zn application on fruit set and retention in the two mango cultivars NMBP-1201 and R2E2. The current study tests the hypothesis that foliar application of B and Zn immediately prior to flowering increases fruit set and subsequent fruit retention in the two mango cultivars.

6.3 Material and methods

6.3.1 Experimental site and trees selection

The experiment was conducted during the 2015 flowering and fruiting season at Walkamin Research Station (17°8′17″S 145°25′41″E; Elevation: 599.23 m), Department of Agriculture and Fisheries, Mareeba. Four-year-old cv. NMBP-1201 and R2E2 mango trees, grafted on ‘Kensington Pride’ rootstock, were used in the experiment. Trees were spaced in a 7 × 6 arrangement and managed according to the practices described in Table 6-1 and Fig. 6-1.

Table 6-1: Horticultural management practices applied to experimental trees

Operation	2014		2015	
	Frequency	**Month**	**Frequency**	**Month**
Irrigation	Per 4 days	June-Dec	Per 4 days	June-Dec
Mowing	Per month	Jan; Feb; March; May	Per month	July; Sep; Nov
Weedicide spray	Per month	Jan; April; May	Per month	Jan

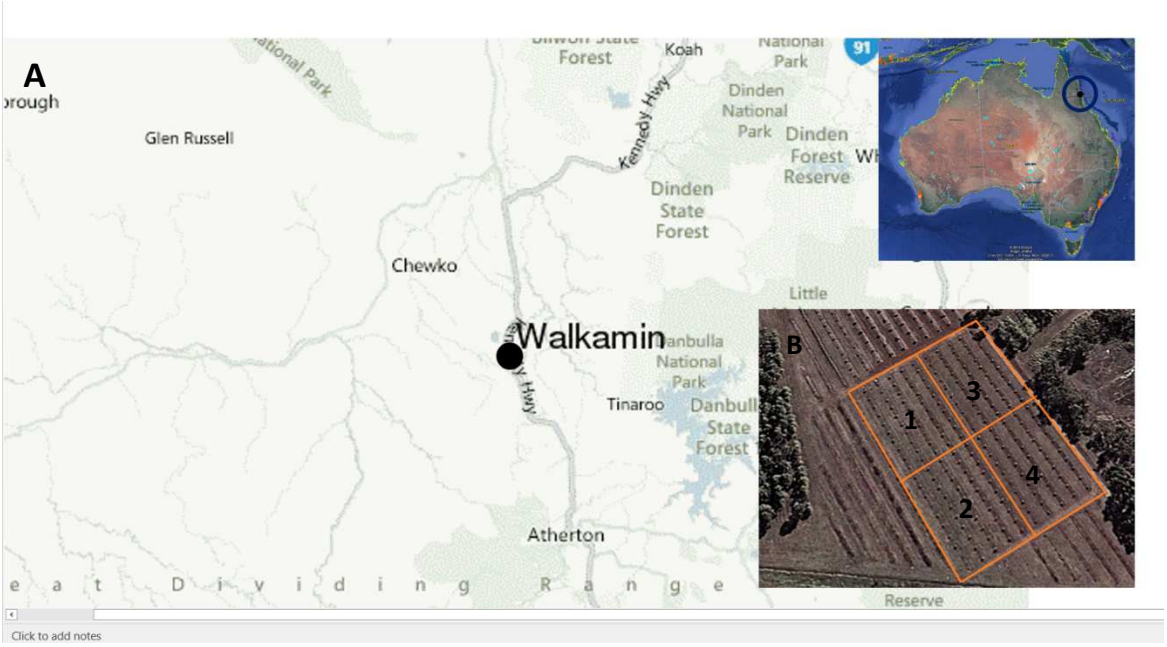

Fig 6-1: (A) Location of experimental site in Queensland, Australia, (B) Aerial view of experimental mango block including (1) NMBP-1243, (2) Keitt, (3) NMBP-1201, and (4) R2E2 cultivars.

6.3.2 Micronutrients treatments

The two micronutrients boron (B; boric acid) and zinc (Zn; zinc sulphate) were selected for analysis in this study, based on data from previous studies describing their contribution towards improved fruit set and retention in different mango cultivars (Daulta *et al.*, 1981; Rajput *et al.*, 1976; Singh and Dhillon, 1987). Each micronutrient treatment was applied at two concentrations, as detailed in Table 6-2.

Table 6-2: Four micronutrient treatment

Treatment	Spray concentration	Details
Control		No spray
Boric acid	0.6%	Boric acid, technical grade, Barmac Chemical Pty. Ltd, Rocklea, Queensland, Australia
	0.8%	
Zinc sulphate	0.25%	Zinc sulphate, technical grade, Barmac Chemical Pty. Ltd, Rocklea, Queensland, Australia
	0.50%	

All micronutrients treatments were applied at the start of bloom (first flower opening; BBCH scale 610; (Delgado *et al.*, 2011; Rajan *et al.*, 2011). Foliar treatments were applied on the 10 September for both varieties, using a knapsack spray (Model CP3, Cooper Pegler & Co. Limited, Burgess Hill, Sussex, England). Micronutrients were diluted in water to each particular treatment concentration (Table 6-2) and sprayed over the whole tree until runoff. Following foliar treatments, a maximum of twenty-five panicles were selected and tagged for the monitoring of fruit set and retention.

6.3.3 Data collection

Fruit set, fruit retention, and fruit quality data were collected as described below.

Assessment of fruit set and retention

Measurement of fruit retention (fruit count) on tagged panicles began at 28 days after full bloom (DAFB) and continued at fortnightly intervals. Fruit count at 28 DAFB was considered as the fruit set per panicle because fruit set occurs approximately 14 DAFB in mango (Notodimedjo, 2000) and early fruit drop occurs during the first three to four weeks of fruit development due to self-incompatibility, failure of pollination or fertilization, or embryo abortion (Davenport and Nunez-Elisea, 1983; Guzman-Estrada, 1997; Notodimedjo, 2000; Singh *et al.*, 2005). Fruit counts continued until harvest, which was at 112 DAFB in both varieties. The fruit set data was expressed as mean fruit number per panicle. The fruit retention data of all tagged panicles of a tree were converted to single fruit retention reading per panicle before statistical analysis, and hence these data are expressed as mean percentage fruit retention per panicle.

Fruit quality measurement

Fruit sampling and post-harvest handling

Four mature, solid green, uniform-sized, healthy, and blemish-free mangoes were harvested with long stems at random from each experimental tree. Fruit were transported in plastic field bins to the horticulture laboratory, Department of Agriculture and Fisheries, Mareeba. Fruit were de-stemmed and de-sapped before they were placed in 400 g 100 L^{-1} Septone Mango Wash® solution (ITW AAMTech, Wetherill Park, NSW, Australia) for 1 minute to neutralise sap residues. Following de-sapping, fruit were removed from the wash solution and rinsed with clean water. Fruit were then dipped in a hot (52°C) 120 mL 100 L^{-1} water Scholar® fungicide solution (Syngenta Australia Pty Ltd, Macquarie Park, NSW, Australia; a.i. 230 g/L fludioxonil) for 5 minutes. Fruit were then air-dried and maintained at 21°C and 90% relative humidity to ripen until they reached the stage suitable for consumption (Hofman *et al.*, 2010).

Fruit weight (g)

Fruit were weighed using a digital balance (Model FZ3000iWP, A&D Company Ltd, Tokyo, Japan) at ripe stage (n=4 fruit).

Fruit size (mm)

The length, width, and depth of fruit were measured (Fig. 6-2) to determine fruit size. Fruit length was measured along the axis from the stalk attachment site to the furthest opposite point. Fruit width was measured at the broadest fruit section perpendicular to fruit length. Fruit measurements were performed using a digital vernier caliper (Model TD2082, Jaycar Electronics, Rydalmere, NSW, Australia) at ripe stage (n=4 fruit).

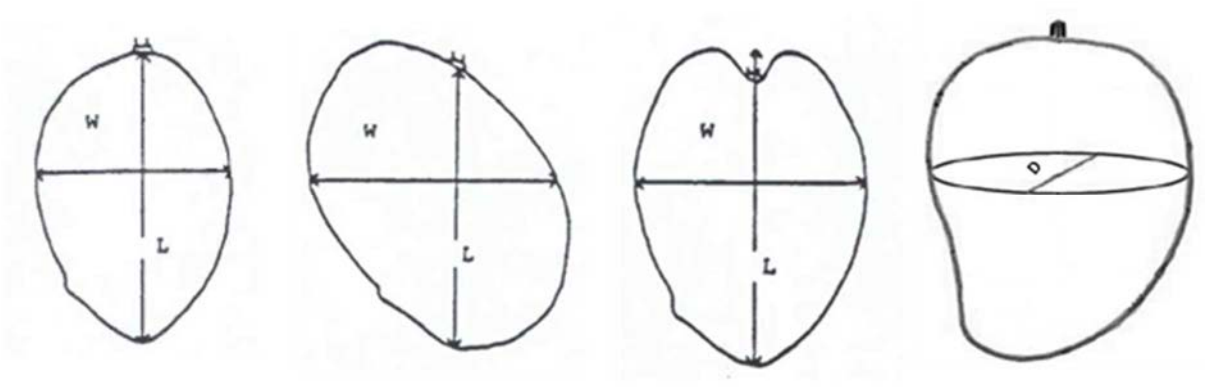

Fig. 6-2: Mango fruit length (L), width (W), and depth (D) measurement, performed as previously described (Dhameliya *et al.*, 2016; UPOV, 2006).

Fruit dry matter contents (%)

Fruit dry matter contents were measured at ripe stage (Hofman *et al.*, 2010). Fruit flesh was weighed before (wet weight) and after (dry weight) drying at 60ºC for 48 h in an oven (Model ODW50, Laboratory Equipment Pty Ltd, Marrickville, NSW, Australia) (n=4 fruit). Fruit dry matter contents were as follows:

$$\text{Fruit dry matter contents (\%)} = \frac{\text{Dry Weight}}{\text{Wet Weight}} \times 100$$

Total Soluble Solids (%)

A digital refractometer (Model DBR-1, Starr Instruments, Dandenong South, Victoria, Australia) was used to estimate fruit total soluble solids (TSS). For this, 2‑3 drops of mesocarp juice were placed on the prism of the refractometer and TSS were recorded as °Brix (n=4 fruit)

6.3.4 Statistical design and analysis

Field layout

The experiment was created following a Completely Randomised Design (RCD) with 5 treatments and 7 replications. Each treatment unit consisted of one tree. A maximum of twenty-five panicles were selected per tree for the evaluation of fruit set and fruit retention.

Statistical Design

The effect of treatments on fruit set and retention were statistically analysed using two methods: (1) residual maximum likelihood (REML) under factorial arrangement with two factors (micronutrient and spray concentration) using Genstat version 18.0 (VSN International Ltd., Hemel Hempstead, UK), and (2) generalised linear model (GLM) using SAS/STAT software version 9.0. (SAS Institute, Cary, NC, USA). Results are presented as treatment means. Analysis of variance (ANOVA) was used to test the significance of micronutrient treatment on the different parameters of fruit quality measured. Least significant difference (LSD) at $p \leq 0.05$ was used to establish significant differences between the treatment means (Singh and Janes, 2000).

