Final report

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Public summary

The Molecular Physiology Toolbox of the National Tree Genomics Program (AS17000) used applied molecular biology, genetics and genomics tools to understand the molecular mechanisms regulating key productivity traits in tree crops. This project collaborated with tree crop physiologists in the National Tree Crop Intensification Program (AS18000) to advance understanding of yield traits in horticultural trees, including determinate and indeterminate flowering in avocado, precocity in macadamia, fruit set and retention in mango and avocado, and the use of dwarfing rootstocks in macadamia.

Determinacy in avocado is associated with a significant crop yield advantage due to increased fruit set and retention. This project applied established girdling techniques to assess whether girdling may enhance determinacy and increase fruit retention in avocado. Trials resulted in a 59% (terminal) and 61% (axillary) increase in determinate inflorescences, and a 19% increase in fruit retention. Further trials are required to confirm these results before a recommendation can be made. Previous results have also demonstrated Paclobutrazol may be used to suppress levels of the plant hormone gibberellin in shoots to reduce shoot vigour and potentially increase determinacy. Results from glasshouse trials showed promising effects, while field results were inconsistent due to poor flowering in the year tested. RNA-seq and gene expression analysis is being completed to identify genes associated with determinacy in avocado.

A significant challenge faced by growers is the lag time between orchard establishment and the time when the orchard becomes cash flow positive. In collaboration with the National Macadamia Breeding Program, selected precocious and non-precocious genotypes were investigated for genetic differences (genes and gene expression) that could be associated with precocity. Candidate genes were identified with differential expression in precocious varieties that could form the basis of future work to identify the mechanism of precocity in macadamia.

In macadamia, the development of dwarfing rootstocks is a key objective of AS18000. This project aimed to identify potential mechanisms through which the rootstock alters scion vigour, focussing on aquaporin proteins which facilitate the movement of water through plant cells. Research by PhD student Pragya Poudel successfully identified the aquaporin gene family in macadamia and through phenotypic and gene expression analysis identified a significant correlation with aquaporin activity and tree vigour.

Fruit set and retention in crops, such as mango and avocado, is regulated by phytohormones. This project developed an improved phytohormone extraction and quantification method to accurately detect and quantify hormones in tree crops, in particular gibberellins, which are present in trace amounts. In collaboration with Dr Harley Smith, we identified changes in phytohormones associated with fruit abscission in avocado. We also investigated the effect of plant growth regulator 1-Naphthaleneacetic acid (NAA) on fruit retention in mango. This research was continued and expanded on in MG21004 investigating the use of plant growth regulators to improve fruit retention in mango.

This project has highlighted the significant benefits in developing collaborative projects between molecular research laboratories and tree crop physiologists to identify the molecular pathways regulating traits of value to the horticultural industry.

Keywords

Macadamia, avocado, mango, fruit set, fruit retention, fruit abscission, determinant inflorescence, flowering, precocity, dwarfing rootstocks.

Introduction

Australia's fruit and nut industry is a highly valuable industry within Australia's agricultural sector and continues to increase in value as the industry maintains a high domestic and international reputation as a producer of premium quality food. Although currently profitable, continual improvement in orchard management and varieties is integral for maintaining profitability in a competitive global market. The Molecular Physiology Toolbox of the National Tree Genomics Program (AS17000) uses applied molecular biology, genetics and genomics tools to understand the molecular mechanisms regulating key productivity traits in tree crops. This project focused on three valuable tree crops within the industry, avocado, mango and macadamia working alongside the AS18000 program – National Tree Crop Intensification in Horticulture - and other Toolboxes within AS17000. The objective of this research was to improve understanding of major traits of interest, including, vigour management, flowering and crop load. High-quality genomes and transcriptomes now available for avocado, mango and macadamia have enabled research into analysis of gene activity associated with traits of interest. A deeper understanding of the relationship between phenotype and the underlying genetic mechanisms has the potential to offer tools and opportunities to rapidly and more efficiently address current and future needs of the Australian horticulture industry.

KEY RESEARCH AREAS

To ensure that this project was targeted to research questions with a genuine potential for impact and for synergies with AS18000, a literature review and consultation process with growers and research leaders from AS18000 for mango, avocado and macadamia, was undertaken. A design thinking workshop run by AS18000 was attended over two days including interviews with Australian growers and industry representatives to identify industry priorities. Four key research areas have been investigated in this project: determinate and indeterminate flowering in avocado; precocity in macadamia; fruit set and retention in mango and avocado; and the use of dwarfing rootstocks in macadamia. The justification and aims for researching these topics are detailed below.

Determinate and indeterminate flowering in avocado

In avocado, data suggests there is a significant crop yield advantage associated with having an increased percentage of determinate flowers. In a determinate inflorescence the tip of the inflorescence ends in a flower, therefore terminating shoot growth. An indeterminate inflorescence will terminate with a vegetative bud thus allowing the continuing of vegetative growth (Arpaia et al. 2020). Reports have shown that avocado plants generally produced a much higher percentage of indeterminate vs determinate inflorescences (approx. 90% indeterminate). However, determinate inflorescences have at least three times higher percentage of fruit set per flower than indeterminate inflorescences (Salazar-Garcia & Lovatt 1998). A potential reason for this is determinate growth is proposed to reduce competition with the developing vegetative apex and allow more reserves for fruit set. However, it is important to maintain an optimal balance between vegetative reproductive resource allocation, as a reasonable level of indeterminate shoots are required to maintain plant vigour, while a higher level of determinate flowering can contribute to alternate bearing.

Phytohormones potentially play a role in regulating determinate and indeterminate flowering. Gibberellin (GA) has been shown to inhibit flowering potential through the regulation of FLOWERING LOCUS T, a phloem mobile protein that initiates flowering, and the floral suppressor gene *TERMINAL FLOWER 1* (*TFL1*) (Nakagawa et al. 2012). In apple, GA application led to increased *TFL1* expression (Zhang et al. 2019). In avocado, GA3 application was shown to increase the number of indeterminate inflorescences and decrease the number of determinate inflorescences (Salazar-Garcia and Lovatt 1998). GA may also inhibit activity of cytokinins (CK), which promote floral initiation.

This study aimed to produce methods to increase determinate inflorescence production in avocado and identify what genes regulate the production of determinate inflorescences. This also involved studying the effects of girdling and inhibiting GA biosynthesis on floral development and gene expression. The work was undertaken in collaboration with Carola Parfitt and the research team at the DPI Bundaberg Research Station.

Precocity in macadamia

A significant challenge faced by growers is the lag time between orchard establishment and the time when the orchard becomes cash flow positive. Obtaining macadamia varieties with reduced time from planting to first harvest is of high importance to the industry. While knowledge is increasing around the genes involved in flowering in macadamia, little is known about the signals that lead to earlier flowering in one variety compared to another.

The transition of the meristem from the vegetative phase to the reproductive phase signals the beginning of the flowering process. Precocity or early transitioning from the juvenile to adult phase is typically regulated by the age flowering pathway, in particular, the activity of two microRNAs: miR156 and miR172. Across diverse species, miR156 is abundant in young development stages but gradually decreases as the plant matures, while the opposite pattern is exhibited by miR172 (Fornara and Coupland 2009). Figure 1 demonstrates the genetic pathway associated with the transition from the juvenile phase, leading to promotion of the floral initiation genes *FT* and *SOC1* and the initiation of flowering.

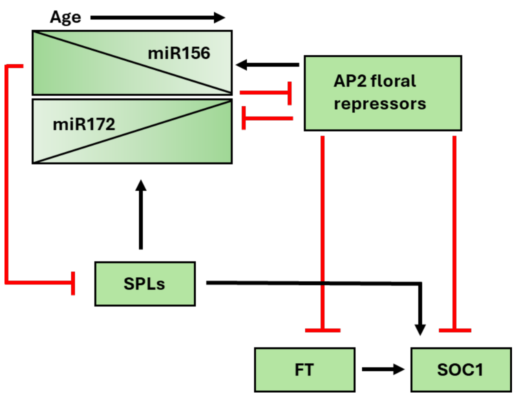

Figure 1: The interaction between genes and miRNA in the age-related flowering pathway in plants leading to floral initiation. The activity of *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*), *APETALA 2* (*AP2*) and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) is outlined with the black arrow indicating promotion of gene activity while the red indicating repression of gene activity. Modified from Leijten et al. (2018)

Very little is known about the conservation of this pathway in macadamia. Building on initial research undertaken in macadamia by Ahsan *et al.* 2019a,b, this project aimed to identify key genes associated with precocity by comparing gene activity during early tree development between precocious and non-precocious varieties developed by the National Macadamia Breeding Program.

Fruit set and retention in mango and avocado

Poor fruit set and poor fruit retention reduce potential yield. The amount of fruit carried to harvest by a mango tree may be limited by hormonal cues and the availability of carbohydrate resources among other factors (Capelli et al., 2021). Fruit drop is triggered by hormones with increased ethylene concentration leading to the development of an abscission zone and to fruit drop (Xie et al. 2013). Enhanced IAA flux in the fruit can counter this through causing decreased ethylene-sensitivity (Xie et al. 2013). Additionally, and most likely linked to this ethylene mechanism, there may be an upper yield limit associated with the total carbohydrate production and storage (Capelli et al., 2021).

The use of plant growth regulators, including the synthetic auxin 1-Naphthaleneacetic acid (NAA), to reduce fruit drop, increase fruit size and increase yield is well established in many crops. This has been tested with variable success in tree crops but limited studies have been undertaken with mango under Australian conditions. This experiment aimed to study the effects of NAA on fruit retention, hormone levels and starch levels in mango. This work was undertaken in collaboration with Dr Ryan Orr and the research team at the DPI Mareeba Research Station.

The molecular processes involved in fruit set and retention remain unclear in mango, macadamia and avocado. Studies in other species have shown the process of fruit abscission is likely regulated by cross-talk between IAA, ethylene, ABA, CK and GA pathways, particular in response to carbohydrate availability. In collaboration with Dr Harley Smith's group at CSIRO this project studied the change in phytohormone activity in avocado in response to carbohydrate limitation. A better understanding of the molecular signals regulating fruit abscission in avocado and mango will help inform decisions on tree management to maximise fruit retention.

Dwarfing rootstocks in macadamia

The management of excessive vigour through pruning and hedging is one of the major expenses of nut production in commercial orchards. As a newly domesticated crop, there are no vigour and size managing rootstocks available in macadamia. Queensland's Department of Primary Industries (DPI) and The University of Queensland (UQ) took initiatives to control scion vigour by using rootstocks through two consecutive Horticulture Innovation (HI) Australia funded projects: "Transforming subtropical/tropical tree crop productivity" (AI 13004) and National Tree Crop Intensification in Horticulture Program (AS18000).

Along with genetic and phenotypic studies being undertaken in AS18000, this project aims to identify potential mechanisms through which the rootstock alters scion vigour. There is evidence of an association between the vascular anatomy of rootstocks and scion vigour (Clearwater et al. 2004, Olmstead et al., 2006, Gonçalves et al., 2007, Toft et al. 2019). Exploring the molecular basis of vigour control, some studies have highlighted the role of aquaporins (AQPs) in plant vigour. AQPs are proteins that are present in the intracellular and plasma membranes, facilitating the movement of water through plant cells (Maurel, 2007). In particular, PLASMA INTRINSIC PROTEINS (PIPs), a sub-class of AQP family, are found to be associated with rootstock mediated vigour control in other horticultural species (Gambetta et al., 2012; Lovisolo et al., 2007). This project aims to identify AQP genes in macadamia using available genome sequences and investigate the role of AQPs in rootstock-mediated vigour control in macadamia. The research was undertaken by PhD student Pragya Poudel as part of her PhD research at UQ.

Methodology

Determinate and indeterminate flowering in avocado

Branch girdling trials in avocado

To identify the molecular signals regulating determinate and indeterminate floral production it is essential to be able to reliably predict whether an inflorescence will be determinate or indeterminate. 'Hass' on 'Ashdot' consistently produces a higher percentage of determinate inflorescences than 'Hass' on 'Velvick'. Six 'Hass' trees on 'Ashdot' rootstock were selected from the DPI Bundaberg Research Station density trials. Four phenotypically similar branches per tree were then selected and two of the four selected branches were girdled per tree in April 2021, predicted to be prior to floral initiation. Samples were harvested from one girdled and one ungirdled branch per tree. The other two branches were used for phenotyping to determine the percentage of determinate and indeterminate inflorescences and fruit retention. Samples were collected once a month in the morning, commencing on the day of girdling. Monthly sampling continued until July and the emergence of the inflorescence. The terminal bud and the three closest axillary buds, leaf and small section of stem closest to the terminal bud were collected from three growth units per branch. Surface roots were also harvested for each tree. Samples were immediately stored on dry ice and transported to UQ for processing. Branches were phenotyped at flowering to determine the percentage of determinate inflorescences and again at fruit maturation to record the number of fruit retained on the tree..

Paclobutrazol trials

Forty avocado trees of Hass scions on Zutano rootstocks were purchased from Anderson Horticulture Pty Ltd and re-potted in 18L pots in 1:1 mix perlite:UQ23 soil with pine bark mulch and placed in a UQ Glasshouse with evaporative cooling in August 2021. The majority of plants were already flowering or close to flowering when received from the nursery. Due to facility damage from the 2022 floods, the plants were moved to outdoor growth space. In August 2022, and at the time of floral initiation in March 2023, paclobutrazol (AuStar), a GA biosynthesis inhibitor, was applied as a root drench at a dilution rate of 0.7% (7L/1000L). Leaf and bud samples were collected monthly from 4 treated and 4 untreated trees from March to July at three hours after sunrise and at dusk when key genes involved in floral initiation and development are predicted to be highly expressed. At flowering the percentage of determinate to indeterminate inflorescences was recorded comparing treated and untreated trees.

Field trials were conducted by the AS18000 team at the DPI Bundaberg Research Station assessing the effects of paclobutrazol on vegetative growth. Paclobutrazol (AuStar) was applied in August at 9.6 mL/tree in 500 mL water as a root drench and samples were collected during floral development in June and July the following year. By August the flowers were between Stage 6 (rounded bud) and Stage 9 (elongation of tertiary axes and vegetative bud at the inflorescence apex is visible) and therefore no further samples were collected. At flowering, the percentage of determinate to indeterminate inflorescences was recorded comparing treated and untreated trees.

Precocity in Macadamia

In collaboration with the National Macadamia Breeding Program, a small trial of 24 trees including four precocious genotypes (three trees each) and four non-precocious genotypes (three trees each) were planted in the field at the Maroochy Research Station in August 2021. As trees were very young, two newly emerged leaves were sampled from the outer top canopy every two months from May 2022 until July 2023 and flowering dates recorded. Six precocious trees from three genotypes and six non-precocious trees from three genotypes were selected for gene expression analysis based on genotype and flowering date.