6.4 Results

6.4.1 Effect of micronutrients on fruit set and retention in mango cv. NMBP-1201

Effect of micronutrients on fruit set in mango cv. NMBP-1201

Fruit set is the retention and growth of an ovary following pollination, and is an important event in fruit tree phenology (Srivastava, 2002). In mango cv. NMBP-1201, fruit set at 28 days after full bloom (DAFB) was not significantly affected by foliar application at full bloom of various micronutrient treatments (Fig. 6-3 and 6-4). Combined data for all micronutrient treatments revealed no significant difference in fruit set between control and treated trees (Fig. 6-5). Furthermore, no significant impact of micronutrient treatment on fruit set in experimental trees was observed using either combined data for individual micronutrient treatments (Fig. 6-6), combined data for micronutrient spray concentration (Fig. 6-7), or data for the interactive effects of micronutrient treatment and spray concentration (Fig. 6-8).

Effect of micronutrients on fruit retention over time in mango cv. NMBP-1201

Foliar application of various micronutrient treatments had a significant effect on fruit retention at 42 DAFB, with highest fruit retention observed in untreated (control) trees, which was statistically comparable with that in trees sprayed with 0.6% boric acid (Fig. 6-3 and 6-4). Trees sprayed with 0.50% zinc sulphate exhibited lowest fruit retention, which was statistically comparable to that in trees sprayed with 0.8% boric acid and 0.25% zinc sulphate (Fig. 6-3 and 6-4). No further significant differences were found regarding fruit retention in trees sprayed

with different micronutrients treatments in the period after 42 DAFB until harvest (Fig. 6-3 and 6-4).

Combined data for all micronutrient treatments revealed that trees sprayed with micronutrients had significantly lower fruit retention at 42 DAFB compared to that in control trees (Fig. 6-5). However, no further significant impacts were observed regarding the effect of micronutrient application on fruit retention during later fruit growth stages until harvest (Fig. 6-5).

Combined data for individual micronutrient treatments showed that trees sprayed with Zn had the lowest fruit retention at 42 DAFB, which was significantly lower than that in control and B-treated trees (Fig. 6-6). However during later fruit growth stages, the experimental trees sprayed with micronutrients and control trees exhibited statistically comparable fruit retention (Fig. 6-6). Micronutrient spray concentrations had no significant effect on fruit retention throughout fruit development until harvest (Fig. 6-7).

The interactive effects of micronutrient treatment and spray concentration revealed no significant effect on fruit retention at 42 DAFB and 56 DAFB; however, during later stages of fruit growth, this data indicated significant differences in fruit retention, but these were too low to be detected by LSD test (Fig. 6-8). The highest fruit retention at 84 DAFB was observed in trees sprayed with low-spray concentration boric acid (22.77%) and the lowest fruit retention was observed in trees sprayed with low-spray concentration zinc sulphate (17.05%). The highest fruit retention at 98 DAFB was found in trees sprayed with low-spray concentration boric acid (17.17%) and the lowest fruit retention was found in the trees sprayed with high-spray concentration boric acid (12.40%). Trees sprayed with low-spray concentration boric acid exhibited the highest fruit retention at 112 DAFB and trees sprayed with high-spray concentration boric acid exhibited the lowest fruit retention at 112 DAFB (Fig. 6-8).

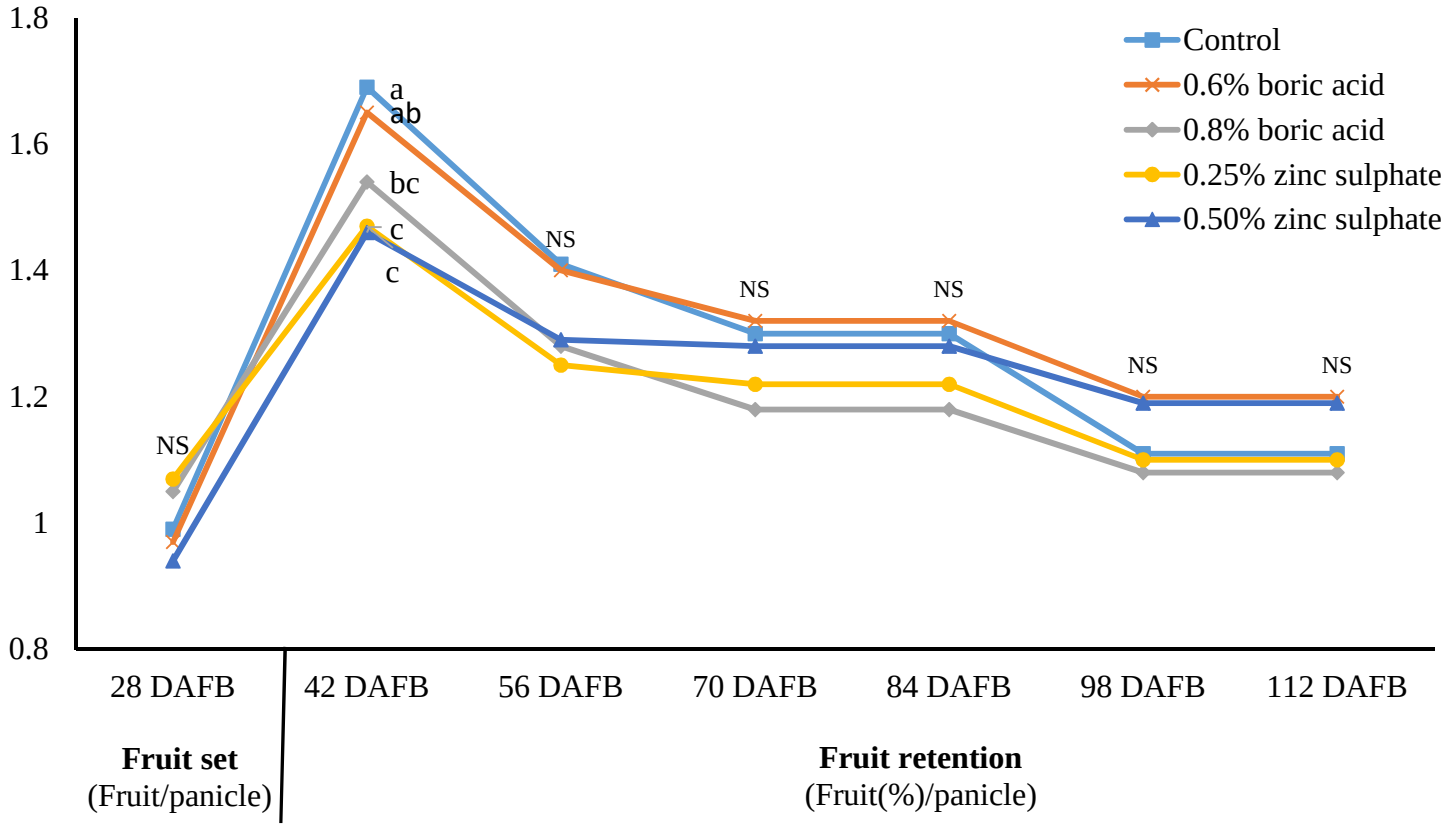

Fig. 6-3: The effect of four foliar-applied micronutrient treatments on fruit set and retention per panicle in mango cv. NMBP-1201. DAFB=days after full bloom. Different letters associated with data points in the same DAFB category indicate a statistically significant difference. NS=Non-significant ($p \leq 0.05$).

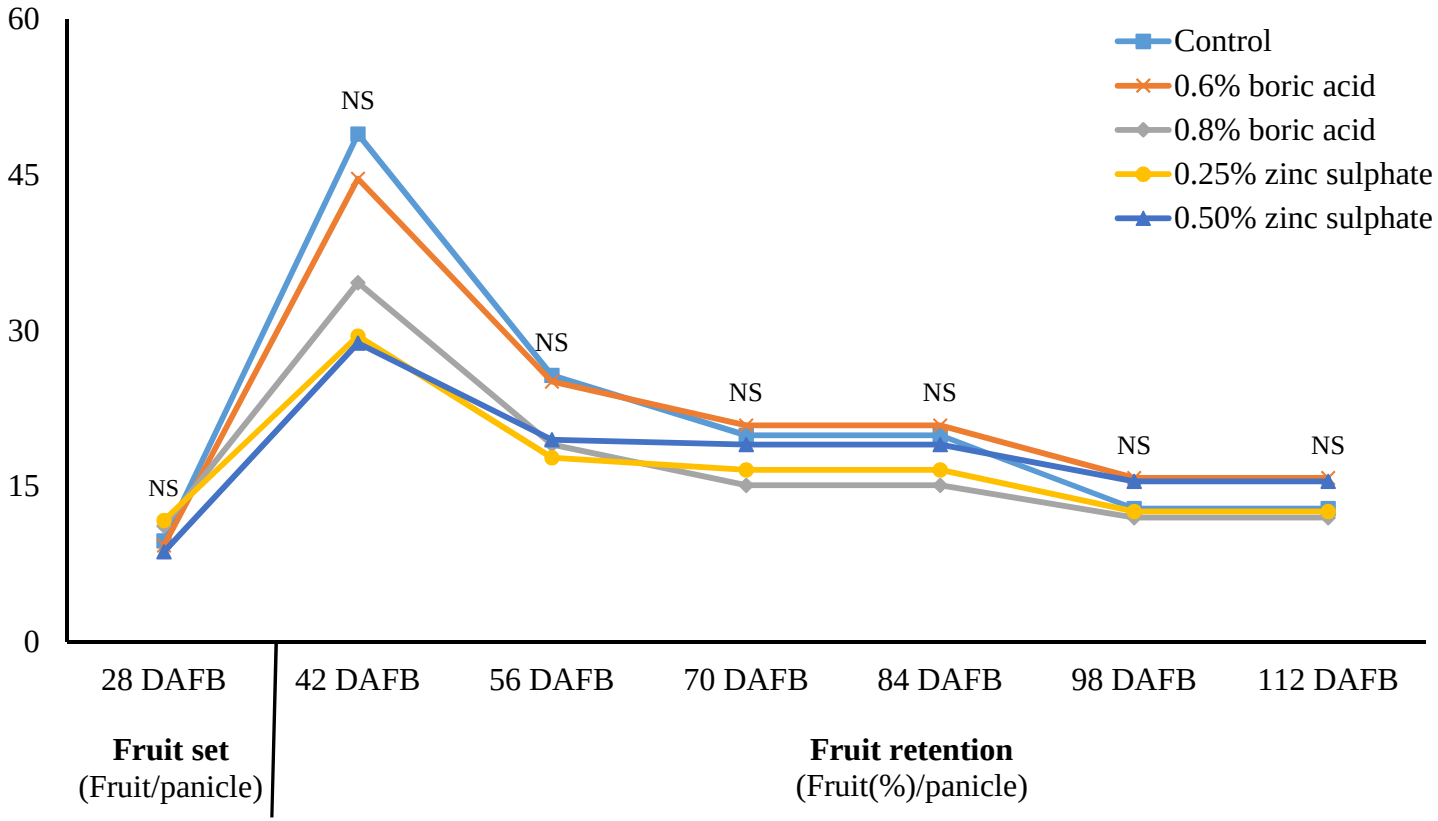

Fig. 6-4: The effects of four foliar-applied micronutrient treatments on fruit set and retention per panicle in mango cv. NMBP-1201 (back transformed treatment means). NS=Non-significant ($p \leq 0.05$).