Fruit set and retention in mango and avocado

Mango NAA trials

Experiments were performed in a mature low density planting mango orchard located at the Walkamin Research Station, Walkamin, Australia (17.1341° S, 145.4271° E). The following two foliar treatments were applied in a randomised block design on 9-year-old Calypso cultivar trees: (*i*) untreated control; (*ii*) 50 mg.L^{-1} NAA (Gro-chem NAA 20™), with ten replicates each. Treatments were applied as a foliar spray until glistening when fruits were approximately pea-sized (late September 2021). Control and NAA treatments included 1 mg.L^{-1} Kendral 600™, a non-ionic surfactant. The volume of solution applied to each tree was measured between replicates, and a large tarpaulin was used to separate adjacent trees to prevent treatment cross-contamination due to spray drift. Netting was placed under five control and five treated trees to collect abscised fruit. Dropped fruit was collected and counted weekly to determine the rate of fruit abscission. In November, four fruit from one panicle, the closest leaf and pedicels were sampled per tree for five treated and five control trees. Samples were immediately stored on dry ice and transported to UQ for processing.

Avocado fruit abscission

Persisting and arresting fruitlets were harvested from 'Hass' avocado trees during summer fruit abscission. Subsequently, the seed coat, embryo, pericarp and pedicel were isolated and frozen on dry ice before storing samples at -80^{0}C. All samples were ground in the Retsch Cryomill at -196C and sent to UQ for hormone quantification. Phytohormones were extracted using the method developed by Cao et al. 2020. Detection of phytohormone internal standards was tested to determine if significant matrix effects were present for avocado tissue due to fruit tissues containing high lipid concentrations and if current concentrations for internal standards of 1ng were comparable to endogenous levels. The following phytohormones were quantified in the four different tissue types in control and arresting fruits; *trans*-zeatin; tZR, *trans*-zeatin riboside; tZMP, trans-zeatin riboside-50 -monophosphate; DZ, dihydrozeatin;; DZR, dihydrozeatin riboside; iPAMP, isopentenyladenosine-50 -Monophosphate; iP, isopentenyladenine; iPA, isopentenyladenosine; ABA, abscisic acid; IAA, indole-3-acetic acid.

Dwarfing rootstocks in Macadamia

The experiment was conducted in 2022, with a subset of 7 rootstock accessions selected from a larger pool of 24 accessions established in 2017 at the Maroochy Research Facility, QLD, Australia (Poudel et al., 2024; Topp, 2019). In 2017, the grafted trees were planted in a randomized incomplete block design with two-plant plots, each comprising a grafted and an ungrafted tree from the same genetic background for comparative analysis. Trees were spaced 4 m between rows and 2 m within rows. In 2022, tree vigour was assessed based on 4 traits: total height (HT), trunk circumference (TC), canopy area (CA) and canopy volume (CV) as part of AS18000. Rootstocks were categorized as dwarfing or vigorous based on their phenotypic traits (Poudel et al., 2024). In February 2022, during the onset of the summer flush, newly emerged leaf and stem tissues were collected from a single branch located in the northern-facing crown within the lower half of the canopy for both grafted and ungrafted trees. Sampling took place within a 2.5-hour period, starting 3.5 hours after sunrise, to minimize potential transcriptional variation due to diurnal effects, as observed in other crops (Henzler et al., 1999; Khan et al., 2019). Upon collection, all the tissues were immediately placed in dry ice and stored at -80°C. Bioinformatic analysis was undertaken to identify all AQP genes in macadamia (Appendix 2). MiPIP genes were selected for gene expression analysis comparing gene activity in scion tissues on dwarfing and vigorous rootstocks.

Disclaimer – Use of Plant Growth Regulators

This report includes the experimental testing of the effect of plant growth regulators on tree physiology. Any commercial in crop use of these chemicals should only be undertaken in line with State and Federal legislation. Please refer to the APVMA website (https://www.apvma.gov.au/) for information on how and when plant growth regulators are permitted for use in specific crops.

Results and discussion

Determinate and indeterminate flowering in avocado

Girdling of tree branches was found to have a significant effect on the percentage of determinate and indeterminate inflorescences in avocado. Branches that were not girdled had on average 90% indeterminate terminal inflorescences and 6% determinate terminal inflorescences. This was consistent with reports which suggests that healthy avocado trees generally have 90% indeterminate inflorescences. The axillary inflorescences showed a lower percentage with an average of 62% being indeterminate and 27% being determinate. On girdled branches, 35% of the terminal inflorescences were indeterminate and 65% were determinate. However, there was significant variation among the girdled trees with the percentage of determinate inflorescences being 95%, 83%, 74%, 57%, 55% and 26%. The first three values (Tree 3, 4 and 7, Figure 2A) showed a higher percentage of determinate inflorescences compared with the ungirdled branches and were selected for further investigation into the molecular differences between determinate and indeterminate inflorescences. The axillary inflorescences also showed a significant change with girdling with 11% of inflorescences being indeterminate and 88% determinate (Figure 2B).

In early November, the number of fruit retained for each tree was recorded for branches selected for phenotyping at start of project (Figure 3A). Consistent with results from previous field trials undertaken in AI13004, terminal determinate inflorescences retained on average 19% more fruit during early fruit development stages (Figure 3B).

Paclobutrazol trials

Field trials conducted by AS18000 at the DPI Bundaberg Research Station looking at the effects of paclobutrazol on vegetative growth observed that application of paclobutrazol (AuStar) by root drench in late August resulted in the majority of flowers being determinate the following year (C. Parfitt, personal communication). Therefore, avocado trees in the glasshouse and field were treated with paclobutrazol (AuStar) and samples collected during floral initiation and floral development to look at the changes in phytohormones and gene expression associated with inhibited GA biosynthesis.

Field trials: Contrary to the previous year, minimal flowering in 2022 occurred across the avocado trees analysed, and among the flowers present no significant differences in determinacy were observed between treated and untreated trees (Figure 4a).

Glasshouse trials: Results have shown that GA inhibits flowering. Therefore, as expected paclobutrazol significantly increased the number of flowers per plant by 82% for plants growing in pots. On average for plants treated with paclobutrazol, 71% of the inflorescences were indeterminate and 29% of the inflorescences were determinate. For untreated control plants 82% of the inflorescences were indeterminate and 18% were determinate (Figure 4b). Therefore, paclobutrazol treatment significantly increased the percentage of determinate inflorescences.

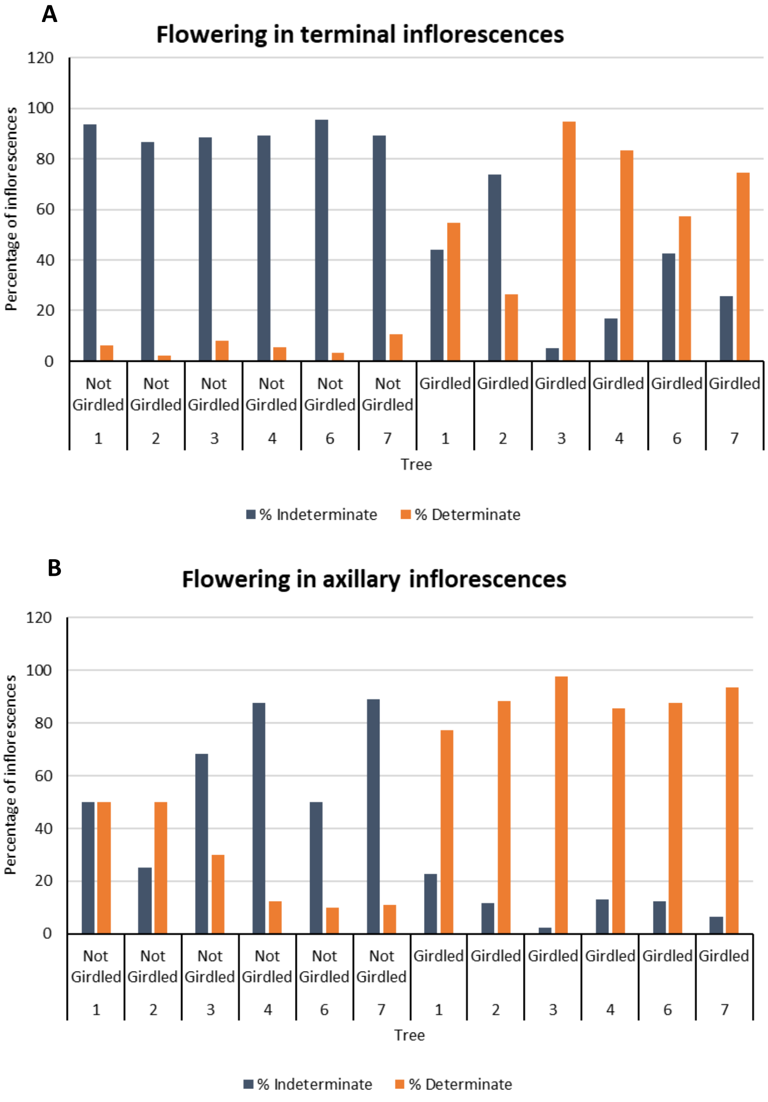

Figure 2: Percentage of indeterminate and determinate terminal inflorescences (A) and axillary inflorescences (B) per tree for girdled and not girdled branches in avocado.

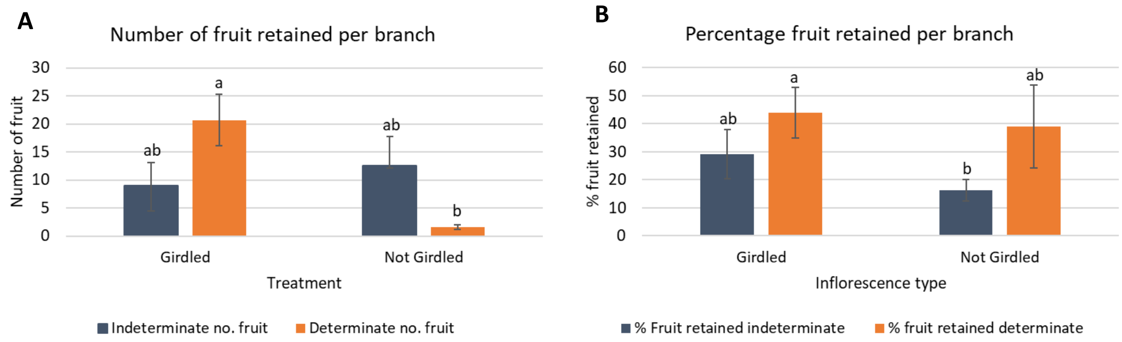

Figure 3: Number of fruit retained (A) and percentage of fruit retained (B) for indeterminate and determinate axillary inflorescences for girdled and ungirdled branches in avocado. Means and standard error are from 6 trees. Statistical significance between treatments and inflorescence type was calculated with the student's T-Test with a significance threshold set at a p-value of <0.05.

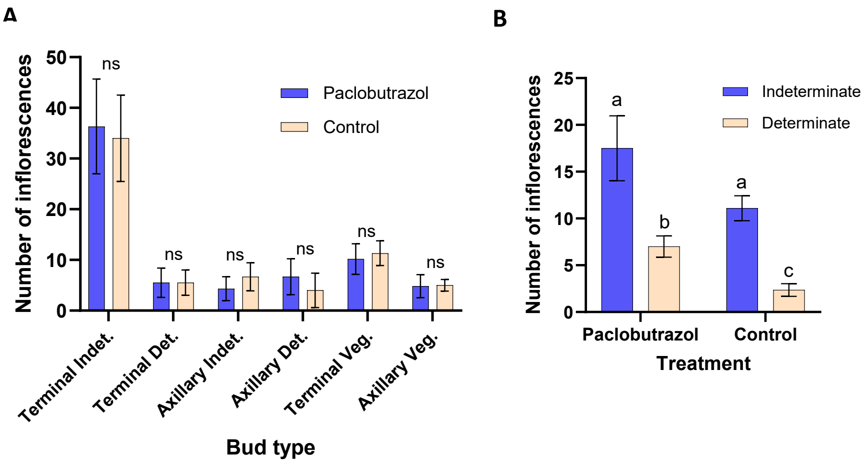

Figure 4: (A) Flower counts in paclobutrazol treated and untreated avocado trees from the field trial. No differences were observed for terminal and axillary inflorescences comparing mean ± SEM (n = 6) (B) Comparison of mean ± SEM of indeterminate and determinate inflorescences between paclobutrazol treated (n=8) and control avocado (n=11) plants in pots in glasshouse trials. Statistical significance was calculated with the student's T-Test with a significance threshold set at a p-value of <0.05.

From this study girdling is identified as an effective method to increase determinate inflorescences and number of fruit retained. Paclobutrazol was also identified to increase flowering and the percentage of determinate inflorescences. However, results were inconsistent in the field and requires further trials. RNA-seq analysis was undertaken to identify candidate genes potentially involved in the differentiation of determinate and indeterminate inflorescence production and will be reported in a subsequent peer-reviewed publication. The activity of candidate genes identified from the girdling trials will be confirmed in samples from the paclobutrazol glasshouse trials.

Fruit set and retention in mango and avocado

The foliar application of NAA at the pea-sized fruiting stage had no significant effect on final fruit retention in cv. Calypso, with 12.69% of fruits retained on untreated control trees and 18.82% of fruits retained on NAA treated trees (Table 1). While not significantly different, NAA treated trees produced fewer fruits per tree, with an average of 188.90 fruits per tree compared to control's 210 fruits and consequently resulted in reduced yield (77.29 kg/tree). This indicates that the NAA was likely taken up by the plant. Due to no significant differences in phenotype, further molecular analysis was not undertaken. This research was expanded upon in MG21004 – Investigating the control of fruit drop in mango to support innovative solutions for Australian growers. While no significant differences were observed with NAA alone, studies in MG21004 have identified promising results with additional PGRs (Appendix 4 & 5).

Table 1: Fruiting attributes of untreated control and NAA treated cv. Calypso mangoes at commercial maturity.

Parameter	Treatment	Final value	Significance
Pre-treatment panicle count	Control	271.20 ± 40.02	
	NAA	216.50 ± 42.43	ns (p = 0.360)
Initial number of fruits per tree	Control	2128.01 ± 280.77	
	NAA	1610.64 ± 335.64	ns (p = 0.252)
Fruit retention (%)	Control	12.69 ± 2.45	
	NAA	18.82 ± 6.02	ns (p = 0.358)
Yield (kg of fruit per tree)	Control	86.46 ± 6.42	
	NAA	77.29 ± 8.48	ns (p = 0.399)
Fruits per tree	Control	210.00 ± 20.21	
	NAA	188.90 ± 25.71	ns (p = 0.526)
Fruits per inflorescence	Control	1.01 ± 0.22	
	NAA	1.19 ± 0.30	ns (p = 0.629)
Average fruit weight (kg)	Control	0.422 ± 0.01	
	NAA	0.429 ± 0.02	ns (p = 0.741)
Individual fruit weight (g)	Control	418.88 ± 12.93	
	NAA	420.83 ± 13.85	ns (p = 0.919)

Values represent the mean ± SEM (n = 10). A student's *t*-test was used to determine statistical significance between control and NAA treated trees, however no significant differences were observed between treatments (ns = not significant, $p > 0.05$).