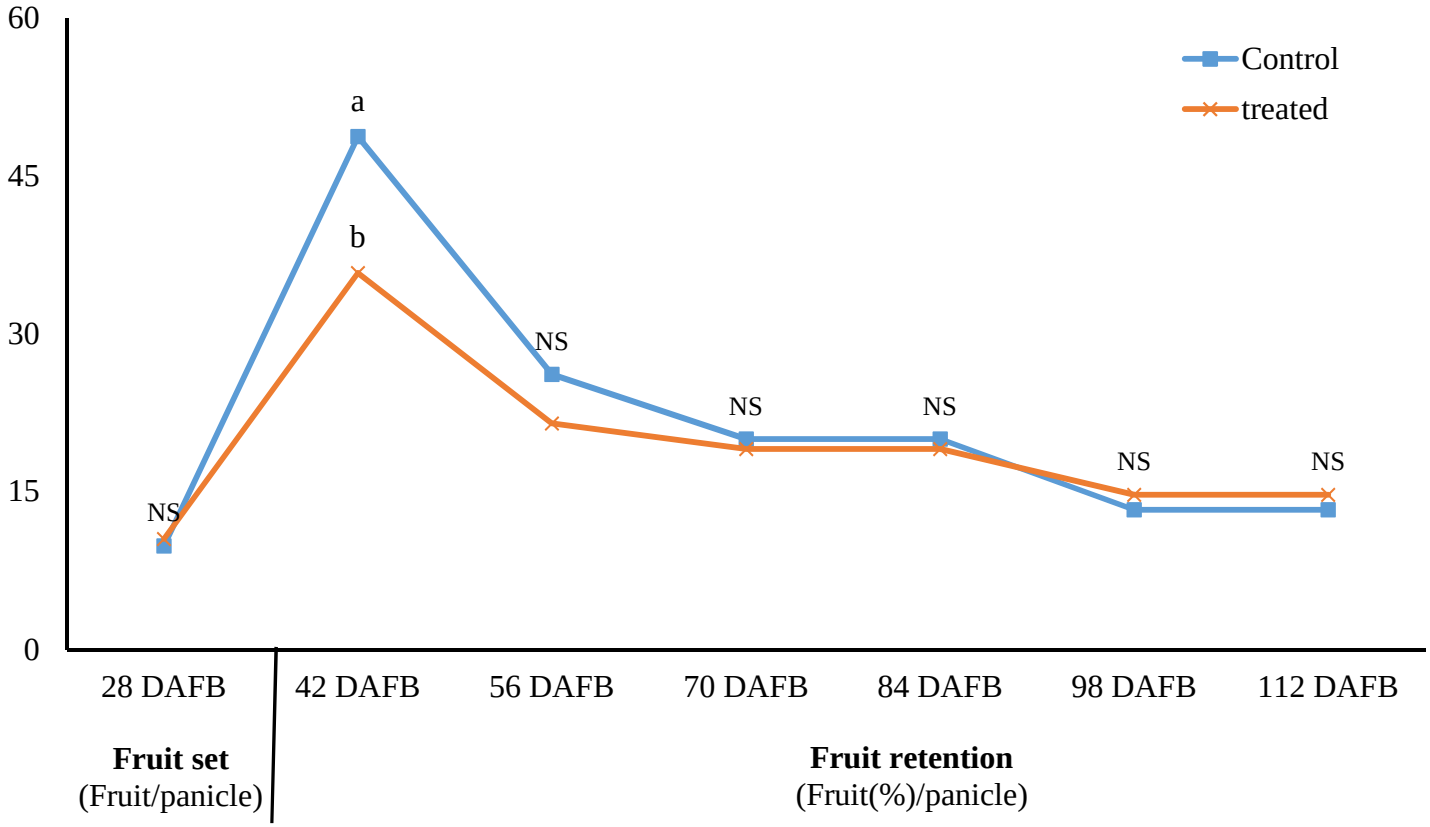

Fig. 6-5: The combined effects of four micronutrient treatments on fruit set and retention per panicle in mango cv. NMBP-1201. DAFB=days after full bloom. Different letters associated with data points in the same DAFB category indicate a statistically significant difference. NS=Non-significant ($p \leq 0.05$).

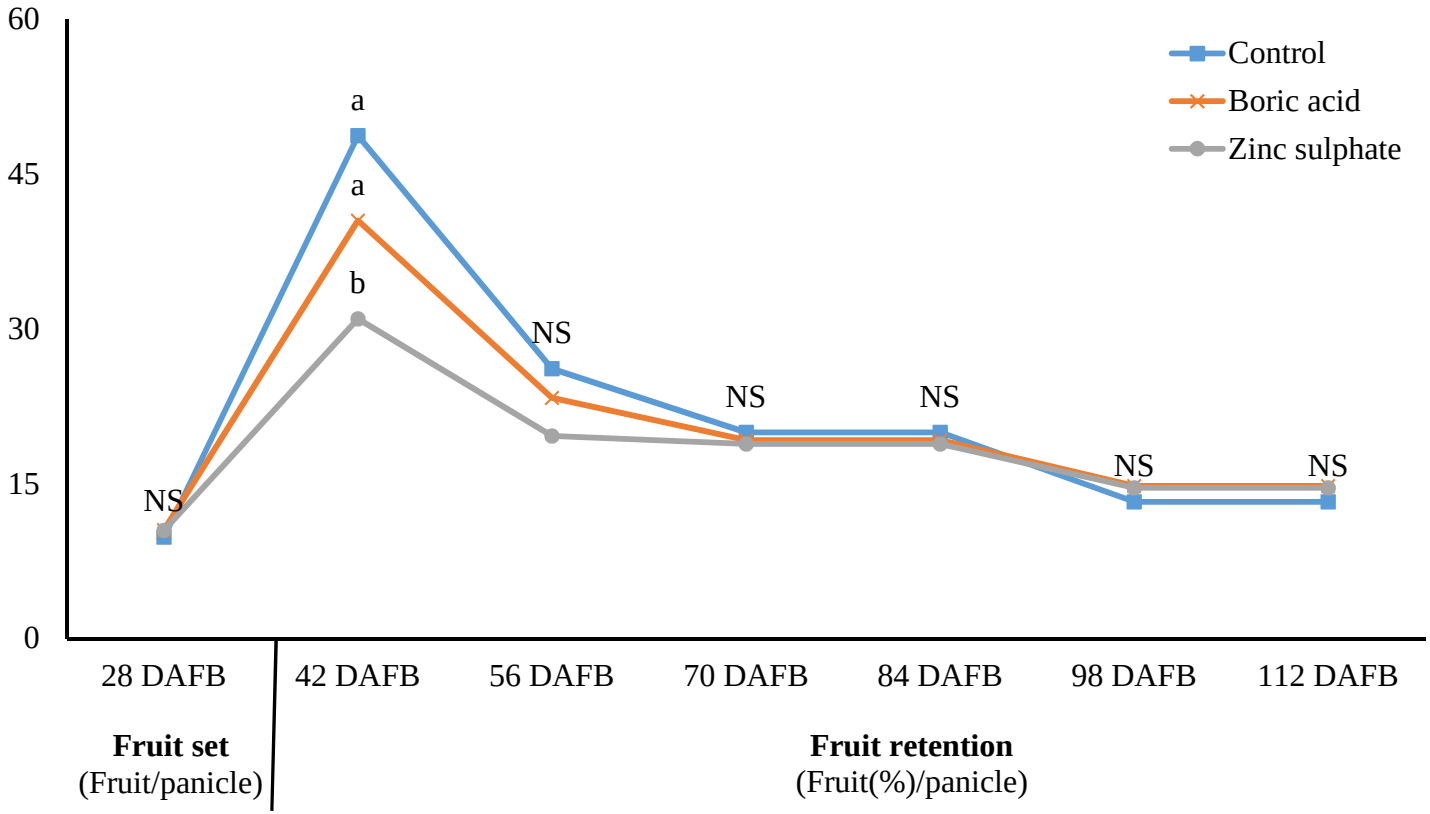

Fig. 6-6: The effects of two micronutrients on fruit set and retention per panicle in mango cv. NMBP-1201. Data are the average of two application rates for each micronutrient. DAFB=days after full bloom. Different letters associated with data points in the same DAFB category indicate a statistically significant difference. NS=Non-significant ($p \leq 0.05$).

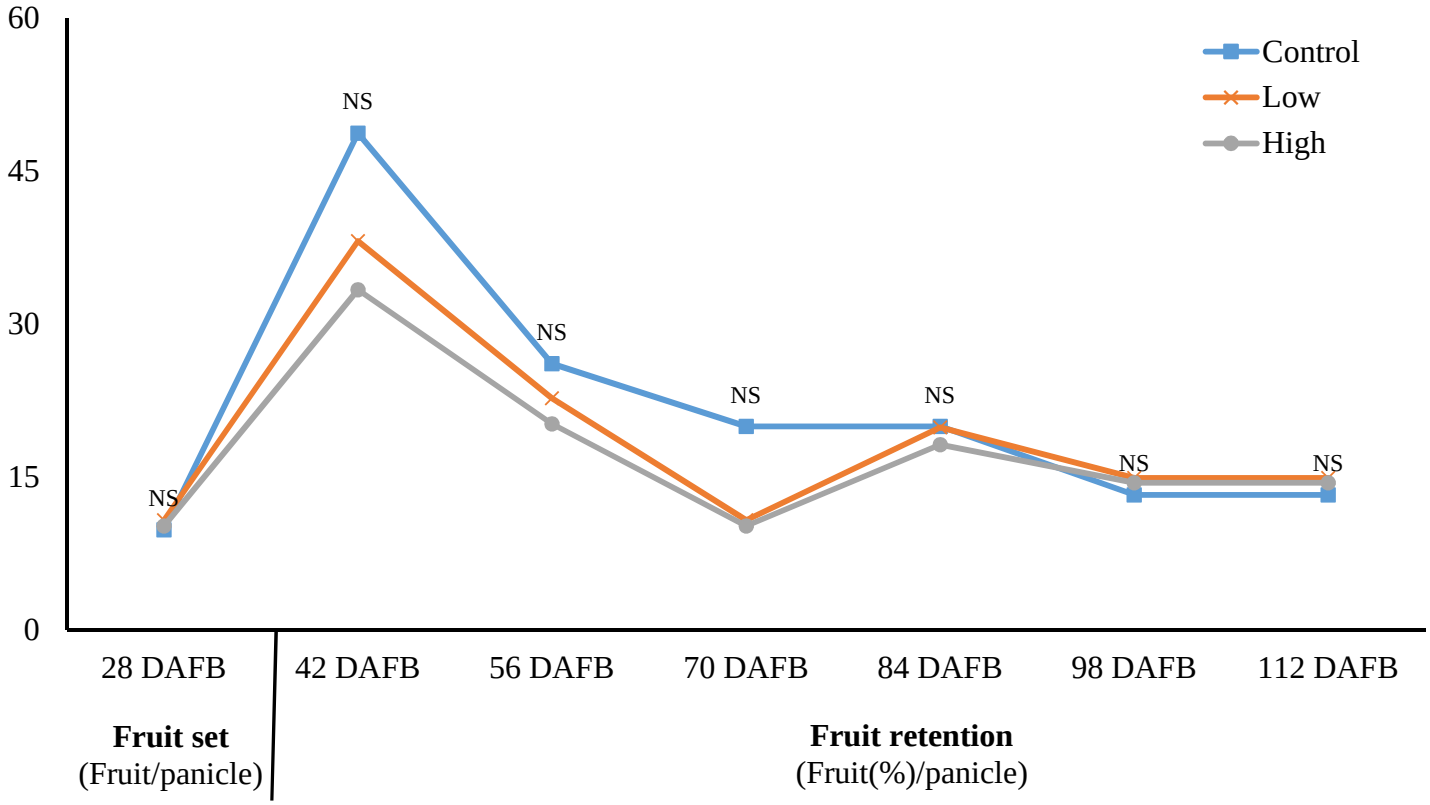

Fig. 6-7: The effects of micronutrient spray concentrations on fruit set and retention per panicles in mango cv. NMBP-1201. DAFB=days after full bloom. NS=Non-significant ($p \leq 0.05$).

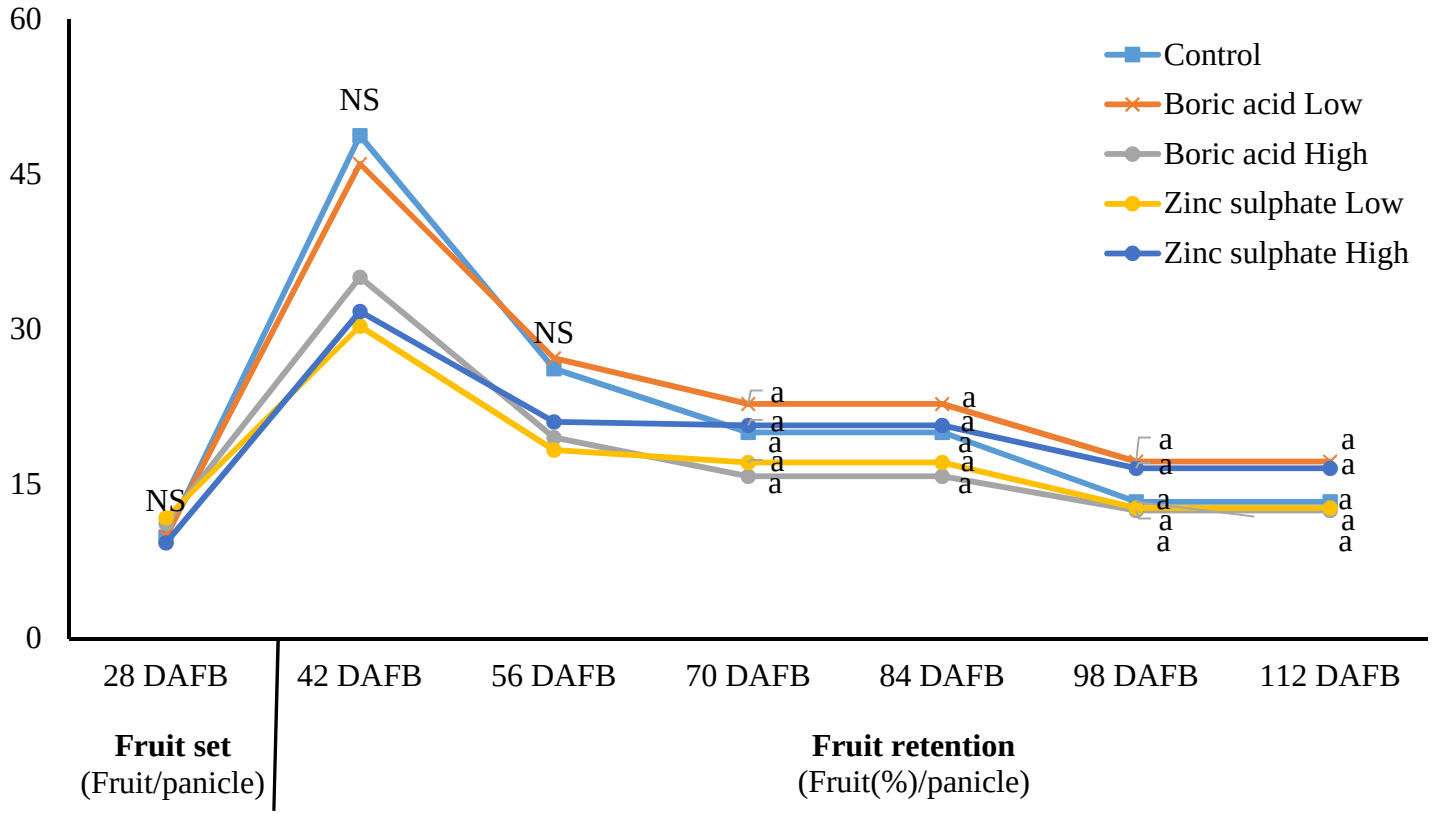

Fig. 6-8: The interactive effects of micronutrient treatment and spray concentration on fruit set and retention per panicle in mango cv. NMBP-1201. DAFB=days after full bloom. NS=Non-significant ($p \leq 0.05$).

Effect of micronutrients on fruit quality of mango cv. NMBP-1201

Effect of micronutrients on fruit weight of mango cv. NMBP-1201

Fruit weight of mango cv. NMBP-1201 was not significantly influenced by foliar application of various micronutrient treatments (Table 6-3). Combined data for all micronutrient treatments also revealed no significant difference in fruit weight between control and sprayed trees (Table 6-4). In addition, no significant difference in fruit weight was observed using either combined data for individual micronutrient treatments (Table 6-5), combined data for micronutrient spray concentration (Table 6-6), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-7).

Effect of micronutrients on fruit length of mango cv. NMBP-1201

Fruit length data detailed in Table 6-3 revealed that foliar application of various micronutrient treatments did not significantly influence fruit length of mango cv. NMBP-1201. Combined data for all micronutrient treatments revealed that control and sprayed trees had statistically similar fruit length (Table 6-4). No significant difference in fruit length was observed using either combined data for individual micronutrient treatments (Table 6-5), combined data for micronutrient spray concentration (Table 6-6), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-7).

Effect of micronutrients on fruit width of mango cv. NMBP-1201

Statistical analysis showed no significant effect of foliar application of various micronutrient treatments on fruit width of mango cv. NMBP-1201 (Table 6-3). Combined data for all micronutrient treatments revealed that control trees displayed statistically similar fruit width as that in trees sprayed with micronutrients (Table 6-4). No significant difference in fruit width was observed using either combined data for individual micronutrient treatments (Table 6-5), combined data for micronutrient spray concentration (Table 6-6), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-7).

Effect of micronutrients on fruit depth of mango cv. NMBP-1201

Fruit depth of mango cv. NMBP-1201 did not differ significantly following foliar application of various micronutrient treatments (Table 6-3). Combined data for all micronutrient treatments

also showed no significant differences in fruit depth between control and sprayed trees (Table 6-4). No significant difference in fruit depth was observed using either combined data for individual micronutrient treatments (Table 6-5), combined data for micronutrient spray concentration (Table 6-6), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-7).

Effect of micronutrients on fruit TSS of mango cv. NMBP-1201

Foliar application of various micronutrient treatments had no significant effects on fruit TSS of mango cv. NMBP-1201 (Table 6-3). Combined data for all micronutrient treatments revealed no significant difference in TSS of control and sprayed trees (Table 6-4). No significant difference in fruit TSS were observed using either combined data for individual micronutrient treatments (Table 6-5), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-7). Fruit harvested from the trees sprayed with high micronutrient spray concentration had the highest fruit TSS, statistically comparable to the fruit from control trees (Table 6-6).

Effect of micronutrients on fruit dry matter contents of mango cv. NMBP-1201

Fruit dry matter contents (DM) of mango cv. NMBP-1201 did not differ significantly as a result of foliar application of various micronutrient treatments (Table 6-3). Combined data for all micronutrient treatments also revealed that statistically similar DM were exhibited by control and treated trees (Table 6-4). No significant difference in fruit DM were observed using either combined data for individual micronutrient treatments (Table 6-5), combined data for micronutrient spray concentration (Table 6-6), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-7).

Table 6-3: The effect of four foliar-applied micronutrient treatments on fruit quality of mango cv. NMBP-1201.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	363.82	88.37	89.26	79.35	13.43	17.64
0.6% boric acid	365.48	89.06	89.97	78.42	13.90	17.54
0.8% boric acid	361.17	89.75	87.81	78.79	13.15	16.99
0.25% zinc sulphate	385.40	90.48	90.05	81.14	13.36	17.11
0.50% zinc sulphate	366.12	91.68	90.04	79.21	12.71	17.18
Level of significance	NS	NS	NS	NS	NS	NS
C.V.	8.98	4.14	3.63	2.95	6.51	4.25

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents C.V.=co-efficient of variation. n=7.

Table 6-4: The combined effects of four micronutrient treatments on fruit quality of mango cv. NMBP-1201.

Treatments	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control	363.8	88.37	89.26	79.35	13.43	17.6
Treated	369.5	90.24	89.47	79.39	13.28	19.0
Level of significance	NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-5: The effect of two micronutrients on fruit quality of mango cv. NMBP-1201. Data are the average of two application rates for each micronutrient.

Treatments	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control	363.8	88.37	89.26	79.35	13.43	17.6
Boric acid	363.3	89.4	88.89	78.6	13.52	20.8
Zinc sulphate	375.80	91.08	90.05	80.18	13.03	17.10
Level of significance	NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-6: The effects of micronutrient spray concentrations on fruit quality of mango cv. NMBP-1201.

Spray concentrations	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control	363.8	88.37	89.26	79.35	13.43^{ab}	17.6
Low	375.4	89.77	90.01	79.78	13.63^{b}	17.3
High	363.6	90.72	88.92	79.00	12.93^{a}	20.7
Level of significance	NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-7: The interactive effects of micronutrient treatment and spray concentration on fruit quality of mango cv. NMBP-1201.

Treatment	Spray concentrations	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control		363.8	88.37	89.26	79.35	13.43	17.6
Boric acid	Low	365.5	89.06	89.97	78.42	13.90	17.5
	High	361.2	89.75	87.8	78.78	13.15	24.1
Zinc sulphate	Low	385.4	90.48	90.05	81.14	13.35	17.1
	High	366.1	91.68	90.04	79.21	12.71	17.2
Level of significance		NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-8: Variation in fruit quality of mango cv. NMBP-1201 as influenced by foliar application of micronutrients.

Variation level	Estimator	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Within Trees	Mean SE	21.93	2.14	1.71	1.64	0.55	1.90
	Mean SD	43.86	4.29	3.43	3.28	1.11	3.81
Between Trees	Mean	368.4	89.87	89.42	79.38	13.3	18.72
	SD	32.31	3.69	3.17	2.40	0.90	8.43
	SEM	5.46	0.62	0.54	0.41	0.15	1.43

SE=standard error. SD=standard deviation. SEM=standard error of means. TSS=total soluble solids. DM=dry matter contents.

6.4.2 Effect of micronutrients on mango cv. R2E2

Effect of micronutrients on fruit set and retention in mango cv. R2E2

Effect of micronutrients on fruit set in mango cv. R2E2

In mango cv. R2E2, fruit set at 28 days after full bloom (DAFB) was not significantly affected by foliar application of various micronutrient treatments at full bloom (Fig. 6-9). Combined data for all micronutrient treatments also showed no significant difference in fruit set between control and treated trees (Fig. 6-10). No significant difference in fruit set was observed using either combined data for individual micronutrient treatments (Fig. 6-11), combined data for micronutrient spray concentration (Fig. 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Fig. 6-13).

Effect of micronutrients on fruit retention over time in mango cv. R2E2

Foliar application of various micronutrient treatments had no significant effect on fruit retention in mango cv. R2E2 during fruit growth until fruit maturation (Fig. 6-9). In agreement, combined data for all micronutrient treatments showed that trees sprayed with micronutrients had statistically similar fruit retention compared to that control trees throughout fruit development (Fig. 6-10). No significant difference in fruit retention during fruit growth and development until harvest stage was observed using either combined data for individual micronutrient treatments (Fig. 6-11), combined data for micronutrient spray concentration (Fig. 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Fig. 6-13).