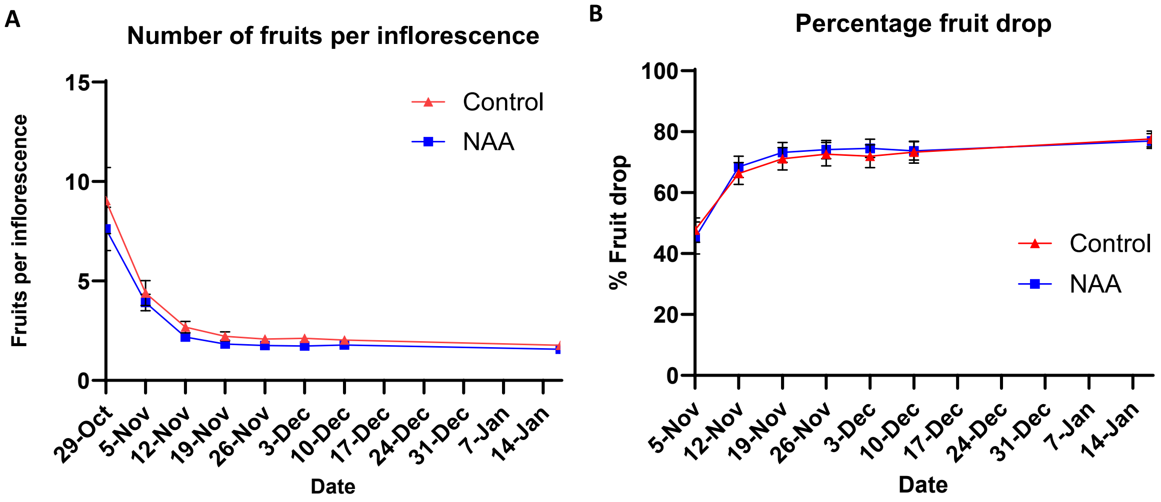

Figure 5: (A) Mean ± SEM (n = 10) number of fruits maintained per panicle for control vs NAA treated plants over the fruit development period. (B) Mean ± SEM (n = 10) percentage of fruit drop throughout fruit development for control vs NAA treated plants.

Role of phytohormones in avocado fruit abscission

Avocado production is limited by poor fruit set and a high rate of fruit abscission during the growing phase of fruit development. Results from AV16005 indicate that immature fruit abscission is initiated by a decrease in carbohydrate availability, which causes fruits to undergo growth arrest before abscising from the tree (AV16005 Final Report)(Appendix 1). To gain insight into the hormonal control of growth arrest and fruit abscission, a collaboration was formed with AV16005 to quantify auxin (IAA), abscisic acid (ABA) and cytokinins (CKs) in persisting and arresting fruit tissues. Consistent with a role for auxin in promoting fruit growth, IAA was detected in the seed coat, embryo and pericarp (Figure 6E). In addition, the seed coat contained the highest levels of IAA compared to the embryo and pericarp, indicating that this maternal organ influences fruit growth and development. In arresting fruitlets, the levels of IAA were substantially reduced in the seed coat and pericarp (Figure 6E). In contrast, the levels of IAA in the arresting embryo were similar to that in persisting fruits (Figure 6E). Therefore, we speculate that fruit growth arrest and abscission is mediated in part by a decline in IAA levels in the maternal organs of the fruit.

Experimental results indicate that ABA regulates the maturation phase of fruit and seed development. In addition, in climacteric fruit, ABA is suggested to activate ethylene production to promote ripening after the fruit undergoes maturation. In persisting avocado fruitlets, ABA is detected in the embryo, seed coat, pericarp and pedicel (Figure 6D). In arresting fruitlets, a substantial increase in ABA levels occurred in the pericarp and seed coat (Figure 6D). In addition, ABA levels also increased in the pedicel (Figure 6D). The overall increase in ABA in the seed coat and pericarp indicates that this hormone may be acting to promote fruit growth arrest and abscission, when the levels of auxin decline in these maternal organs.

While cytokinin is also associated with fruit growth and development, the overall low levels isopentenyladenosine-5’-Monophosphate, isopentenyladenosine and isopentenyladenine suggest that these cytokinin metabolites may not be involved in late fruit growth and development. However, we present the data for *trans*-zeatin, *trans*-zeatin riboside (TZR) and dihydrozeatin (DZ) (Figure 6A-C). In arresting fruitlets, the levels of *trans*-zeatin decreased in the seed coat and embryo (Figure 6A), however, differences were not statistically significant. In contrast, there was an increase in TZR in the seed coat of arresting fruitlets (Figure 6B). In normal growing persisting and arresting fruitlets, the levels of DZ were similar in all tissues except the pericarp where DZ was present at extremely low levels and not present in arresting fruits (Figure 6C). Taken together these results suggest that cytokinin metabolites may play a role in growth arrest in abscission.

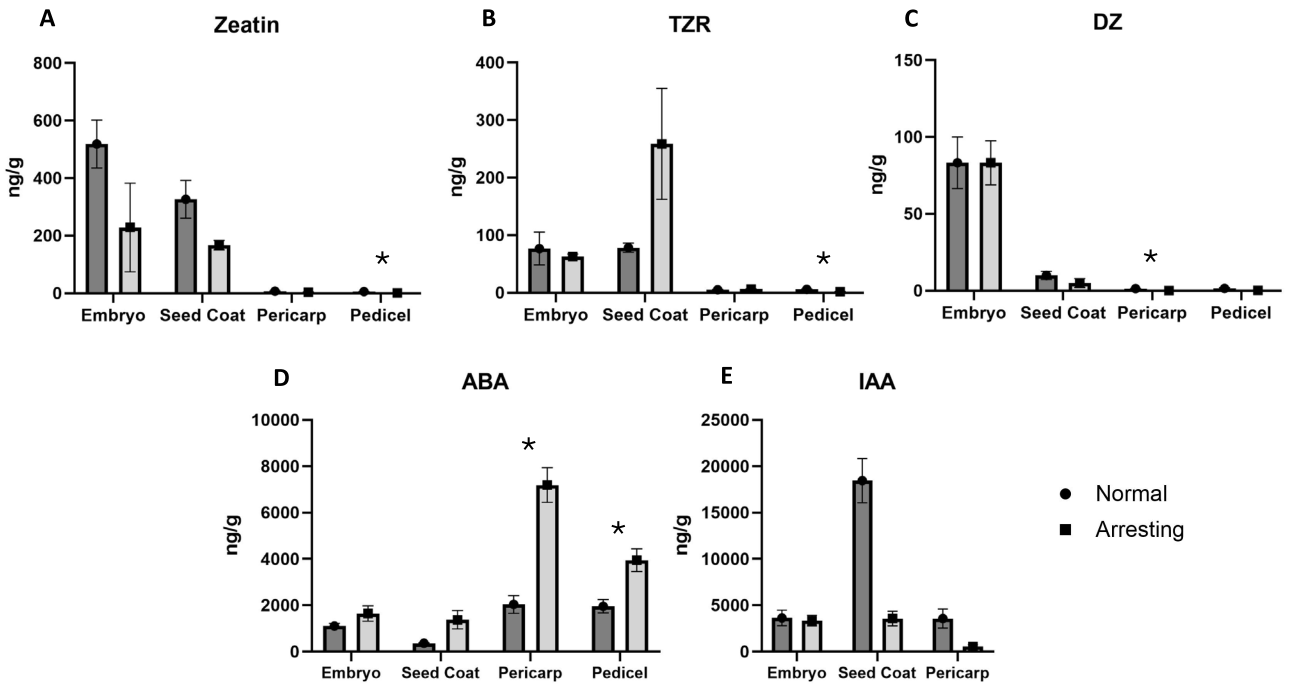

Figure 6: Quantification of hormones in arresting (light grey) avocado fruits compared to normal growth persisting fruits (dark grey) in late fruit growth. Data are presented as mean ± SEM, *n* = 3 in embryo, seed coat, pericarp and pedicel fruit tissues. Phytohormones quantified include (A) *trans*-zeatin, (B) *t*ZR, *trans*-zeatin riboside, (C) dihydrozeatin (DZ), (D) abscisic acid (ABA), (E) indole-3-acetic acid (IAA)

Dwarfing rootstocks in Macadamia

A total of 37 putative AQP genes were identified in the genome of the *M. integrifolia* cultivar 'HAES 741' (Appendix 2). Phylogenetic analysis classified the *MiAQPs* into five distinct subfamilies, namely, PIPs, TIPs, NIPs, SIPs and XIPs, with 11, 10, 10, 2 and 4 members in each subfamily, respectively. The nomenclature of individual genes was based on phylogenetic relationships and sequence similarity to AQPs in other plant species.

The expression of *MiPIP* genes was compared between scions grafted onto dwarfing and vigorous rootstocks. In the grafted trees, all but two of the *MiPIP* family members presented significantly higher expression in the leaf tissues of the 'HAES 741' scion grafted onto the dwarfing rootstocks than in those grafted onto vigorous rootstocks (Figure 7). A heatmap showing the relative expression levels of *MiPIP* genes in the leaf tissues in dwarfing and vigorous rootstock accessions was generated using TBtools (Chen et al., 2020) (Figure 7). Among these rootstocks, the leaf tissues of 'A268S', one of the dwarfing rootstocks, presented the highest expression levels for most *MiPIP* genes, except *MiPIP2-5a,* which had higher expression in the vigorous rootstocks. A similar comparison in ungrafted rootstock varieties revealed greater expression of 7 out of 11 *MiPIP* genes in the leaf tissues of dwarfing rootstocks compared vigorous rootstocks. The expression levels of *MiPIP* family members in the leaf tissues of grafted trees were significantly negatively correlated with 4 vigour traits (height, trunk circumference, canopy area and canopy volume) for all PIP genes (r_p = -0.63 to -0.87, $p < 0.05$), except for *MiPIP1-4b*, whose expression did not correlate significantly ($p > 0.05$) with any of the vigour traits. Notably, *MiPIP2-5a*, exhibited a significant positive correlation (r_p = 0.69 to 0.78, $p < 0.05$) with all vigour traits (Figure 7).

Genes	Height	Trunk circumference	Canopy Area	Canopy Volume
MiPIP1-1a	-0.68*	-0.70*	-0.72*	-0.73*
MiPIP1-1b	-0.79**	-0.65*	-0.50^{ns}	-0.54^{ns}
MiPIP1-4a	-0.78**	-0.70*	-0.80**	-0.83**
MiPIP1-4b	-0.53^{ns}	-0.37^{ns}	-0.48^{ns}	-0.51^{ns}
MiPIP2-1a	-0.70*	-0.65*	-0.63*	-0.64*
MiPIP2-1b	-0.82**	-0.85**	-0.79**	-0.77**
MiPIP2-1c	-0.81**	-0.79**	-0.76*	-0.75*
MiPIP2-2	-0.81**	-0.82**	-0.77**	-0.76*
MiPIP2-5a	0.69*	0.75*	0.78*	0.77*
MiPIP2-5b	-0.87**	-0.87***	-0.78**	-0.79**
MiPIP2-8	-0.76*	-0.65*	-0.63*	-0.65*

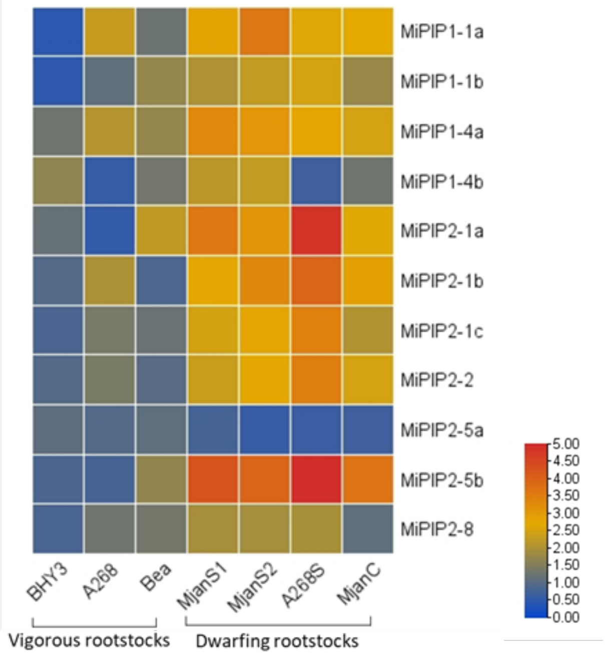

Figure 7. Phenotypic correlations of scion vigour traits with the relative expression of MiPIP genes in the leaf tissues of grafted trees for height, trunk circumference, canopy area and canopy volume. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, ^{ns} Not significant. Relative expression of *MiPIP* genes in the leaf tissues of the 'HAES 741' scion grafted on 7 rootstock genotypes. The log-transformed relative expression values normalized to those of ACTIN were used for heatmap construction in TBTools (Chen et al., 2020). Significantly higher expression (orange colour) of *MiPIP* genes occurred in the scions on dwarfing rootstocks compared to lower expression in the vigorous rootstocks (blue colour).

Dwarfing rootstocks are often characterised by reduced root biomass, fewer and smaller xylem vessels, and lower overall water transport capacity (An et al., 2017; Gonçalves et al., 2007; Olmstead et al., 2006). The higher expression of PIP genes in dwarfing rootstocks suggests a compensatory mechanism that maintains water transport efficiency despite reduced hydraulic capacity. The upregulation of AQP genes under water stress in other woody crops supports this hypothesis (Perez-Martin et al., 2014; Yamada et al., 1997, Lovisolo et al., 2007; Shabalala et al., 2022). Higher *MiPIP* expression in dwarfing rootstocks likely reflects a role in hydraulic regulation and consequent effects on shoot vigour in macadamia.

Method development

Phytohormone quantification

Phytohormones play an essential role in regulating all aspects of tree development. Therefore, it is essential to have a method to accurately quantify phytohormones in tree crops. Matrix effects are the changes observed in the detection or quantification of an analyte when other substances are present in the sample and can be substantial in tree crops due to the high sugar and lipid content. A new method for phytohormone quantification was developed to improve the sensitivity of measuring these compounds and will be disseminated to the research community more broadly via a peer-reviewed publication that is currently in preparation and is expected to be published in 2026. The ultra trace levels of gibberellins makes quantification difficult. This optimised method can significantly improve the detection of phytohormones across the four major classes, cytokinins, indole-3-acetic acid (IAA), abscisic acid (ABA) and gibberellins (GA1, GA3, GA4, GA20 and GA29) (Figure 8).

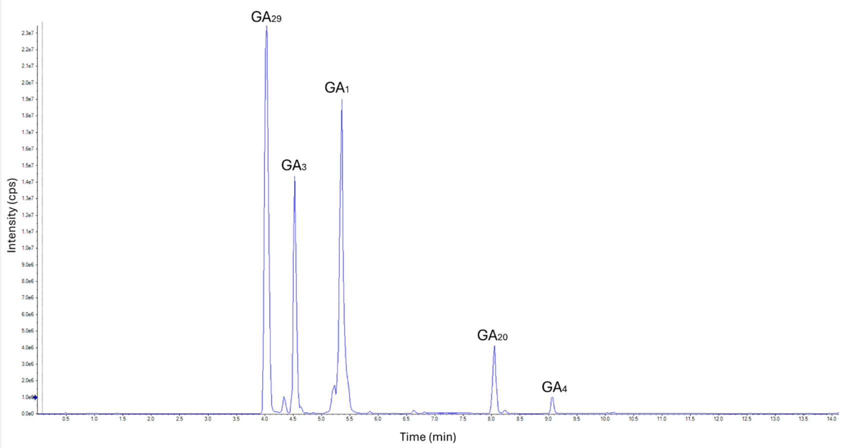

Figure 8: LC-MS chromatogram demonstrating the separation and detection of the five deuterated gibberellin internal standards, GA1, GA3, GA4, GA20 and GA29 after derivatisation.