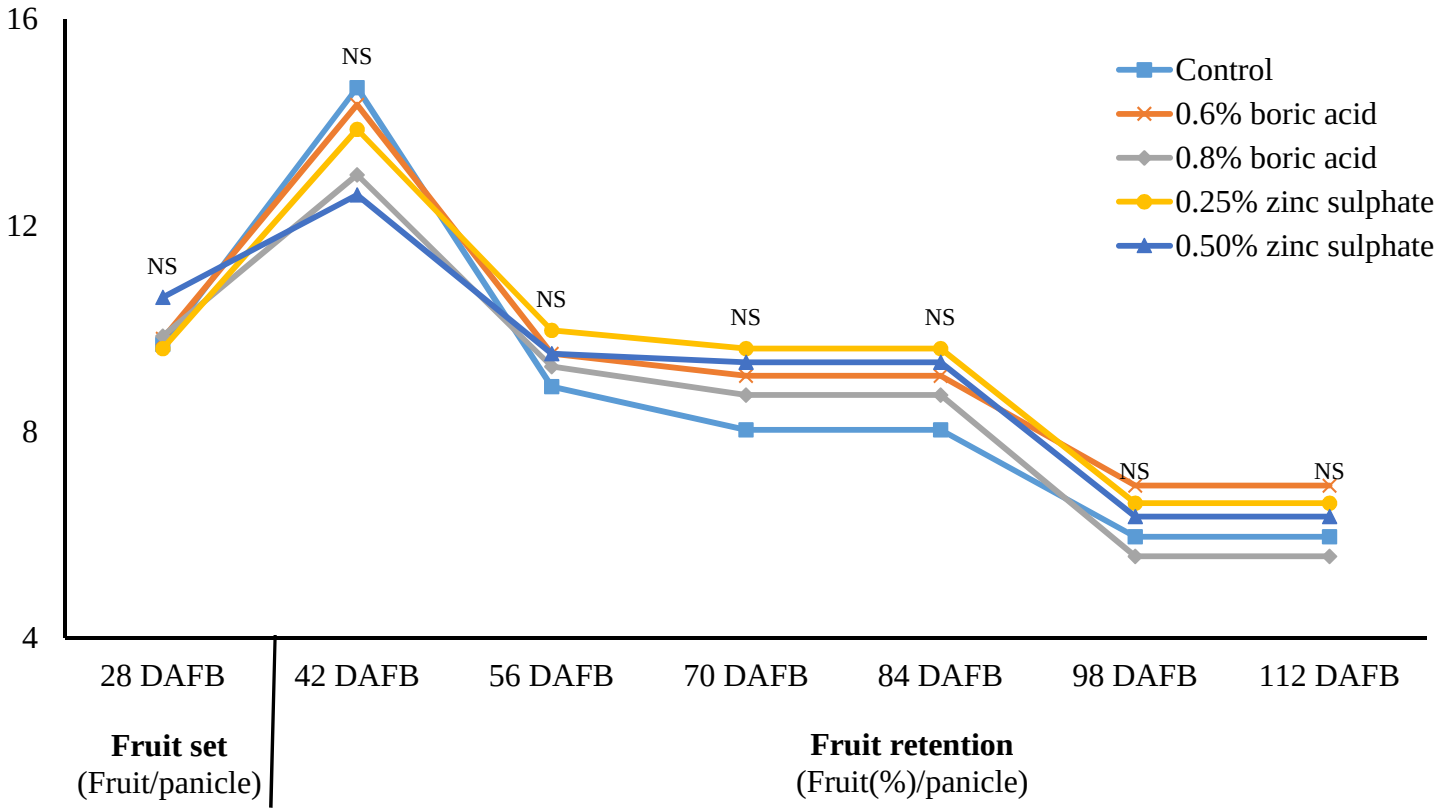

Fig. 6-9: The effect of four foliar-applied micronutrient treatments on fruit set and retention per panicle in mango cv. R2E2. DAFB=days after full bloom. NS=Non-significant ($p \leq 0.05$).

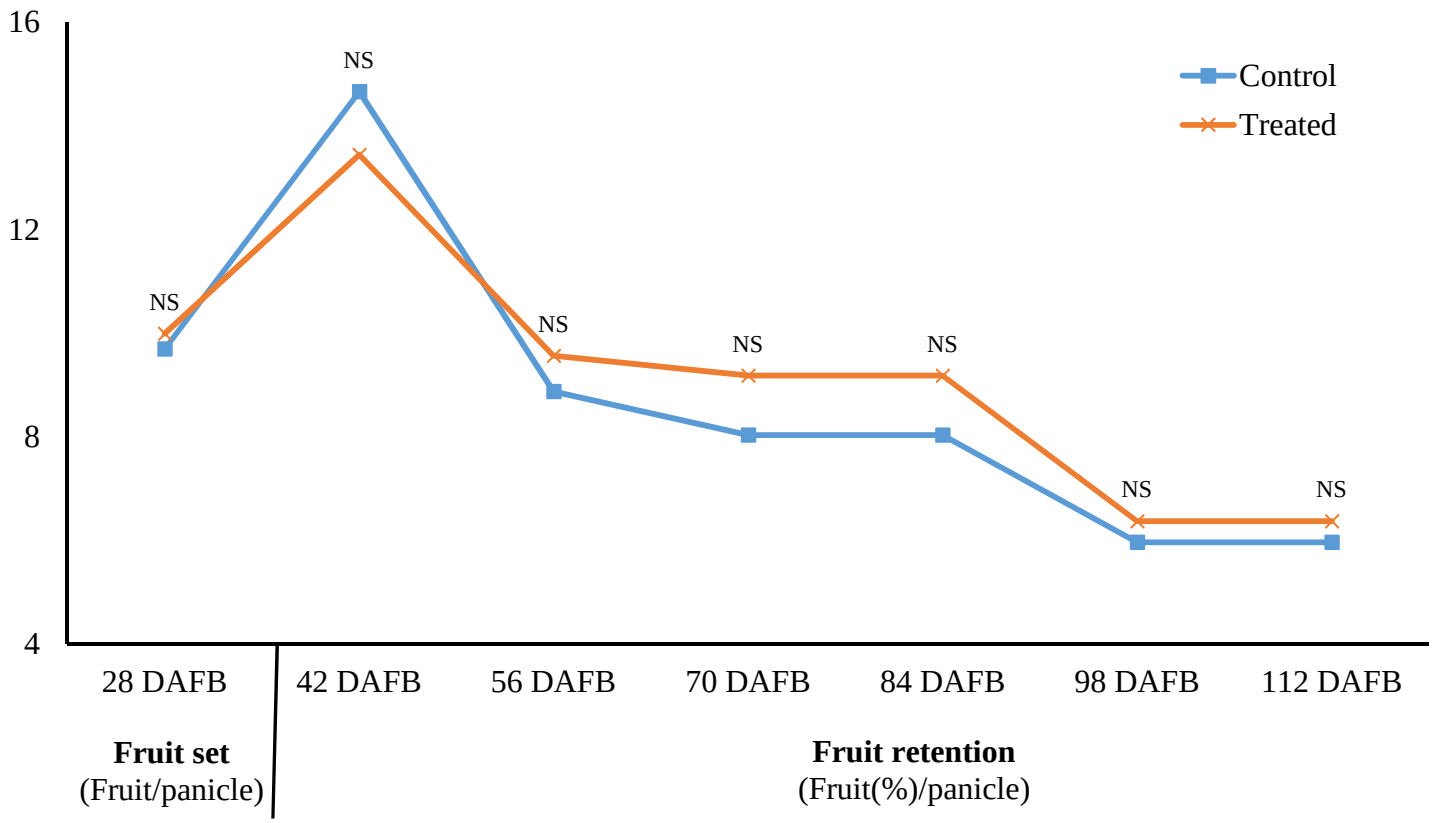

Fig. 6-10: The combined effects of four micronutrients treatments on fruit set and retention per panicle in mango cv. R2E2. DAFB=days after full bloom. NS=Non-significant ($p \leq 0.05$).

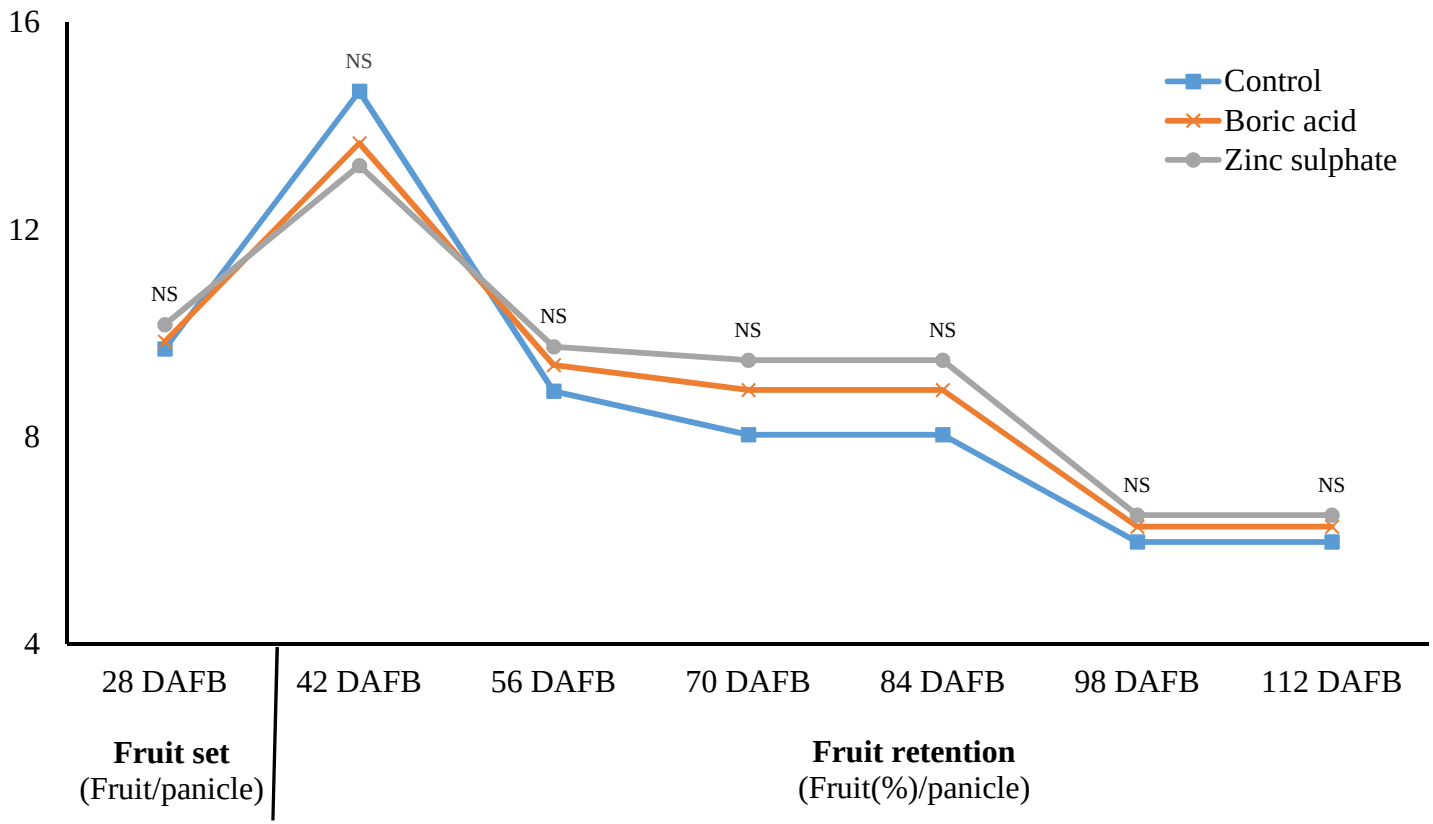

Fig. 6-11: The effects of two micronutrients on fruit set and retention per panicle in mango cv. R2E2. Data are average of two application rates for each micronutrient. DAFB=days after full bloom. NS=Non-significant ($p \leq 0.05$).