Outputs

Table 2. Output summary

Output	Description	Detail
Articles for industry	Articles published in Mango Matters, Talking Avocados and AMS News Bulletin	Managing early fruit drop in mango (Mango Matters, 2023) (Appendix 4); How research is looking for solutions (Mango Matters, 2024) (Appendix 5). Developing a physiological framework for managing summer fruit abscission in avocado (Talking Avocados 2022) (Appendix 1) Genetic insights into rootstocks for vigour control. (AMS News Bulletin 2024) (Appendix 3)
Participation at events with industry	Conference participation including presentations	Research presented at international scientific conferences in Cairns, Perth and Hawaii (IPMB, PAG, ASHS) as well as industry events including Australian Mangoes Scientific symposium and AusMac 2024.
Publications for academia	Peer-reviewed journal articles	Six papers published in peer reviewed journals and two manuscripts prepared and submitted for publication. One book chapter published.
New method developed for plant hormone extraction and UPLC-MS/MS analysis, including for gibberellins	New method developed for phytohormone quantification. Method will be used in future research projects, including AS23003, and made available publicly.	Manuscript has been prepared for publication detailing the method and is already reported in PhD milestone documents (Sophie Jones; Supervisor Lindsay Shaw). Method has been utilised in MG21004, AV16005 and to generate results in this report. Extraction method was applied to quantify changes in phytohormones associated with

		premature fruit abscission in avocado.
Methods identified for successful extraction of RNA from avocado buds and macadamia tissues	Spectrum Plant Total RNA kit (Sigma-Aldrich) and modified CTAB extraction method developed by V. Tsai (unpublished) identified to be suitable for freeze dried tissue for all sample types in this project.	Modified CTAB extraction method will be published by V. Tsai and L. Shaw. Manuscript being prepared.
Proof of principle method for automatic field measurement of traits	Part of a short student project - in house programable automation systems were produced to acquire data, i.e., photographs and temperature at reduced cost. This system provides flexibility in terms of customization and capabilities that commercial cameras do not provide.	After testing phase, the first prototype is available for use. The second prototype requires further development and testing. Follow up would depend on the level of detail required for the monitored traits (Appendix 6).
Established experimental field techniques for application of plant growth regulators, timing of girdling and sample collection.	Experimental design will be applied in future research projects and shared in publications.	In-house know-how was developed for how to manage the harvest of tissues (RNA,DNA, hormones) in remote locations. These approaches were shared across the collaborators to ensure sample collections are of high quality and do not lose integrity during transport. Methods were established for timing of girdling and application of plant growth regulators. This capability will extend beyond this project.
Data sets generated for flowering genes in precocious and non-precocious macadamia trees	Data was produced on changes in flowering related gene expression over two seasons comparing precocious and non-precocious genotypes in macadamia.	Flowering genes were identified which are potentially associated with precocity.
Aquaporin genes were identified and data sets generated looking at aquaporin gene activity between vigorous and dwarfing rootstocks.	Candidate aquaporin genes can potentially be utilised as markers for early screening of dwarfing rootstocks; the genes identified could assist researchers in identifying other molecular mechanisms conferring dwarfism.	Aquaporin genes correlated with reduced vigour in macadamia and a potential role in regulating water transport were identified through bioinformatics and phylogenetic analysis and then differential gene expression analysis of grafted and non-grafted plants of vigorous and dwarfing rootstocks. These results have been submitted for publication and have been presented at conferences and industry meetings to growers and other researchers.
Field trials completed demonstrating that girdling affects determinacy in avocado.	Field trials at the DPI Bundaberg research station demonstrated that girdling prior to estimated molecular floral initiation promoted determinacy with a 59% increase in determinate terminal inflorescences and 61% increase in determinate axillary inflorescences. Girdling resulted in a 19% increase in fruit retention.	This method highlights the potential of girdling as an approach to enhance fruit retention. Molecular factors regulating this process are being investigated using time-courses collected from girdled and paclobutrazol treated plants. Results will be published and reported to the research community.

Field trials completed on the effects of plant growth regulators NAA and paclobutrazol on fruit retention and flowering. - No evidence of an effect of NAA (auxin) on fruit retention on mango - Paclobutrazol might affect determinacy in avocado	Foliar application of NAA at pea-sized fruiting stage had no effect on fruit retention, despite indirect evidence that this auxin was taken up by the plant. Further research is needed to determine if paclobutrazol could be effective in affecting determinate/indeterminate inflorescences – no effect was seen in the season tested in the field but promising results were seen in a glasshouse study.	Further trials are being undertaken by AS18000 investigating the effect of paclobutrazol on flowering and yield.
Training researchers for the horticultural industry	This project enabled leadership development for the Research Fellow and development of continuing collaborations between DPI, CSIRO, QUT and WSU.	This project involved collaborations with the DPI Mareeba, Bundaberg and Maroochy Research Stations, CSIRO and QUT. The research fellow, Dr Lindsay Shaw developed collaborations across AS18000 and AS17000 and these collaborations have been extended into two additional funded projects with Hort Innovation, MG21004 and AS23003. Multiple staff and students have been trained in phytohormone quantification and molecular biology developing emerging researchers in horticultural research.

Outcomes

Table 3. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Knowledge: New method developed to quantify plant hormones in tree crops, including for gibberellins, using UPLC-MS/MS. Methods identified for RNA extraction in difficult tissues eg. buds and roots.	Methods established for molecular analysis	Methods developed to undertake phytohormone quantification and gene expression analysis in tree crops.	Availability of protocols and data reported on these molecular traits.
Knowledge: Identification of genes and correlative evidence for their function in precocity and dwarfing in macadamia.	Improved understanding of genetic mechanisms underlying traits of interest.	Identification of candidate genes potentially involved in regulating precocity and vigour can be incorporated as markers for early identification in breeding programs.	Data sets generated for gene expression changes comparing dwarfing and vigorous rootstocks and precocious and non-precocious genotypes. Results presented to industry and research community.
Improved integration of research from lab to field including codesign, sample collection, analysis and interpretation.	Gain a deeper understanding of the relationship between phenotype and the underlying genetic mechanisms associated with productivity traits in avocado, mango and macadamia tree crops.	Experiments were codesigned with AS18000 researchers at Bundaberg, Mareeba and Maroochy research stations, CSIRO and with researchers at UQ, implementing analyses in the lab.	Cohesion and delivery from broad team members from lab to field and regular meetings and reporting across the teams. Ability to associate molecular processes with traits in the field.

Monitoring and evaluation

Key Evaluation Question	Project performance	Continuous improvement opportunities
All questions	All aspects	General comment relevant to improvement and future development: Natural disasters including extreme weather events and Covid-19 have hampered this project across most areas. Moving forward we have developed resilience in terms of online research and communication capacities, improved glasshouse infrastructure and deeper relationships with researchers across the team and with growers.
To what extent has the project increased understanding of the genetic mechanisms associated with traits of importance to the horticulture industry?	Candidate genes were identified, and experiments were completed to associate genes and metabolite and/or hormone changes with management practices or traits of interest. This included genes associated with precocity and rootstock control of scion vigour. Whole expressed genome analysis of determinant and indeterminate flowering (results forthcoming). Field experiments indicated that (i) girdling improves determinacy and yield for avocado and (ii) paclobutrazol potentially improved determinacy and yield in avocado (iii) NAA treatment did not affect fruit retention in mango	This was an ambitious project relative to its budget, this is especially so given the lag time to commencement of the project. This project emphasized the benefits and importance of collaboration between lab and field stations. Projects of longer duration will allow for variation in field phenotypes across seasons e.g. poor flowering years.
To what extent has the project provided information to researchers and industry partners which can be used to offer tools to address industry needs?	Results from this project have been communicated through publication/preparation of 9 journal articles and 4 industry articles. This project has developed methods for phytohormone quantification and identified candidate genes of interest for precocity and dwarfing which may be potential genetic markers for breeding programs.	
Have regular project updates and outcomes been communicated to collaborating project teams, researchers and industry?	Dr Shaw provided monthly updates to AS17000 toolbox leaders and to the Genotype Prediction and Molecular Physiology Toolboxes. Dr Shaw presented at annual forums for AS17000 and AS18000. Dr Shaw also participated in the AS18000 Program Leadership Group (PLG) meeting to report on AS17000 results. The project has been regularly communicated at conferences and over webinars.	Future projects of this type should ensure travel funding is provided to allow for increased attendance at industry events and conferences.

Did the project engage with relevant parties through their preferred learning style?	Yes. Dr Shaw regularly visited field sites throughout the duration of the project to meet with collaborators and design field trials. Dr Shaw also attended annual forums for AS18000 and AS17000 in person to communicate results from the project and the National Tree Genomics Program to ensure communication of results between the programs. Experiments were codesigned for: a) Mango – NAA trials b) Macadamia rootstock trials c) Macadamia precocity trials d) Avocado girdling trials e) Avocado PGR field trials	Regular online and in-person meetings are essential. These were established at an agreed frequency depending on need (usually monthly for this research team). Emphasis is given to enable leadership opportunities for early and mid-career researchers to enable them to develop skills and this should continue.
What efforts did the project make to improve efficiency?	Methods have been established that enable plant hormones and RNA to be extracted and quantified across the full range of tissues required for macadamia, avocado and mango. These methods were developed with accuracy and throughput in mind, enhancing efficiency. Key successful modifications were the Spectrum™ Plant Total RNA Kit – Sigma Aldrich kit and modified CTAB extraction method with freeze dried tissues, and the QuEChERS (UCT) and EDC treatment in plant hormone quantification.	Method improvements focused on quality and efficiency, should continue where appropriate.

Recommendations

The following recommendations followed from the project:

- Girdling and paclobutrazol were identified as promising treatments for enhancing determinacy and fruit retention in avocado – however further field trials are required with different cultivars, seasons and across multiple sequential years.
- Genes were identified potentially associated with precocity or rootstock control of scion vigour in macadamia - further research could enhance knowledge and seek potential benefit of these findings.
- Plant growth regulators provide a promising avenue for on-farm management of fruit retention and research is continuing in MG21004 and AS23003.
- Researchers should use the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich) kit for RNA extractions from buds with freeze dried tissues and the plant hormone extraction and purification methods should incorporate QuEChERS (UCT) and EDC treatment.
- Proof of principle automation of photography and temperature measurements for within trees should be followed up within a future research field study.

Refereed scientific publications

Journal article

Nath O., Fletcher S.J., **Hayward A., Shaw L.M.,** Agarwal R., Furtado A., Henry R.J., **Mitter N**., 2022. A comprehensive high-quality DNA and RNA extraction protocol for a range of cultivars and tissue types of the woody crop avocado. Plants 11(3):242.

Nath O., Fletcher S., **Hayward A.**, **Shaw L.M.**, Masouleh A.K., Furtado A., Henry R. and **Mitter N.,** 2022. A haplotype resolved chromosomal level avocado genome allows analysis of novel avocado genes. Horticulture Research doi.org/10.1093/hr/uhac157

Rupawalla Z., **Shaw L.M.**, Ross I.L., Schmidt S., Hankamer B. and Wolf J., 2022. Germination screen for microalgae-generated plant growth biostimulants. Algal Research 66: 102784.

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Kerr S., Shehnaz S., Paudel L., Manivannan M., **Shaw L.**, Johnson A., Velasquez J., Tanurdzic M., Cazzonelli C., Varkonyi-Gasic E. and Prentis P., 2024. Advancing tree genomics to future proof next generation orchard production. Frontiers in Plant Science 14: 1321555.

Poudel P., Ahmed B., **Shaw L.**, Topp B., Alam M., 2025., Molecular mechanism of rootstock-mediated vigour control in *Macadamia integrifolia*: Investigating the role of aquaporin genes. Submitted for publication.

Jones S., Orr R., Rossouw G., Smith H., **Beveridge C.**, and **Shaw L.**, 2025., Insights into the molecular regulation of premature fruit drop – what we have learned from mango (*Mangifera indica*) and other fruit crops. Scientia Horticulturae .

Dun E., Jones S., Kerr S., Amoo O., Cao D., Fichtner F., Dudley C., Barbier F., Rameau C., **Shaw L.**, Weller J., **Beveridge C.,** 2025. The circadian clock modulates branching in pea via the strigolactone signalling pathway. Submitted to New Phytologist. Under requested revision.

Shehnaz S., Kerr S., **Shaw L.**, Manivannan S., Cazzonelli C., Tanurdzic M., 2025. Current understanding of the regulation of bud dormancy and bud break in perennial crops. Manuscript near completion.

Chapter in a book or paper in conference proceedings

Book Chapter

Shaw L., Nath O., Flether S., Henry R., **Mitter N.**, **Hayward A.**, 2021. Avocado transcriptomic resources. In: Cifuentes A. (Editor), Comprehensive Foodomics. Elsevier, Amsterdam, Netherlands, P544-557.

Conference proceedings

Jones S., Orr R., Rossouw G., Smith H., **Beveridge C.** and **Shaw L.** From signals to shedding: Investigating the molecular interactions associated with mango premature fruit drop. Australian Mangoes Scientific Symposium, Cairns, QLD, Australia, 20 May 2024

Jones S., Orr R., Rossouw G., Smith H., **Beveridge C.** and **Shaw L.** Understanding the molecular signals regulating mango fruit abscission as a basis to increase fruit retention. International Congress on Plant Molecular Biology (IPMB), Cairns, QLD, Australia, 24-28 June 2024

Poudel P., Ahmed B., **Shaw L.**, Topp B. and Alam M., 2024. Genome-wide identification and characterization of aquaporin genes in Macadamia integrifolia. Plant and Animal Genome, Perth, WA, Australia, 18-20 September 2024

Poudel P., Ahmed B., **Shaw L.**, Topp B. and Alam M. Exploring the molecular basis of rootstock-mediated vigour in macadamia: Unravelling aquaporin gene dynamics. American society of Horticulture Science annual Conference, Honolulu Hawaii, United States, 23-27 September 2024.

Poudel. P., **Shaw L.**, Topp B. and Alam M. 2024. Vigour in macadamia: Genetic and physiological insights into rootstocks. AusMac 2024, Gold Coast, QLD, Australia, 29-31 October 2024

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Intellectual property

We have not identified findings for consideration as intellectual property at this stage.

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Appendices

Appendix 1: Bottcher C., Shaw L., Haberman A., Goetz M., Beveridge C. and Smith H., 2022. Developing a physiological framework for managing summer fruit abscission in avocado. Talking Avocados 33 (3), 57-62.

Appendix 2: Posters on aquaporin activity in macadamia

- Poudel P., Ahmed B., Shaw L., Topp B. and Alam M., 2024. Genome-wide identification and characterization of aquaporin genes in Macadamia integrifolia. Plant and Animal Genome, Perth, WA, Australia, 18-20 September 2024
- Poudel P., Ahmed B., Shaw L., Topp B. and Alam M. Exploring the molecular basis of rootstock-mediated vigour in macadamia: Unravelling aquaporin gene dynamics. American society of Horticulture Science annual Conference, Honolulu Hawaii, United States, 23-27 September 2024.
- Poudel. P., Shaw L., Topp B. and Alam M. 2024. Vigour in macadamia: Genetic and physiological insights into rootstocks. AusMac 2024, Gold Coast, QLD, Australia, 29-31 October 2024

Appendix 3: Poudel P. 2024. Genetic insights into rootstocks for vigour control. AMS News Bulletin Winter 2024

Appendix 4: Jones S., Orr R., Rossouw G., Smith H., Beveridge C. and Shaw L., 2023. Managing early fruit drop in mango. Mango Matters, 52, 14-15.

Appendix 5: Orr R., Shaw L., Jones S., Rossouw G., Smith H. and Dickinson G. (2024) Mango fruit drop: How research is looking for solutions. Mango Matters 57, p24-25

Developing a physiological framework for managing summer fruit abscission in avocado

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Avocado is a semi-domesticated tree crop with a theoretical yield potential of 32 t/ha1. Due to the irregular bearing behaviour of avocado, which is primarily driven by low levels of fruit set and high rates of immature fruit abscission, as well as biennial bearing, average annual production levels across Australia and the world are well below the theoretical yield potential. Moreover, establishing overseas export markets to manage current and future oversupply issues will require effective mitigation tools that limit irregular bearing. Methods to sufficiently improve avocado fruit set and reduce immature fruit abscission are currently lacking due to a limited understanding of the physiological and developmental basis of immature fruit abscission and fruit set. Therefore, to effectively mitigate irregular bearing, an in-depth understanding of immature fruit abscission and fruit set is required, which can be leveraged to identify potential intervention strategies in order to develop new methods to maximize yield and reduce seasonal variation.

Summer Fruit Abscission is a major target for management intervention

Fruit abscission is the major factor that negatively impacts production in coastal regions of south western, WA, Tristate area (Riverland) and central QLD. In these regions, 20% or more of the crop will drop during the summer, which is further increased by hot and dry conditions2,3. Given that trees have invested up to 40% dry matter into fruits that drop during the summer2, mitigating summer fruit abscission would have a considerable outcome for mitigating irregular bearing. Research derived from AV16005 supports the hypothesis that seasonal and temporal fluctuations in the carbohydrate status of a tree combined with dominance interactions between fruits and vegetative flushes, as well as among fruits, limits carbohydrate availability to a subset of fruits, resulting in abscission. Further, crop load, environmental conditions and management inputs, which influence tree carbohydrate status and dominance interactions, also impact fruit abscission.

Fruit growth arrest is the primary developmental event of summer fruit abscission

In Hass, experimental studies showed that abscised fruits were statistically smaller than retained fruits, during the summer fruit abscission event in California4. Further, in Fuerte, smaller sized fruits have a higher tendency to abscise compared to larger sized fruits, after the first wave of fruit drop5. Based on these studies, we investigated the possibility that fruits under growth arrest prior to abscission. To test this hypothesis, the diameter of developing fruits was measured at regular time intervals using hand-held digital callipers, as well as fruit dendrometers. Subsequently, these measurements were used to calculate the average growth rates of persisting and abscising fruits. At the start of the trial, the average growth rate of persisting fruits was approximately 0.6 mm/day, which declined to 0.36 mm/day at the end of the trial (Figure 1A). Growth rates for individual fruits that abscised were calculated and collated to better understand if a change in growth rate precedes abscission. Results from this analysis showed that fruits underwent growth arrest prior to abscission during the summer growing period (Figure 1B). Therefore, to mitigate summer fruit abscission, practical strategies must be aimed at managing fruit growth arrest, rather than abscission.

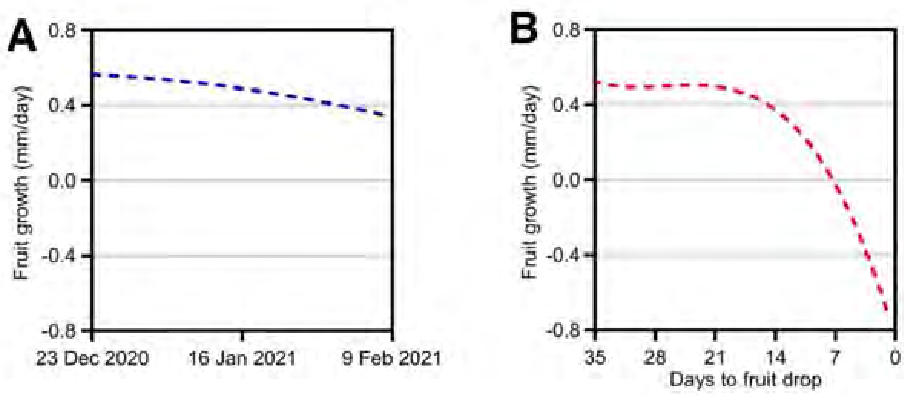

Figure 1.

Physiological drivers of fruit growth arrest

The AV16005 project developed hypotheses aimed at predicting the physiological drivers of summer fruit growth arrest and abscission. The first two of the hypotheses tested had the potential to have an immediate impact for the development of a practical application(s), while the remaining hypotheses require additional basic research due to the lack of understanding of immature fruit abscission.

A change in water potential is not the primary driver of summer fruit growth arrest and abscission

As water stress conditions reduce yields, irrigation strategies were proposed as a means to increase the reproductive potential of a tree 6. Therefore, we addressed whether a change in fruit water potential is a driver of summer fruit growth arrest and abscission. If so, new irrigation management strategies could be developed to manage summer fruit growth arrest and abscission. However, our results showed that summer fruit arrest is not associated with changes in fruit water potential. Therefore, modified irrigation regimes in well managed orchards don't appear to be a feasible solution to manage summer fruit abscission (Figure 2).

A deficiency in a mineral element(s) is not a key driver of summer fruit growth arrest and abscission

Mineral elements acquired from the soil are essential for plant growth and development, as well as yield7,8. A previous study examined whether the nutrient status of the tree influences avocado fruit abscission9. Tree nutrient status was determined by measuring nitrogen and other essential mineral elements in leaves. Results from this study indicate fruit abscission was not associated with changes in leaf nutrient status9. To further determine whether nutrient deficiency may be involved in summer fruit abscission, the levels of key mineral elements were measured in arresting and control fruits with a normal growth rate. Results indicate that mineral element deficiency is not a physiological driver of summer fruit abscission in well-managed orchards (Figure 2). Therefore, new plant nutrition strategies do not appear to be a feasible solution to mitigate summer fruit abscission in well managed orchards.

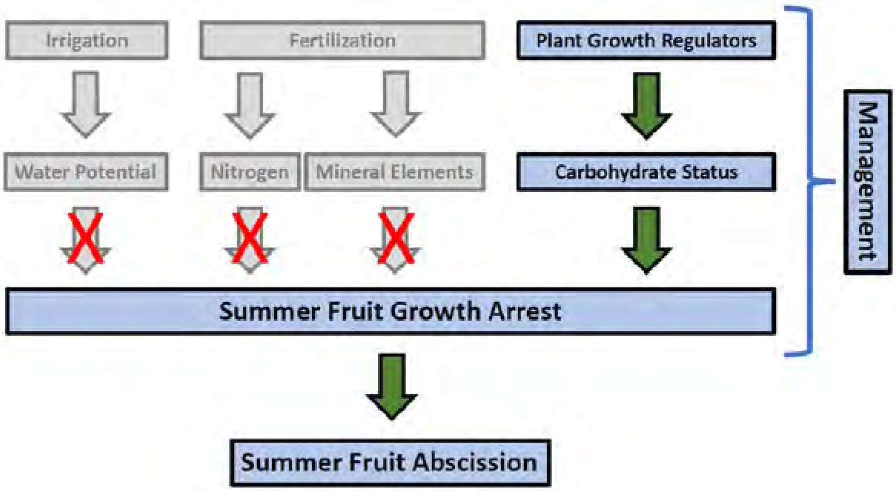

Figure 2.

A change in the carbohydrate status of the fruit is associated with summer growth arrest and abscission

Experimental studies indicate that fruit abscission is mediated by reduced carbohydrate availability, signaling and availability in citrus10,11 and apple12. Given that carbohydrates are key substances required for growth, we hypothesized that reduced carbohydrate status of the fruit would be associated with fruit growth arrest during summer fruit abscission. To test this hypothesis, sugar metabolites and starch were measured in the seed and pericarp of normal growing and arresting fruits during the summer. In addition, gene expression studies were also performed to further examine the impact of growth arrest on carbohydrate metabolism. Results from our studies indicate that carbohydrate supply and metabolism is severely reduced in the seed. In addition, carbohydrate metabolism is also altered in the pericarp. Taken together, a decrease in the carbohydrate status of the fruit appears to be the primary driver of summer fruit growth arrest and abscission (Figure 2). Given the interplay between hormones and carbohydrate signaling and metabolism, managing fruit growth arrest will require an understanding of the hormonal control of fruit growth arrest, which can be leveraged to develop a plant growth regulator (PGR) application(s) to limit summer fruit growth cessation and abscission (Figure 2).

A change in the hormone status of the seed is associated with summer growth arrest and abscission

To understand the hormonal changes associated with fruit growth arrest, key hormone profiles were determined for arresting and persisting fruits (note: the hormone profiles in arresting fruits shown were derived from a late stage of fruit growth arrest; Figure 3). Auxin is a fundamental growth promoting hormone that acts to coordinate the growth of seed and fruit tissues during development13-15. In persisting fruits, preliminary results indicate that auxin levels were high in seeds relative to the pericarp. However, during growth arrest, the levels of auxin in the seed and pericarp was significantly reduced (Figure 3). Cytokinin is another hormone implicated in fruit growth and development13. Preliminary results suggest that the levels of cytokinin were similar between persisting and arresting fruits. However, gene expression studies indicated that growth arrest involved changes in cytokinin response rather than mechanisms that control the levels of this hormone (Figure 3). Currently, we are evaluating hormone profiles for active gibberellins and brassinosteroids to determine if these hormones change in response to summer fruit growth arrest.

In apple, experimental studies indicate that abscisic acid and ethylene mediate immature fruit abscission12,16-18. Consistent with studies in apple, preliminary results indicate that abscisic acid levels primarily increased in the pericarp during avocado fruit growth arrest (Figure 3). In addition, gene expression studies suggest that ethylene plays a role in avocado summer fruit growth arrest and abscission (Figure 3), as the expression of ethylene biosynthesis and signaling genes were induced in the seed and pericarp during growth arrest. We are currently developing a method to quantify aminocyclopropane-1-carboxylic acid (ACC), the metabolite converted to ethylene by ACC oxidase19, in order to validate a role for ethylene in summer fruit growth arrest.

Summary

Managing irregular bearing is a major challenge for the Australian Avocado Industry as this impacts supply and the ability to establish overseas export markets required to increase profitability in years when there is an oversupply of avocados. Research from AV16005 shows that mitigation of summer fruit abscission requires the ability to manage fruit growth arrest in order to reduce fruit drop. As summer fruit growth arrest is not associated with a reduction in fruit water potential or mineral element deficiency, modification of irrigation and fertilization practices will likely not provide a feasible pathway to manage summer fruit drop. Our results showing that the carbohydrate potential of arresting fruits is reduced indicate that growth arrest must be managed via methods to alter carbohydrate supply and metabolism, which is regulated in part via plant hormones. Preliminary hormone quantification studies indicate that changes in the levels of auxin, abscisic acid and ethylene, as well as cytokinin response, are associated with the later stage of growth arrest. Current research is focused on validating the preliminary studies. To build a pathway for impact, the next step of the research project is to identify hormonal changes associated with early growth arrest

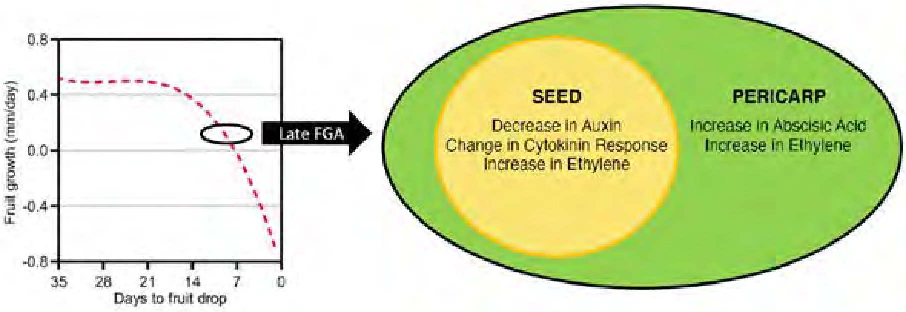

Figure 3.

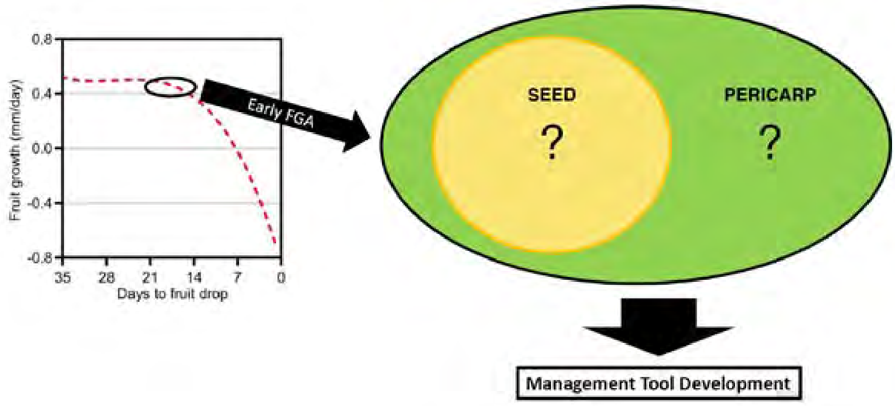

Figure 4.

(Figure 4). This step is absolutely key for developing plant growth regulator application(s) to manipulate hormonal changes in order to allow fruits with a lower growth and carbohydrate potential to persist on the tree until maturity is reached.

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More Information

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Hort Innovation Strategic levy investment | AVOCADO FUND

Exploring the molecular basis of rootstock-mediated vigour in macadamia: Unravelling aquaporin gene dynamics

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INTRODUCTION

- **Rootstocks** play a crucial role in shaping plant growth and vigour, driven by a complex interplay of physiological, molecular, and genetic factors.
- **Aquaporins (AQPs)**, as key water transport proteins, are emerging as pivotal component in understanding rootstock-mediated vigour.
- Evidence for rootstock effects in macadamia remains limited with no exploration into the mechanisms of **rootstock-mediated vigour control**, hindering progress in rootstock development for this crop.
- **Objectives:** To explore the role of **Plasma Intrinsic Proteins (PIPs)**, a sub-family of AQPs in rootstock mediated vigour control in macadamia.