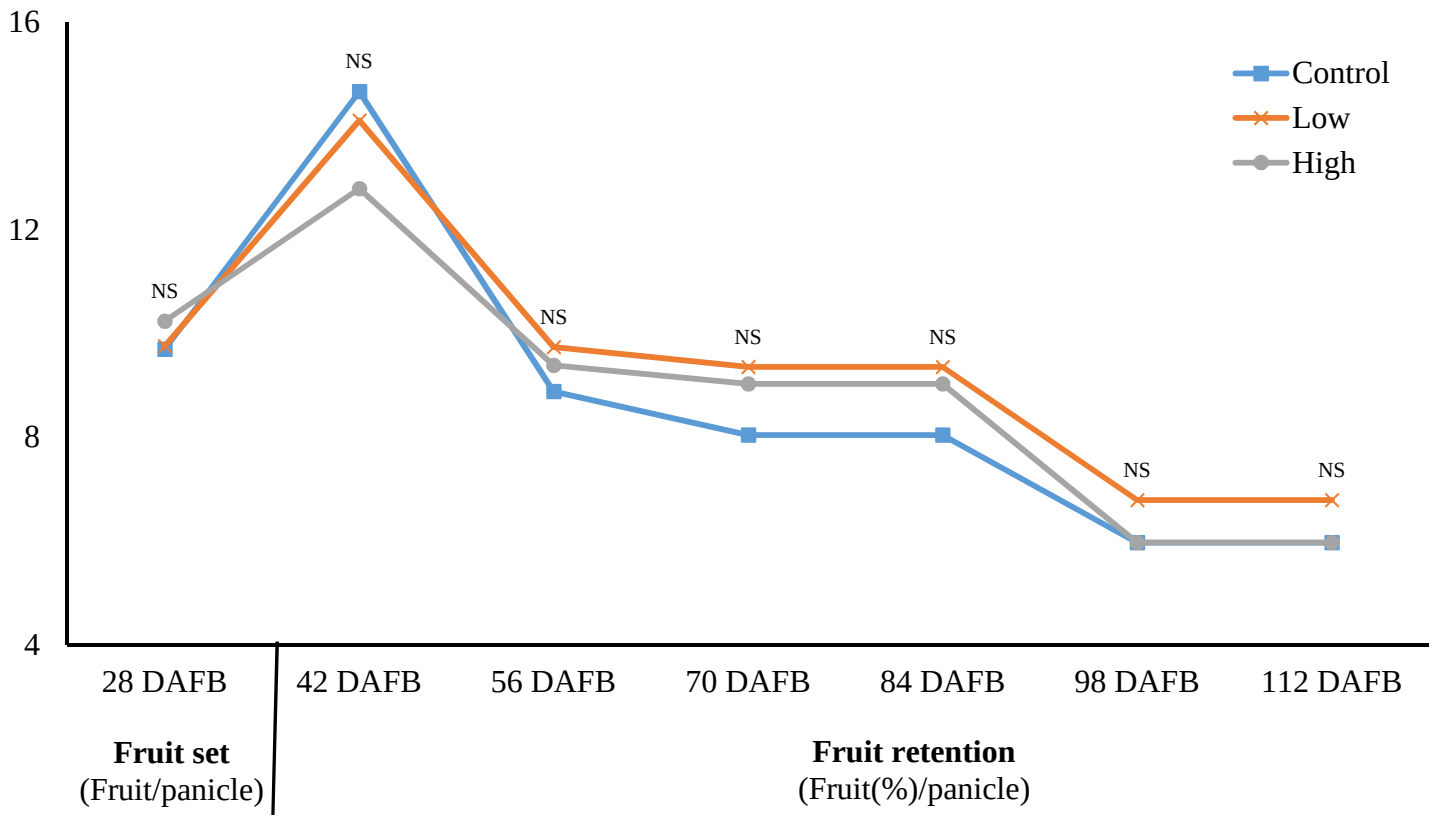

Fig. 6-12: The effects of micronutrient spray concentrations on fruit set and retention per panicles in mango cv. R2E2. DAFB=days after full bloom. NS=Non-significant ($p \leq 0.05$).

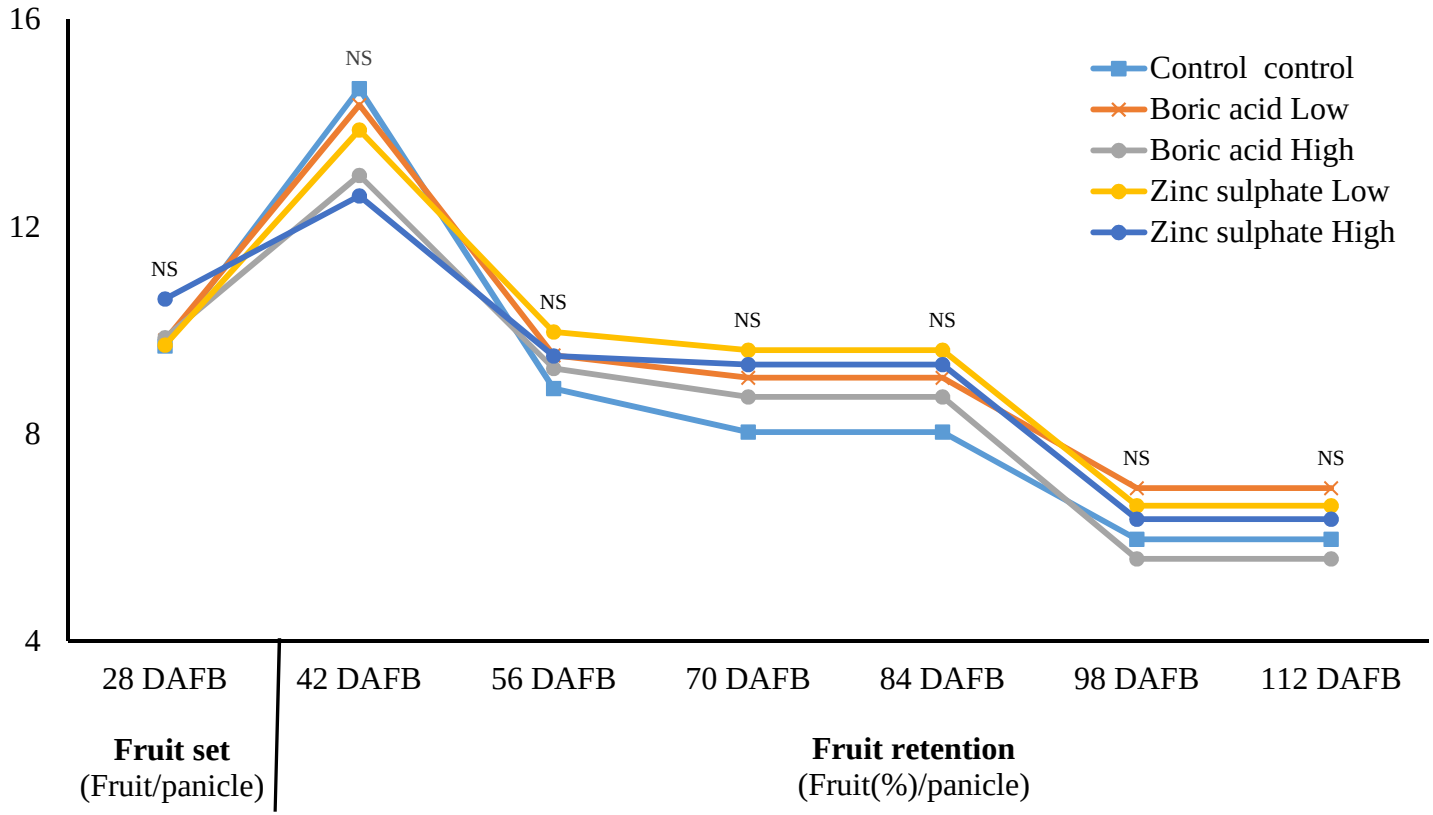

Fig. 6-13: The interactive effects of micronutrient treatment and spray concentration on fruit set and retention per panicle in mango cv. R2E2. DAFB=days after full bloom. NS=Non-significant ($p \leq 0.05$).

Effect of micronutrients on fruit quality of mango cv. R2E2

Effect of micronutrients on fruit weight of mango cv. R2E2

Fruit weight of mango cv. R2E2 was not significantly influenced by foliar application of various micronutrient treatments (Table 6-9). Combined data for all micronutrient treatments revealed no significant difference between fruit weight of control and sprayed trees (Table 6-10). No significant difference in fruit weight was observed using either combined data for individual micronutrient treatments (Table 6-11), combined data for micronutrient spray concentration (Table 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-13).

Effect of micronutrients on fruit length of mango cv. R2E2

Fruit length of mango cv. R2E2 was not significantly influenced by foliar application of various micronutrient treatments (Table 6-9). Combined data for all micronutrient treatments showed control and sprayed trees had statistically comparable fruit length (Table 6-10). No significant difference in fruit length was observed using either combined data for individual micronutrient treatments (Table 6-11), combined data for micronutrient spray concentration (Table 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-13).

Effect of micronutrients on fruit width of mango cv. R2E2

Statistical analysis shows non-significant effect of foliar application of various micronutrient treatments on fruit width of mango cv. R2E2 (Table 6-9). Combined data for all micronutrient treatments revealed that control trees had statistically comparable fruit width as that in trees sprayed with micronutrients (Table 6-10). No significant difference in fruit width was observed using either combined data for individual micronutrient treatments (Table 6-11), combined data for micronutrient spray concentration (Table 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-13).

Effect of micronutrients on fruit depth of mango cv. R2E2

Fruit depth of mango cv. R2E2 did not differ significantly following foliar application of various micronutrient treatments (Table 6-9). Combined data for all micronutrient treatments also showed that fruit depth was not significantly different between control and sprayed trees

(Table 6-10). No significant difference in fruit depth was observed using either combined data for individual micronutrient treatments (Table 6-11), combined data for micronutrient spray concentration (Table 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-13).

Effect of micronutrients on fruit TSS of mango cv. R2E2

Foliar application of various micronutrient treatments did not significantly affect fruit TSS of mango cv. R2E2 (Table 6-9). Combined data for all micronutrient treatments indicated no significant difference in fruit TSS between control and sprayed trees (Table 6-10). No significant difference in fruit TSS were observed using either combined data for individual micronutrient treatments (Table 6-11), combined data for micronutrient spray concentration (Table 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-13).

Effect of micronutrients on fruit dry matter contents of mango cv. R2E2

Fruit dry matter contents (DM) of mango cv. R2E2 did not differ significantly following foliar application of various micronutrient treatments (Table 6-9). Combined data for all micronutrient treatments exhibited statistically comparable DM between control and treated trees (Table 6-10). No significant difference in DM were observed using either combined data for individual micronutrient treatments (Table 6-11), combined data for micronutrient spray concentration (Table 6-12), or data for the interactive effects of micronutrient treatment and spray concentration (Table 6-13).

Table 6-9: The effect of four foliar-applied micronutrients on fruit quality of mango cv. R2E2.

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Depth (mm)	TSS (%)	DM (%)
Control	763.68	120.37	113.15	100.59	11.93	17.17
0.6% boric acid	753.22	121.35	112.42	98.77	11.74	17.10
0.8% boric acid	700.55	116.49	109.92	93.73	11.67	16.80
0.25% zinc sulphate	722.01	120.42	110.83	97.43	12.32	17.27
0.50% zinc sulphate	809.61	123.94	114.80	100.71	12.01	17.37
Level of significance	NS	NS	NS	NS	NS	NS
C.V.	12.97	5.46	4.34	7.11	4.49	3.44

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents C.V.=co-efficient of variation. n=7.

Table 6-10: The combined effect of four micronutrient treatments on fruit quality of mango cv. R2E2.