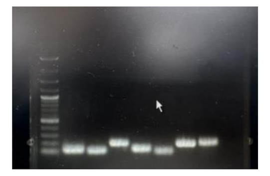
METHODOLOGY

Macadamia rootstock trial at Nambour, Queensland

Plant materials:
- Sub-set of 7 rootstocks with a single scion 'HAES 741' selected
- 4 dwarfing and 3 vigorous rootstocks
- Corresponding ungrafted trees from same rootstock genotype

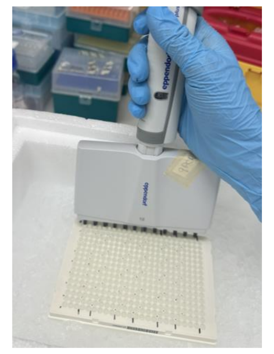
RNA extracted form leaf and stem tissues

RNA quality and quantity check



Primer pairs specific to 11 PIP genes designed

Primer specificity assessed
Primer efficiency calculated



cDNA synthesis and qRT-PCR performed

Gene expression analysis with *ACTIN* as a reference gene

RESULTS AND DISCUSSIONS

Table 1. Phenotypic correlations of scion vigour traits with relative expression of *MiPIP* genes in leaf tissues of grafted and ungrafted trees. Significant correlations (p<0.05) are highlighted in blue.

	Canopy Volume	Height	Trunk Circumference	Canopy Area
MiPIP1-1a	-0.40	-0.21	-0.34	-0.57
MiPIP1-1b	0.06	0.07	0.04	0.03
MiPIP1-4a	-0.57	-0.26	-0.41	-0.69
MiPIP1-4b	0.20	0.07	0.14	0.27
MiPIP2-1a	-0.45	-0.32	-0.39	-0.46
MiPIP2-1b	-0.46	-0.28	-0.41	-0.47
MiPIP2-1c	-0.44	-0.36	-0.47	-0.46
MiPIP2-2	-0.40	-0.40	-0.44	-0.30
MiPIP2-5a	0.55	0.45	0.51	0.49
MiPIP2-5b	-0.65	-0.61	-0.65	-0.58
MiPIP2-8	-0.46	-0.35	-0.39	-0.45

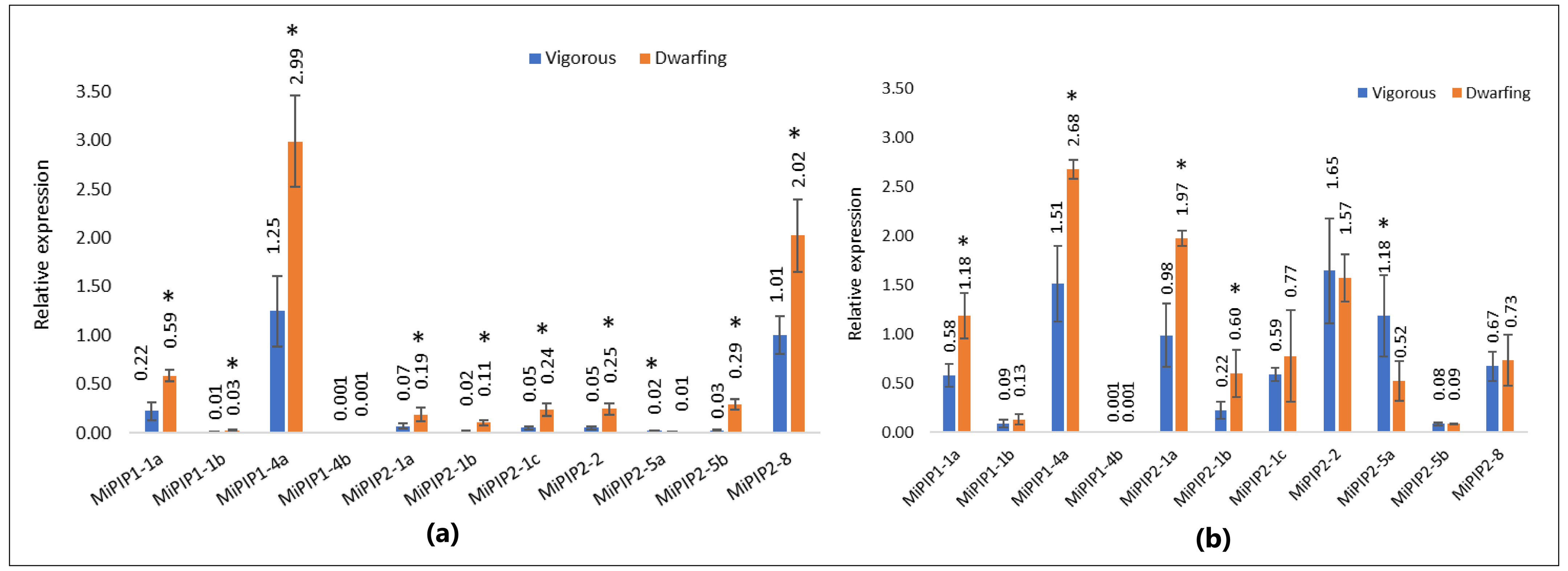


Fig 1. Relative expression of *MiPIP* genes in (a) leaf and (b) stem tissues of 'HAES 741' grafted on dwarfing (n=4) and vigorous (n=6) rootstocks. Bars indicate means of relative expression values, normalized to *ACT*. Values on the top of the bars indicate the average relative expression of each group. Asterisks (*) indicate significant differences between dwarfing and vigorous rootstocks (p<0.05) according to welch's t-test.

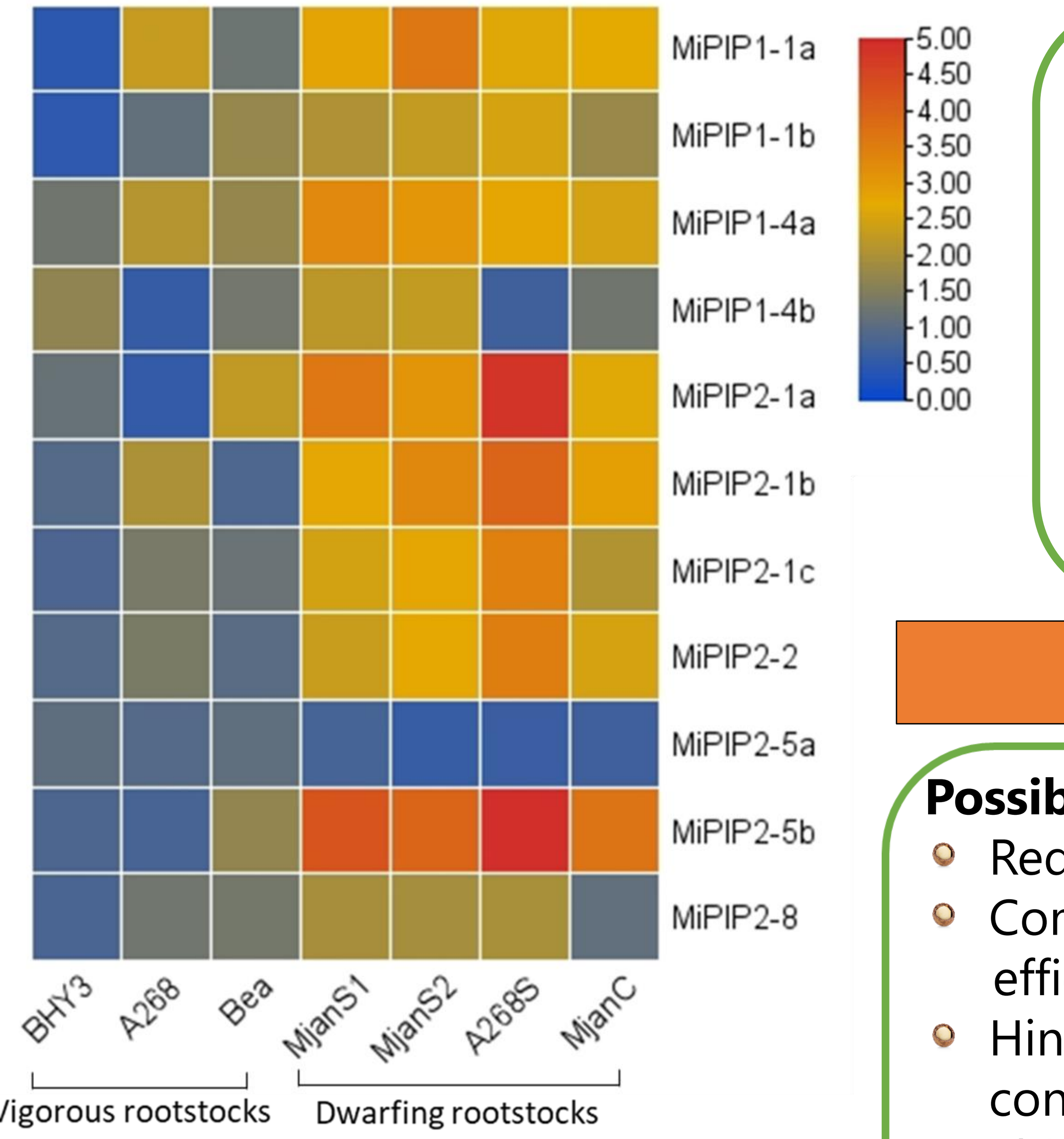


Fig 2. Relative expression of MIPIP genes in leaf tissues of 'HAES 741' scion on 7 rootstock genotypes. The log transformed relative expression values normalized with that of *ACT* were used for heat map construction

- In grafted trees, scions on **dwarfing rootstocks had higher expression** of most of the PIP genes than vigorous rootstocks (Fig 1 and 2)
- Similar results in leaf and stem tissues of ungrafted trees.
- Expression of **PIP genes correlated negatively with vigour traits**, except *PIP2-5a* with positive correlations (Table 1).

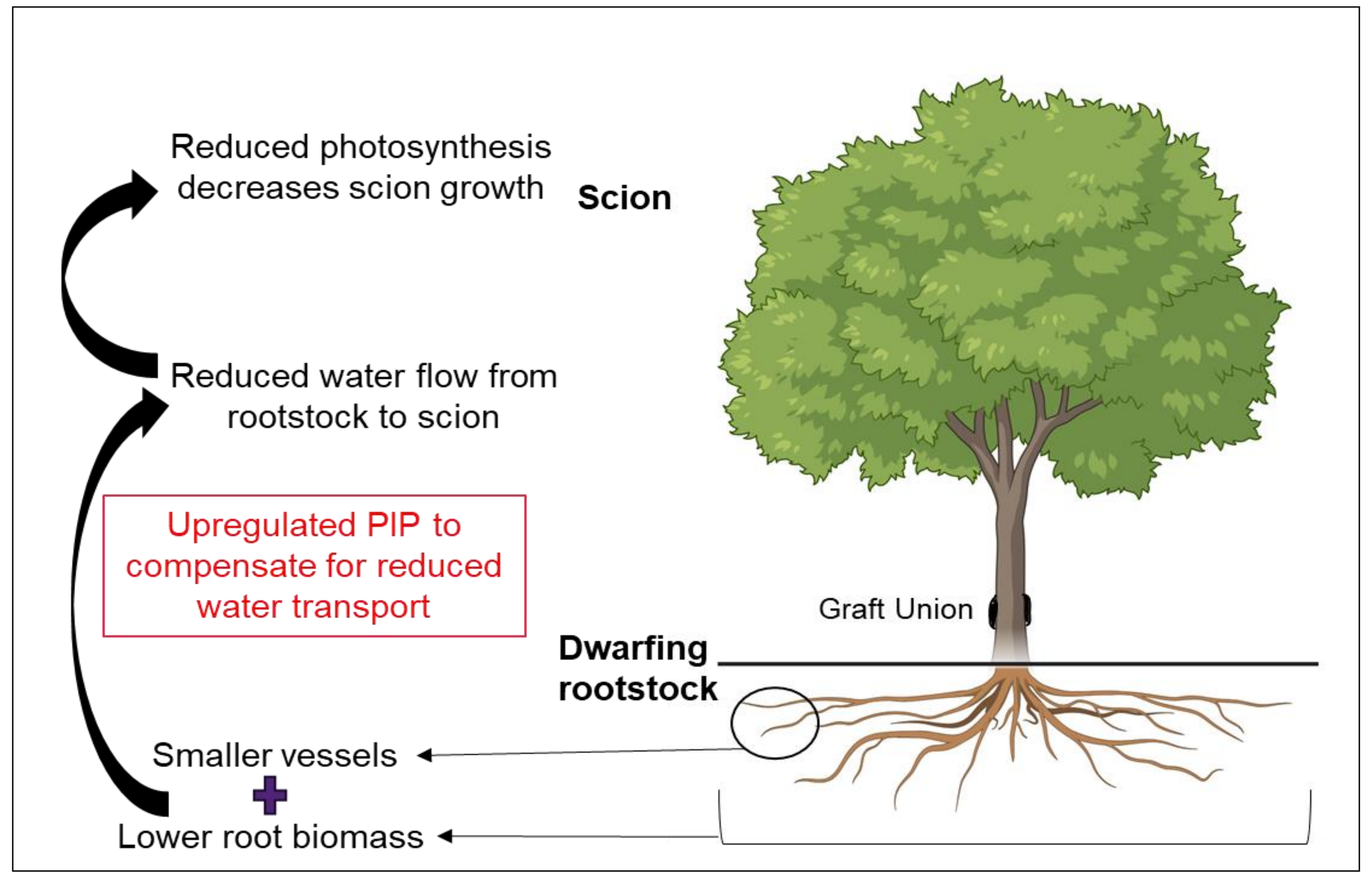


Fig 3. Possible hypothesis of rootstock mediated vigour control in macadamia

CONCLUSIONS

Possible hypothesis (Fig 3)
- Reduced water transport ability of dwarfing rootstocks.
- Compensatory mechanism to maintain water transport efficiency despite this reduced water transport capacity.
- Hints towards **improved resilience and performance of dwarfing rootstocks** under challenging environmental conditions.
- This research provides a base for understanding molecular and physiological **mechanisms of vigour control** in macadamia.
- Further research involving detailed analysis of root biomass, vessel structure and photosynthetic characteristics is suggested.

The University of Queensland Australia — Create Change

QAAFI Queensland Alliance for Agriculture and Food Innovation

Queensland Government

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Genome-Wide Identification and Characterization of Aquaporin Genes in *Macadamia integrifolia*

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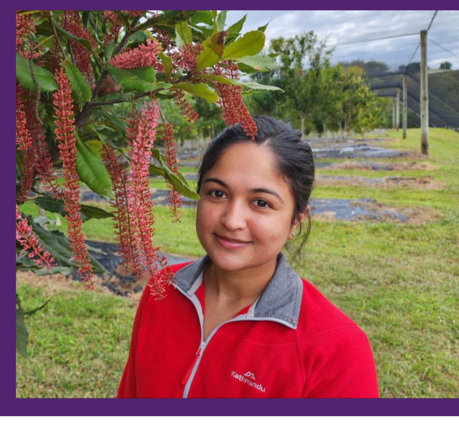


Introduction

Aquaporins
Membrane proteins that facilitate water transport across cell membranes, essential for plant water balance.

Role in Stress Response
Crucial for plant adaptation to drought, salinity, and other environmental stresses by regulating water flow.

Water-Use Efficiency
Improving aquaporin function can enhance water-use efficiency, vital for sustainable agriculture.

Relevance to *Macadamia integrifolia*
Aquaporins may influence vigour control and stress tolerance, important for macadamia breeding.

Objective
To identify and characterize aquaporin genes in *M. integrifolia* for future research on water-use efficiency and vigour control.

Methodology

Whole genome sequence of *M. integrifolia*
Reference genome

AQP protein sequences of *Arabidopsis (AtAQPs)* and *Populus (PtAQPs)*
Query sequences

Homology search in BLASTP

Putative AQP genes identified

Gene information
- NCBI
- ExPaSy server

Gene structure
- Gene Structure Display Server

Conserved residues: sequence alignment

Validation: Conserved Domain Database

Multiple sequence alignment and phylogenetic tree in MEGA 11

Conserved domains: MEME discovery server

Chromosomal position: NCBI database, visualization in TBTools

Results

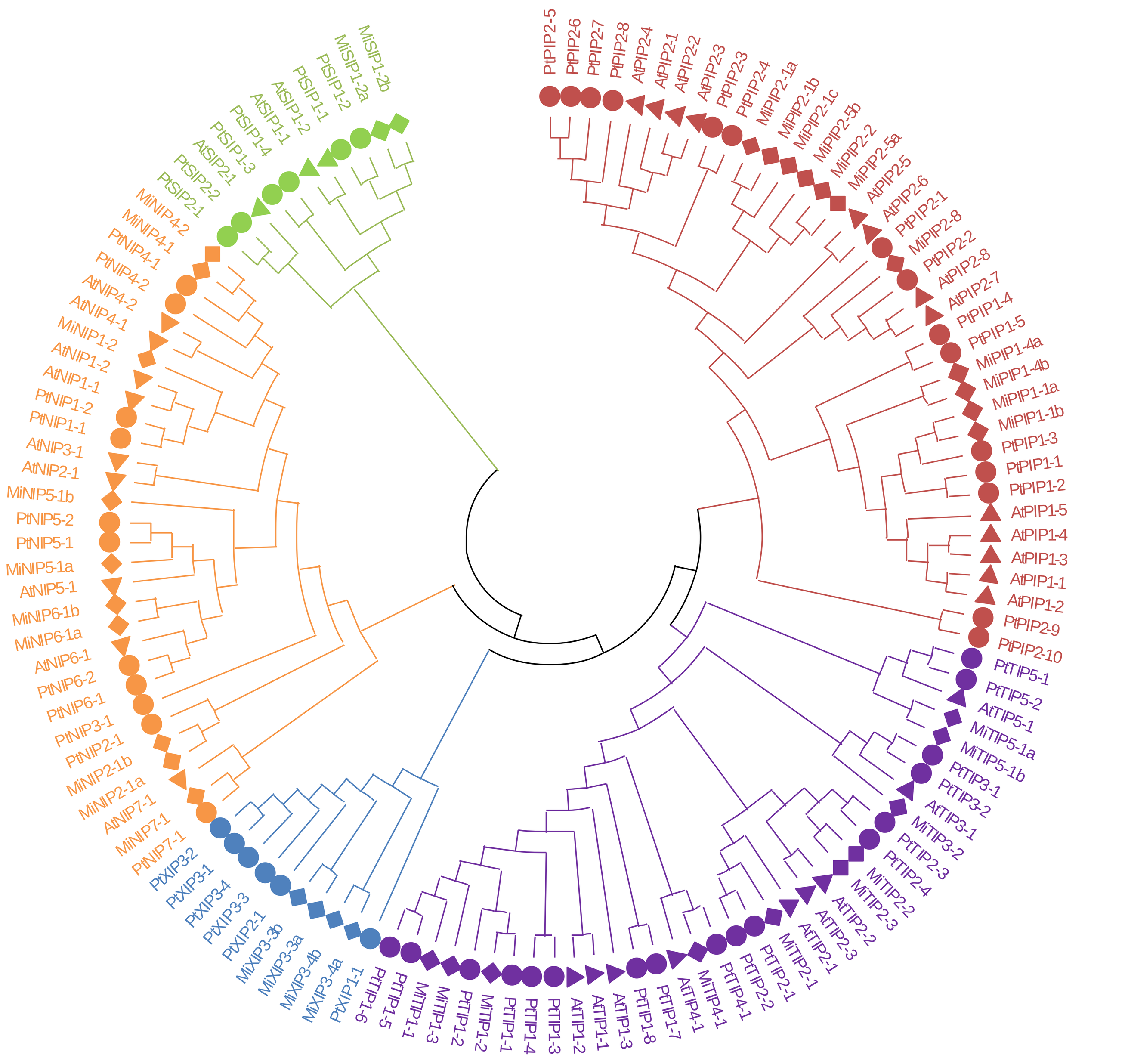


Fig 1. Phylogenetic relationships between the *MiAQPs, AtAQPs* and *PtAQPs* sequences. Sub-families of AQPs are marked by different colours, brown for PIPs, purple for TIPs, orange for NIPs, Green for SIPs and blue for XIPs. Different shapes following protein names represent AQPs of different species, circle for *PtAQPs*, triangle for *AtAQPs* and diamond for *MiAQPs.*

- **37 putative AQP** (*MiAQPs*) genes identified (Fig 1)
- **5 sub-families**:
  - 11 Plasma Intrinsic Proteins (PIPs)
  - 10 Tonoplast Intrinsic Proteins (TIPs)
  - 10 Nod-26 like Intrinsic Proteins (NIPs)
  - 2 Small and basic Intrinsic Proteins (SIPs)
  - 4 Unclassified X Intrinsic Proteins (XIPs)
- Distributed across **11 out of 14** chromosomes (Fig 2)
- **No. of Exons** ranged from 2 to 6
- **3 conserved sequences** among all AQP families
- **Conserved amino acid residues** at the NPA motif, AR/r selectivity filter and Froger's position within subfamilies (Fig 3)

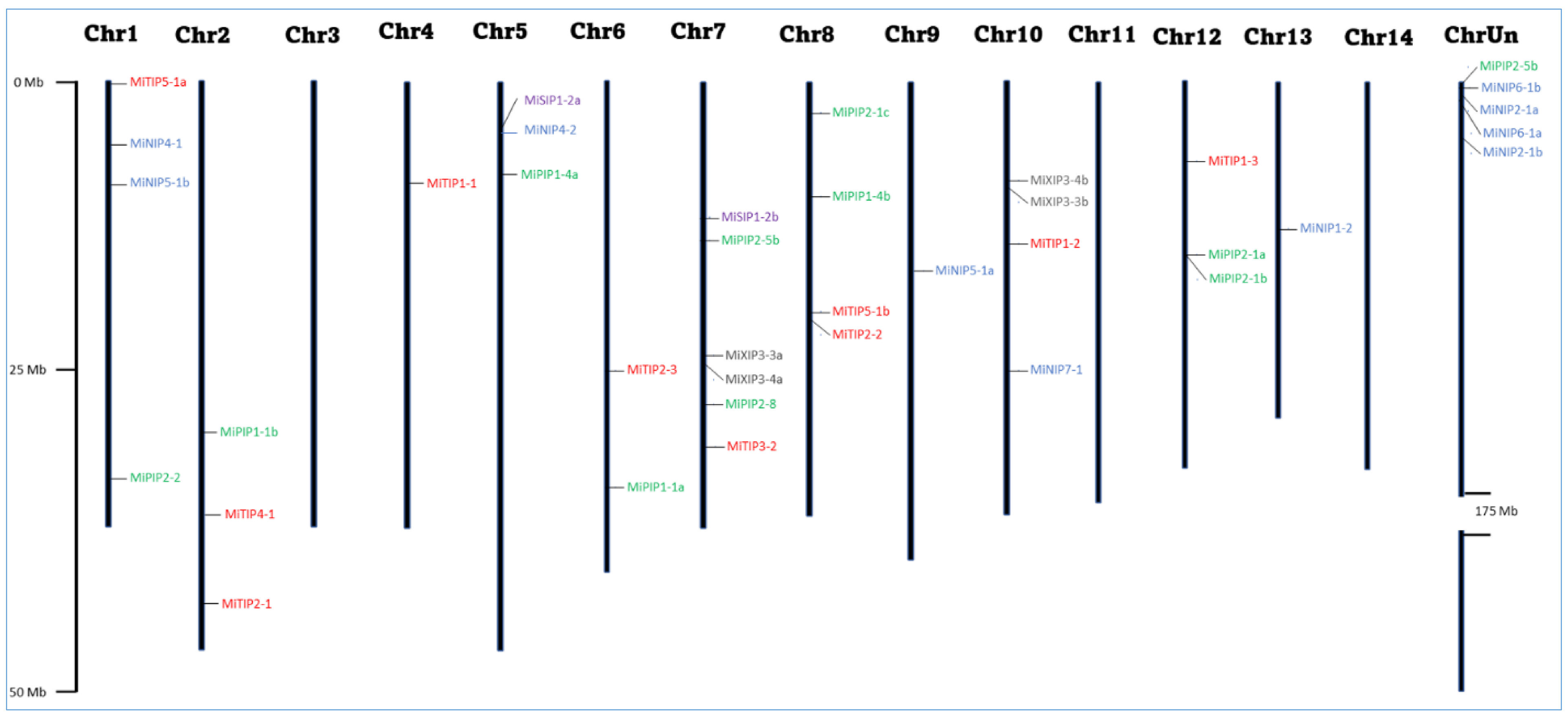
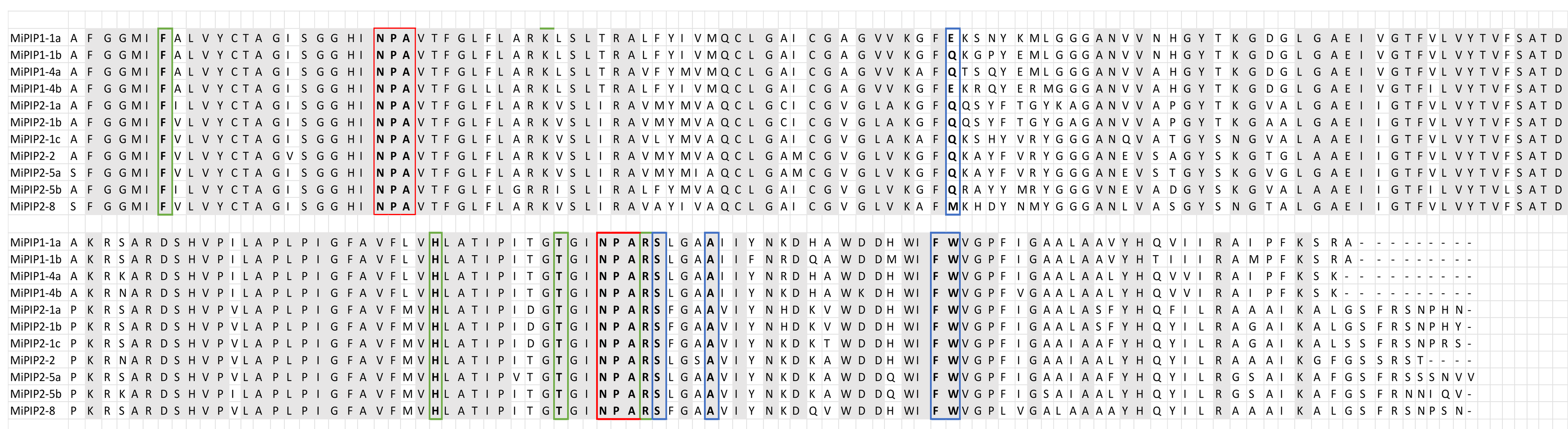


Fig 2. Distribution of *MiAQP* genes on the 14 chromosomes of *M. integrifolia*



NPA motif
Ar/R filter
Froger's residue

Fig 3. Multiple sequence alignment of the deduced amino acid sequences of *MiPIPs* showing the amino acids at NPA domains, ar/R selectivity filters, and Froger's residues

Conclusions and Future Research

This study represents the first genome-wide identification of AQPs in *M. integrifolia*, offering a detailed characterization of their structural and functional diversity. The identified gene sequences and their annotations will serve as valuable resources for future research aimed at enhancing water-use efficiency, stress tolerance, and investigating physiological mechanisms of growth and development in macadamia.

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Vigour in macadamia: Genetic and physiological insights into rootstocks

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The University Of Queensland AUSTRALIA

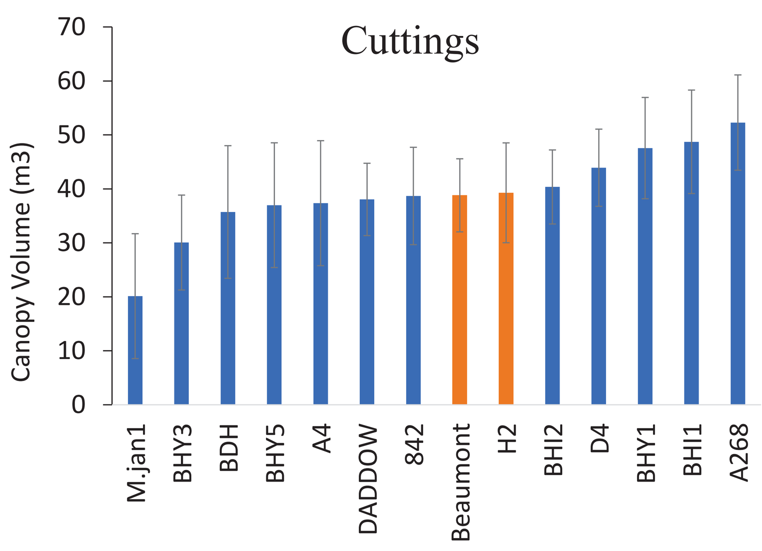
Rootstock study 1: Evaluating rootstocks for vigour

Methodology

- A rootstock trial was established in Maroochy Research Facility, Nambour in 2017.
- **24** rootstock genotypes with a common scion: 'HAES741'.
- Rootstocks were propagated as cutting (14) and seedling (20).
- Vigour traits measured since 2017 and still ongoing.
- Statistical analysis was performed.