Treatment	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control	764.4	120.4	113.15	100.6	11.932	17.166
Treated	746.1	120.5	111.99	97.7	11.932	17.135
Level of significance	NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-11: The effect of two micronutrients on fruit quality of mango cv. R2E2. Data are the average of two application rates for each micronutrient.

Treatment	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control	764.4	120.4	113.15	100.6	11.93ab	17.166
Boric acid	727.3	118.9	111.17	96.2	11.70a	16.951
Zinc sulphate	766.1	122.2	112.81	99.1	12.16b	17.319
Level of significance	NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-12: The effects of micronutrient spray concentrations on fruit quality of mango cv. R2E2.

Spray concentrations	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control	764.4	120.4	113.15	100.6	11.932	17.166
Low	738.4	120.9	111.62	98.1	12.027	17.187
High	755.6	120.2	112.36	97.2	11.838	17.084
Level of significance	NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-13: The interactive effects of micronutrient treatment and spray concentration on fruit quality of mango cv. R2E2.

Treatments	Spray concentrations	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Control		764.4	120.4	113.15	100.6	11.932	17.166
Boric acid	Low	753.1	121.3	112.42	98.8	11.732	17.101
	High	701.4	116.5	109.92	93.7	11.668	16.801
Zinc sulphate	Low	722.3	120.4	110.83	97.4	12.321	17.273
	High	810.5	123.9	114.79	100.7	12.007	17.366
Level of significance		NS	NS	NS	NS	NS	NS

NS=Non-significant ($p \leq 0.05$). TSS=total soluble solids. DM=dry matter contents. n=7.

Table 6-14: Variation in fruit quality of mango cv. R2E2 as influenced by foliar application of micronutrients.

Variation level	Estimator	Weight (g)	Length (mm)	Width (mm)	Depth (mm)	TSS (%)	DM (%)
Within Trees	SE Mean	50.77	3.48	2.56	2.61	0.49	0.49
	SD Mean	101.5	6.96	5.12	5.22	0.99	0.98
Between Trees	Mean	749.8	120.50	112.20	98.24	11.93	17.14
	SD	98.88	6.65	4.89	7.06	0.55	0.59
	SEM	16.71	1.12	0.83	1.19	0.09	0.10

SE=standard error. SD=standard deviation. SEM=standard error of means. TSS=total soluble solids. DM=dry matter contents.

6.5 Discussion

The foliar micronutrient treatments in this study were designed to eliminate any transitory or localised flower B or Zn deficiency that was present because of limited B and Zn mobility in the plant (Brown and Shelp, 1997). Treatments were performed with the intention of improving fruit set and retention in experimental trees. However, in this study, foliar application of B and Zn at the start of bloom had no significant impact on fruit set and retention during most stages of fruit growth and development in either of the tested cultivars. This lack of a response is inconsistent with previous studies that report such micronutrient treatments improve fruit set in multiple crops, including almond (Nyomora *et al.*, 1997), apples (Davison, 1971), olives (Saadati *et al.*, 2016), and mango (Daulta *et al.*, 1981; Masroor *et al.*, 2016; Rajput *et al.*, 1976). Furthermore, foliar-applied B and Zn have been shown to improve fruit retention in various crops, including citrus (Razzaq *et al.*, 2013) and mango (Bhowmick *et al.*, 2012; Singh and Maurya, 2004). Foliar B and Zn application in this study also had no significant effect on fruit quality parameters such as fruit weight, length, width, depth, TSS, and dry matter contents in either experimental cultivar, and fruit quality exhibited no significant variation within and between tree levels as a result of micronutrient treatment (Table 6-7 and 6-14). Many studies on the effects of foliar-applied micronutrients report positive impacts of B and Zn treatment on fruit quality parameters, such as fruit size and TSS in citrus (Boaretto *et al.*, 2002; Razzaq *et al.*, 2013), olive (Saadati *et al.*, 2016), and mango (Bhowmick *et al.*, 2012; Masroor *et al.*, 2016; Rajput *et al.*, 1976).

The findings of this study do not support the hypothesis that foliar application of B and improves fruit set and retention by optimising flower B and Zn content. In addition to poor tree nutritional status that may undermine the effect of micronutrient treatment, the lack of a response to B and Zn foliar application may be attributed to several factors. For example, in the experimental trees in this study, there may have been sufficient endogenous B and Zn available in the flowers for pollination and fruit set. Therefore, extra micronutrient supply from foliar treatment would not have improved fruit set and subsequent fruit retention compared to that in control trees (Usenik and Stampar, 2002). Inappropriate timing and frequency of the foliar applications may also have contributed towards the lack of a response to micronutrient treatment. In mango, several studies have revealed that the timing and frequency of foliar B and Zn treatments have significant impacts on fruit set and retention. For example, when experimental trees were treated before bud burst, then again at panicle emergence and once

more following fruit set (Chauhan *et al.*, 2014), fruit set and retention differed compared to that in trees treated only two times (Masroor *et al.*, 2016). Micronutrient source (fertiliser type) may also affect the response of experimental trees to micronutrient treatments (Boaretto *et al.*, 2002). Previous work showed significantly improved fruit set and retention using different fertiliser types such as B sourced from solubor ($Na_2B_8O_3$(Nyomora *et al.*, 1997) and sodium polyborate (Davison, 1971); however, other reports describe improved fruit set using the same micronutrient sources as employed in this study, namely boric acid and zinc sulphate (Chauhan *et al.*, 2014; Saadati *et al.*, 2016).

In this study foliar micronutrient application did not have a significant effect on fruit set. However, past studies imply that foliar-applied Zn and B improve fruit set, retention, and quality in fruit crops, although not in all instances. The important functions of micronutrients such as B and Zn in the reproductive physiology of fruit crops are well established. Micronutrients contribute towards stamen and pollen formation, pollen quality, pollen tube growth, and fruit set and subsequent fruit retention (Marschner, 2012). However, the lack of a response to micronutrient treatment in the experimental trees of this study may be attributed to prevailing poor tree conditions, poor soil management, and experimental design. The experimental trees used in this study undergo alternate bearing, with the "off-crop" season in 2014 and the "on-crop" season in 2015. This alternate bearing may have caused the experimental trees to have higher carbohydrate reserves during the study period.

Plant and soil nutritional status should be well-managed because poor soil quality or plant health has the potential to mask the effects of micronutrient treatments. In future research aiming to improve fruit set and retention via foliar micronutrient treatment, it would be necessary to include a wider range of B and Zn treatment concentrations from multiple chemical sources, variation in the timing and frequency of micronutrient applications, and the use of multiple study sites and mango cultivars in order to develop effective strategies for use in mango breeding programs and commercial orchards. Destructive examination of the dropped fruit may also further the understanding of factors that influence fruit drop, such as a lack of pollination/fertilization and embryo abortion, which may assist in evaluating the effectiveness of micronutrients foliar sprays to improve fruit set and retention.

Chapter 7: General discussion and conclusions

In the majority of mango-producing countries, the mango industry has historically been based on very few commercial cultivars (Bally *et al.*, 2000b; Hardner *et al.*, 2012; Honsho *et al.*, 2013; Pinto *et al.*, 2004b; Whiley *et al.*, 1993). Most of the commercially grown cultivars worldwide are either the product of chance seedlings selected following natural cross-pollination (Bally *et al.*, 2000a; Mukherjee *et al.*, 1968; Sharma, 1987) or the product of classical breeding techniques (Mukherjee *et al.*, 1961; Mukherjee *et al.*, 1968; Sauco, 1993). Commercial cultivars typically exhibit several undesirable horticultural traits such as vigorous vegetative growth, erratic fruit bearing, short postharvest fruit life, and susceptibility to physiological disorders and diseases (Human *et al.*, 2006; Tomer *et al.*, 1997). While these traditional cultivars have historically sustained the mango industry (Sharma, 1987), they are increasingly poorly suited to the modern mango industry because they produce fruit with low resilience during transport, low shelf life, and poor post-harvest quarantine treatment responses (Pinto *et al.*, 2004b). To ensure the long-term sustainability of the mango industry, there is a critical need to develop cultivars that are acceptable for growers and the modern global mango market (Bally *et al.*, 2009b; Iyer and Subramanyam, 1991).

The modern mango market demands efficient and sustainable production of quality fruit, which can potentially be achieved through mango crop genetic improvement using suitable breeding techniques (Hardner *et al.*, 2012). The majority of such endeavours make use of the classical breeding technique (Bally *et al.*, 2009a; Iyer and Dinesh, 1997; Mukherjee *et al.*, 1961; Mukherjee *et al.*, 1968). Classical breeding employs hand pollination to achieve crossing; however, the hand pollination technique is inefficient because it is time-consuming, labour intensive, associated with low fruit set rates, and promotes extended juvenile duration (Pinto *et al.*, 2004b; Sharma, 1987). Several modifications to the hand pollination technique have been investigated in an effort to improve its efficiency, such as the availability of quality pollen, parental selection based on better understanding of trait inheritance, improved fruit set, and higher retention of the hybridised fruit. The present project aimed to improve the efficiency of hand pollination by three approaches: (1) improving the accessibility of quality pollen available for crossing in a single mango season (Chapter 3), (2) improving the decision-making process regarding parental selection by using the 'breeding values' approach (Chapter 4), and (3)

improving fruit set and retention by foliar application of plant growth regulators (PGRs) (Chapter 5) and micronutrients such as boron and zinc (Chapter 6).

There were two general aims of the work detailed in Chapter 3: (1) to optimise the mango pollen germination test, and (2) to develop a simple pollen storage and retrieval method for use in routine breeding work. Different pollen germination media recipes and pollen storage techniques were trialled to achieve these aims, which, if realised, contribute towards overcome the barrier of crossing individuals with asynchronous flowering. In mango, flowering times may differ significantly among genotypes, cultivars, and environmental conditions (Bally *et al.*, 2009a). This flowering time variation poses a limitation on mango breeding because pollen may not be available for crosses between asynchronously flowering individuals. Successful pollen storage assists in hand pollination by extending the time during which quality mango pollen is available. Organic solvents and mineral oil have been shown to be effective storage media for the short-term storage of pollen in different plant species (Iwanami and Nakamura, 1972; Iwanami, 1973; Jain and Shivanna, 1990; Jain *et al.*, 1990). In particular, organic solvents are favourable for pollen storage because they do not require maintenance of specific relative humidity and are therefore useful for pollen transport without refrigeration or dry ice (Stanley and Linskens, 2012).

In the present study, different pollen storage regimes were tested to develop a simple method for use in routine breeding work. In Chapter 3, two storage media, namely hexane and paraffin oil, and different storage temperatures were investigated for their effects on pollen viability. Pollen stored for one week in hexane at room temperature retained the highest germination rate, which was comparable to that in pollen stored at −80ºC without any storage medium. The viability of the mango pollen stored in different media was significantly lower than that of pollen from other crops stored in different organic solvents (Iwanami and Nakamura, 1972; Iwanami, 1973; Jain and Shivanna, 1990; Jain *et al.*, 1990).

The findings of Chapter 3 suggest that mango pollen cannot tolerate longer that one-week exposure to hexane or paraffin oil. Chaudhury *et al.* (2010) reported that pollination with mango pollen that displayed approximately 50% viability resulted in fruit set that was statistically comparable to that resulting from the use of fresh mango pollen. Mango pollen stored for one week in hexane at room temperature maintained a pollen germination rate of 40.77%, which is sufficiently high to use in routine breeding crosses. This finding has practical

importance for mango breeders working with spatially and temporally separated parental lines in locations that experience energy shortages, such as Pakistan (Asif, 2009).

Other factors that contribute substantially to the success of hand pollination include the timing of hand pollination and number of flowers per panicle that are pollinated. The receptive period of the mango stigma is short and thus, ensuring that hand pollination is performed within that short receptive window may result in high-level fruit set and retention as a result of increased fertilisation (Geetha *et al.*, 2016). A pollination strategy that includes fewer flowers per panicle and more panicles overall also improves the success rate of hand pollination (Bally *et al.*, 2000a; Mukherjee *et al.*, 1961; Mukherjee *et al.*, 1968).

The selection of parents for crosses is important for fruit breeders (Lavi *et al.*, 1998). In classical breeding, parents are generally selected on the basis of phenotypic expression of desired traits (Hansche, 1983). In mango breeding, the highly heterozygous nature of the crop is considered a handicap to generating recombinant progeny from desirable parental lines (Dinesh *et al.*, 2013; Lavi *et al.*, 1989b). The creation of useful progeny can be achieved by generating a large hybrid population (Iyer, 1989). However, the selection of appropriate parents from large mango populations is often limited by poor selection techniques that rely on phenotypic expressions of desirable traits, which does not guarantee that the offspring will inherit these traits due to the highly heterozygous nature of mango as described above. Advanced knowledge of mango genetics and the inheritance of target traits improves the breeding efficiency in fruit crops (Dinesh *et al.*, 2013; Hardner *et al.*, 2012; Lavi *et al.*, 1998). This advanced knowledge allows the breeder to predict genetic potential and response to selection, and hence avoid unnecessary crosses using low-quality parents (Hardner *et al.*, 2016).

Chapter 4 focused on methods for parental selection for breeding low-vigour mango. Developing low-vigour trees is one of the main objectives of mango breeding programs worldwide (Bally *et al.*, 2000a; Campbell and Ledesma, 2013; Cilliers *et al.*, 1997; Kulkarni *et al.*, 2002; Lavi *et al.*, 1989a; Whiley *et al.*, 1993). Control of tree vigour improves orchard productivity, labour efficiency, and promotes cost-effective orchard management by reducing labour costs and pesticide consumption (Fideghelli *et al.*, 2003; Olmstead *et al.*, 2006). The need for cost-effective orchard management is the driving force behind research into ways to

alter tree size and growth (Byrne, 2012). Chapter 4 evaluated multiple tree morphological traits to identify the trait that exhibited the highest correlation with vegetative vigour.

The findings of Chapter 4 indicate that trunk cross-sectional area (TCA) displays the strongest correlation with tree vigour. Similar results were reported in a number of previous studies on various woody fruit species (Barden *et al.*, 2002; Guxiong *et al.*, 1987; Khatamian and Hilton, 1977; Nesme *et al.*, 2005; Strong and Azarenko, 2000). These studies identified TCA as an accurate and efficient tool to evaluate tree vigour. However, because of orchard management practices such as regular pruning, TCA ceases to predict tree vigour better than other tree morphological traits after a certain tree age, as reported by Nesme *et al.* (2005). This notion is also supported by the work of Westwood and Roberts (1970), who observed a linear relationship between TCA and plant vigour in apple assuming the plants were not heavily pruned. TCA can be employed to assess vigour in breeding programs where plant vigour is assessed an early age.

Parent selection is important because it ensures individuals with desirable features contribute towards the genetic makeup of the following generation (Bauer *et al.*, 2006). Narrow-sense heritability (h^2) was used to assess the TCA heritability within a mango breeding population located across two research stations in north Queensland. This revealed that TCA exhibits a low narrow-sense heritability value (0.23), indicating that TCA is subject to weak additive genetic control and is a poorly heritable trait. Thus, genotype only weakly determines the phenotypic expression of TCA in mango breeding populations as a result of higher non-additive variance. This is consistent with the sentiments of Lavi *et al.* (1998), who described how high non-additive genetic variation is found in most agriculturally important traits. However, this idea does not hold true for traits such as average fruit weight, which was identified by Hardner *et al.* (2012) as under strong additive genetic control. The significant difference in h^2 observed between the two research stations is the result of the large difference in additive variance in the mango populations at either research station. The higher non-additive variance at Southedge Research Station is due to the large proportion of open-pollinated individuals in that particular breeding population.

Best linear unbiased predictions (BLUPs) were used in this study to estimate breeding values to use in determining the most suitable family or individual progeny for inclusion in future crosses. Despite relatively low TCA narrow-sense heritability, breeding values for this trait

were statistically significantly different among breeding families and progenies. Parental selection based on breeding values increases the likelihood of generating progeny that exhibit phenotypic expression of desired traits to a higher degree.

The efficiency of classical mango breeding is significantly reduced by low fruit set and retention following hand pollination. Fruit set rates in the progeny of mango breeding crosses are reported to range between 0% and 18.6% (Iyer and Dinesh, 1997; Lavi *et al.*, 1993b), which leads to low hybrid numbers per panicle crossed (Bally *et al.*, 2009a). In fruit tree crops, fruit set is regulated by endogenous hormones (De Jong *et al.*, 2015), including auxins, gibberellins, and cytokinins (Kumar *et al.*, 2014). Foliar application of synthetic PGRs can correct hormonal imbalances, and is a widespread practice that leads to increased fruit retention and reduced pre-harvest fruit drop in various crops (Anthony and Coggins, 1999; Anthony and Coggins, 2001; Singh and Lal, 1980; Tuan and Chung-Ruey, 2013b).

Chapter 5 investigated the effects of PGRs and micronutrients on fruit set and retention. In this chapter, foliar-applied PGRs were tested for their ability to improve the rate of fruit set and retention. However, contrary to multiple prior reports of higher fruit set following foliar application of auxins including 2,4-D (García-Martínez and García-Papí, 1979; Tuan and Chung-Ruey, 2013b; Tuan and Chung-Ruey, 2013a), the data within Chapter 5 indicated that foliar application of PGRs at full bloom stage did not significantly increase fruit set. The trees sprayed with 2,4-D exhibited lower fruit set compared to that in control trees and trees treated with other PGRs. This result supports a previous study that reported lower fruit set in Dusehri mango trees sprayed with 2,4-D compared to that in trees sprayed with NAA and GA_3 (Ahmed *et al.*, 2012). This same study also showed that cashew trees sprayed with 2,4-D had lower fruit set than that in trees treated with other PGRs (Ahmed *et al.*, 2012). The lower fruit set in 2,4-D-treated trees described in Chapter 5 may be because of poor soil nutrient status (soil macro- and micronutrient content) at time of PGR application, which can result from poor soil management in the experimental plots. Low soil nutrients in conjunction with 2,4-D treatment potentially cause plant nutrient deficiencies which prevent accelerated growth and the development of the ovary into fruit, which underlies the fruit setting process. In commercial settings, where soil is well-managed and adequate soil nutrients are available to the plant to facilitate mango fruitlet development, fruit set may be improved by foliar-applied PGRs. Foliar application of PGRs combined with the application of micronutrients such as zinc and boron at full bloom may increase fruit set and retention. Foliar application of zinc and 2,4-D

significantly increased the fruit retention rates and final fruit number in Kinnow mandarin (Gurjar and Rana, 2014).

The effects of PGRs on fruit retention differed in the experimental varieties in both seasons. In general, auxins had a strong positive effect on fruit retention in experimental varieties in both seasons, which is consistent with past reports (Ahmed *et al.*, 2012; Arteca, 1996; Chattha *et al.*, 1999; El-Otmani *et al.*, 1990; Guardiola and García-Luis, 2000; Nawaz *et al.*, 2008). Higher fruit retention in trees treated with foliar-applied auxin may be due to the negative effect of auxins on development of the abscission zone in fruit crops (Arteca, 1996). Poor management of the experimental trees in this study may have caused the poor combined performance of PGR-treated trees, making their rate of fruit retention comparable to that in control trees. This hypothesis is supported by a previous report that concluded that fruit drop may be affected by cultural practices (Anthony and Coggins, 1999).

The results presented in Chapter 5 suggest that foliar application of 2,4-D or NAA on panicles prior to hand pollination may increase subsequent fruit retention up to two fold. In commercial settings under effective soil management, adequate nutrients are available in the plant for rapidly growing mango fruitlets. Higher tree productivity and farm yields are thus expected following foliar application of PGRs in commercial orchards. However, foliar application of 2,4-D at flowering stage may reduce fruitlet growth rate, delay fruit abscission, and decrease final fruit size and weight (Guardiola and García-Luis, 2000). Poor nutrient management may also play a role in decreased fruit weight and size, as growing fruitlets under the influence of PGR treatment may have insufficient nutrients and thus higher competition from adjacent fruit, which limits their development (Guardiola *et al.*, 1993). Fruit weight and size are not critical factors in mango breeding as long as a healthy stone is produced. However, fruit weight and size is a major concern in commercial mango production. Improved soil management may overcome the problem of small fruit size resulting from foliar-applied PGRs.

Another strategy for improving fruit set and retention in mango is the elimination of boron or zinc deficiencies in flowers by foliar application of these micronutrients. Chapter 6 explores the effect of foliar micronutrient treatments on fruit set and retention in mango. The data detailed in Chapter 6 indicate that micronutrient treatment had no significant impact on either fruit set and retention or fruit quality in two mango cultivars. This result can be attributed to several reasons. Firstly, the experimental plants may not have been initially deficient in boron

and zinc, and therefore supplemental micronutrient treatments did not improve fruit set compared to rates of fruit set observed in control trees (Usenik and Stampar, 2002). Secondly, the application timing, treatment concentration, and micronutrients source (fertilizer type) may have been inappropriate (Boaretto *et al.*, 2002) for successful foliar uptake of micronutrients. Finally, experimental trees may have had poor nutritional status due to inadequate soil nutritional management, which may have undermined the positive effects of micronutrient treatment. In similar conditions, the combined application of PGRs and micronutrients may be more effective in improving fruit set and retention to ultimately increase yield. Similar results were reported by Gawande *et al.* (2012) and Farahat *et al.* (2016), who found that the combined application of PGRs and micronutrients significantly increased yield and fruit quality in mango and olive trees.

The experiments undertaken for this thesis were subject to a number of limitations. These include the investigation of pollen storage with only one experimental genotype (Chapter 3), an unbalanced number of breeding families and disparities in their age categories at either research station (Chapter 4), and poorly managed experimental trees (Chapters 5 and 6). Different measures can be recommended for future research to test the results presented here. To investigate the findings of the pollen storage experiments, further research should include several promising organic solvents and storage periods of less than one week, as well as evaluate different mango genotypes. The use of a well-structured breeding population on multiple sites would improve the breeding value data and is important for calculating Genotype × Environment interaction for improved site-specific recommendations. In terms of fruit set and retention, well-managed experimental trees may respond more favourably to foliar-applied PGRs and micronutrients. To determine the most influential means of using foliar-applied PGRs and micronutrients, multiple application periods and frequencies as well as multiple genotypes, experimental locations, and PGR/micronutrient types should be included in further experiments. Performing comparable research across seasons is important in horticulture to account for seasonal variations in climate and plant phenology. The studies described in Chapters 3, 5, and 6 could be extended by further experimental replication across seasons in future years; however, such an endeavour was not possible during the limited time period of a PhD.

The research work in this thesis has demonstrated the significant potential in using the tested approaches, aside from foliar-applied micronutrients, to improve the efficiency of the classical

hand pollination technique in mango. The parents selected on the basis of breeding values estimated in Chapter 4 can be crossed using pollen stored and retrieved following the methodology optimised in Chapter 3. The fruit set and retention of these crosses can be improved by applying effective treatments described in Chapters 5 and 6 to ultimately increase hybrid number. The findings described in this thesis can be used to improve the efficiency of classical mango breeding as well as commercial mango production.

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