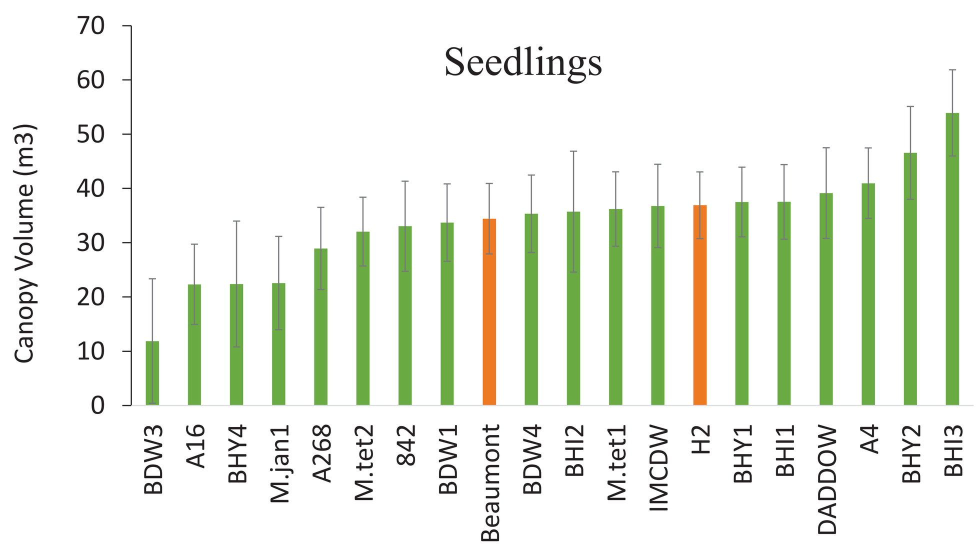
Fig 1. Macadamia rootstock trial at Maroochy Research Facility, Nambour established in 2017

Results



Early vigour reducing rootstocks

- Cuttings and seedlings of wild *M. jansenii* (M.jan1)
- 'BDW3' seedlings - potential dwarf selection from National breeding program
- 'BHY4' seedlings – high yield efficiency selection from National breeding program
- 'A16' seedlings



Early vigour promoting rootstocks

- Early vigour important for orchard establishment.
- High early vigour and controlled vigour at maturity?
- Evaluation is ongoing

Fig 2. Canopy volume at age 4 (2021) of scion 'HAES 741' as affected by different cutting and seedling propagated rootstocks. Commercial rootstocks are highlighted in orange.

Rootstock study 2: Evaluating rootstocks for stomatal traits

Methodology

- 150 grafted trees (seedlings and cuttings) from the rootstock trial were phenotyped for stomatal density and area.

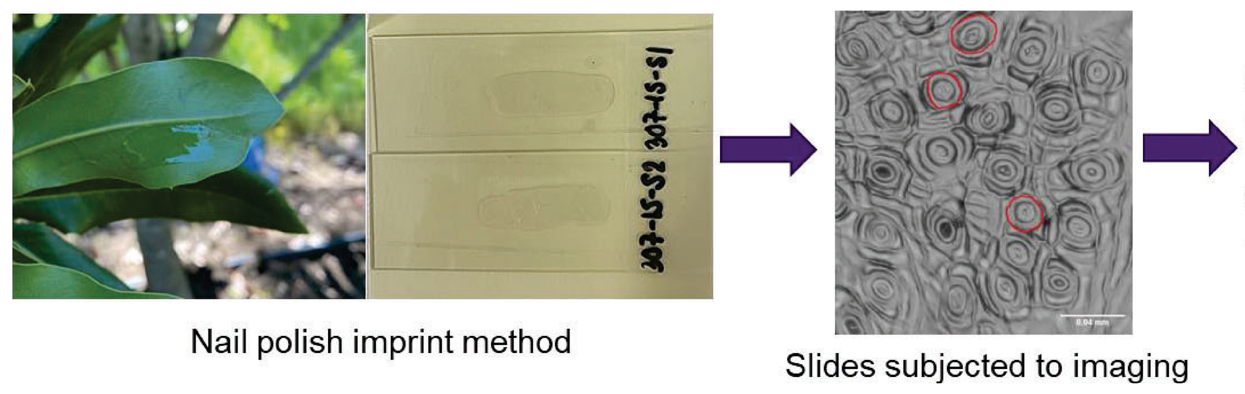
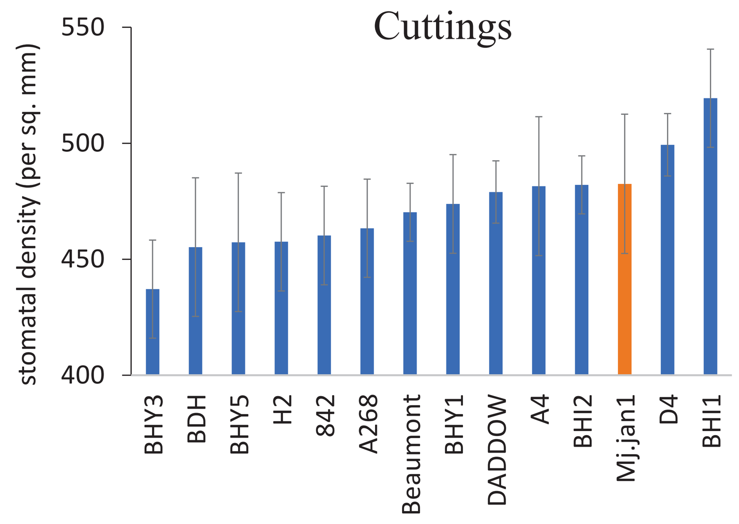


Fig 3. Flowchart showing phenotyping procedure for stomatal density and area

Results

- Rootstocks affected stomatal density but not stomatal area.
- Stomatal area and density had inverse relationship (phenotypic correlation = -0.39).
- Few vigour reducing rootstocks (Fig 4, highlighted in orange) led to an adjustment in stomatal density as the tree attempts to balance its reduced vigour with sufficient gas exchange.
- Ideal rootstocks for sustainable orchard system where excessive vigour is supressed while ensuring sustainable growth and productivity.



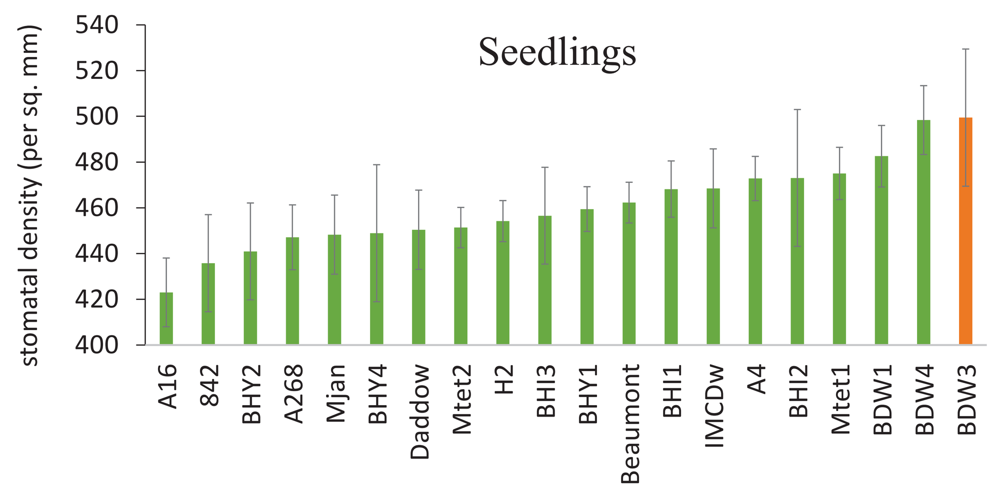


Fig 4. Variation in stomatal density of scion 'HAES 741' as affected by different cutting and seedling propagated rootstocks.

Background

- Managing tree vigour is crucial for productivity and profitability in macadamia cultivation.
- **Excessive vigour** leads to orchard crowding, shading, and restricted machinery access.
- Traditional management methods are labour-intensive and costly with reported yield loss.
- While other tree crops benefit from **vigour-controlling rootstocks**, macadamia lacks suitable options .

Key objectives

- Investigate the **genetic aspects of rootstocks** for vigour control in macadamia.
- **Evaluate rootstocks** for their effects on scion vigour and stomatal traits.
- Investigate possible molecular **mechanisms of rootstock** mediated vigour control.
- Identify **candidate genes** associated with rootstock mediated vigour.

Rootstock study 3: Vigour control mechanism by rootstocks

- **Aquaporins -** channel proteins that form pores in the cell membrane mainly facilitating transport of water between cells.
- **Plasma intrinsic proteins (PIP)** -AQP subfamily with highest water transport activity.

Methodology

- Sub-set of 7 rootstocks with a single scion 'HAES 741' selected: 4 dwarfing and 3 vigorous rootstocks.
- RNA was extracted from leaf and stem tissues.
- Expression of 11 PIP genes was observed in leaf and stem tissues.

Results

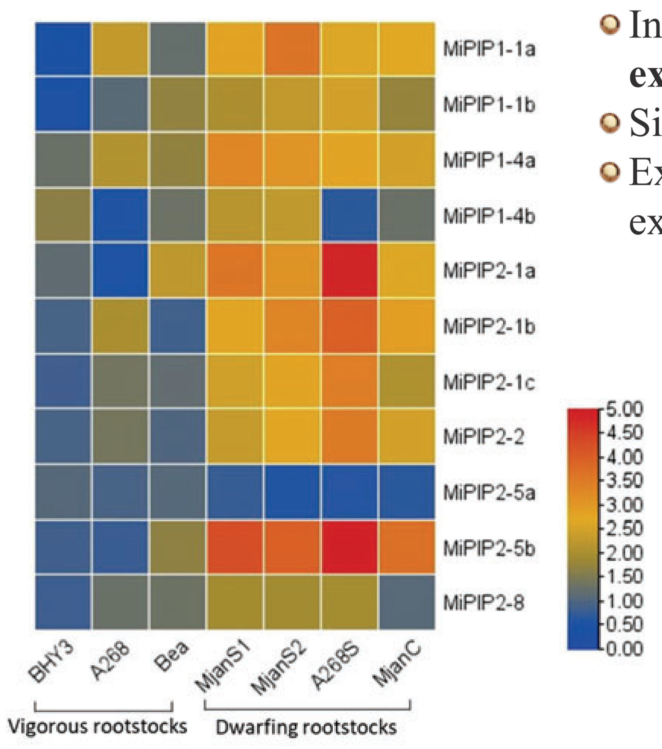


Fig 5. Relative expression of PIP genes in leaf tissues of 'HAES 741' scion on 7 rootstock genotypes.

- In grafted trees, scions on **dwarfing rootstocks had higher expression** of most of the PIP genes than vigorous rootstocks (Fig 5)
- Similar results in leaf and stem tissues of ungrafted trees.
- Expression of **PIP genes correlated negatively with vigour traits**, except *PIP2-5a* with positive correlations.

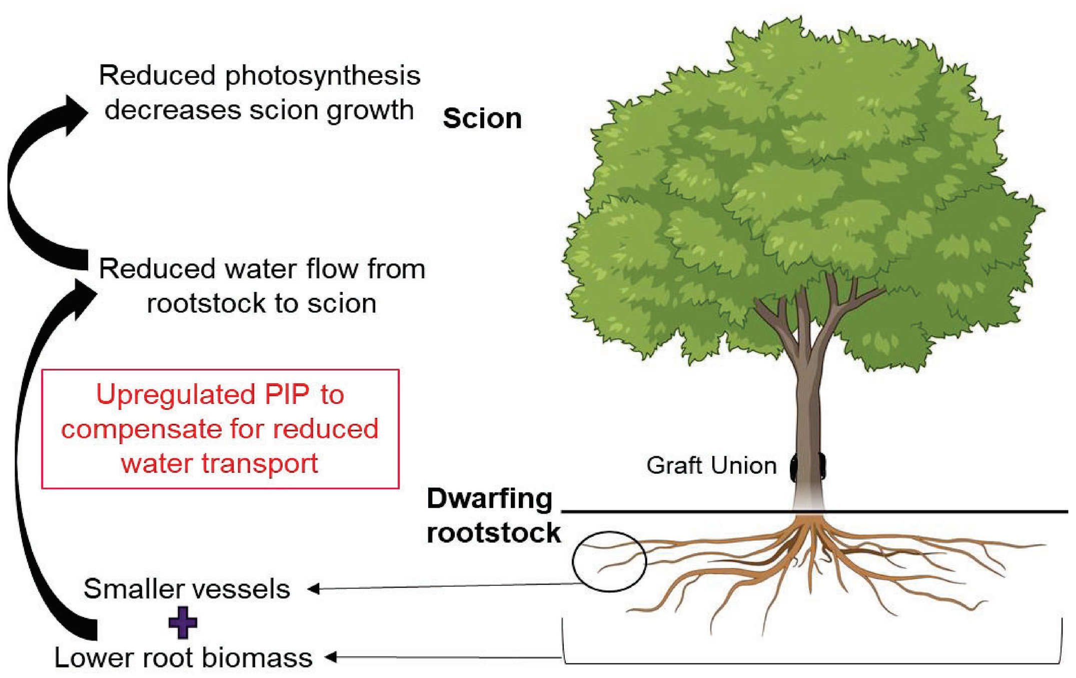


Fig 6. Possible hypothesis of rootstock mediated vigour control in macadamia.

- Higher expression of these water channel proteins in scions on dwarfing rootstocks suggests a compensatory mechanism to maintain water transport efficiency despite their reduced water transport capacity.
- Hints towards **improved resilience and performance of dwarfing rootstocks** under challenging environmental conditions.

Future studies

1. Continuous evaluation of rootstocks based on scion vigour until age 7 before recommendations are made**.**
2. **Genomic assisted rootstock breeding for scion vigour management in macadamia**

- All the rootstocks have been genotyped for ~4000 SNP (Single Nucleotide Polymorphism) markers through Diversity Array Technology.
- The genetic and phenotypic data (growth measurements) will be integrated to perform a genome-wide association study which allows the identification of markers associated with rootstock mediated vigour.
- Candidate genes associated with rootstock vigour will be identified.
- This will allow selection of rootstocks based on desired candidate genes and therefore accelerate rootstock development.
- This combined data also informs genomic prediction models. These models, once validated, can be utilized in predicting vigour of rootstocks based on their genetic data.

Fig 7. Collecting cambium tissues below the graft union for rootstock genotyping.

Key Takeaways

- Rootstocks such as the wild *M. jansenii,* A4 and some selections from the National breeding program can be further explored for their potential use as dwarfing rootstocks.
- Rootstocks that reduce vigour but also have higher stomatal density such as the wild M.jan1, A4 and BDW3 have the potential improved water-use efficiency.
- Vigour reducing rootstocks seem to have higher expression of a water transporting protein family (PIP), which could be one of the molecular mechanisms by which vigour is controlled.
- PIP genes are involved in overcoming water-stress, and hence vigour reducing rootstocks may have improved performance during stress.
- Future rootstock studies from the trial will help accelerate rootstock breeding using genomic techniques to identify vigour controlling rootstocks.
- The outcome from this project provides an important guide for the development of rootstocks, thereby supporting vigour management for efficient and productive orchards.

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This project has been funded by Hort Innovation, using the macadamia research and development levy and contributions from the Australian Government and Queensland Government funded project "Advance Queensland Industry Research fellowship: Integrating technologies for rapid domestication in macadamia".

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Genetic insights into rootstocks for vigour control

Pragya Dhakal Poudel, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland

In Australia, managing tree vigour often requires labour-intensive practices like machine hedging, heavy hand pruning and limb bending. These practices require a lot of work and expensive equipment, driving up costs. While hedging and pruning enhance light penetration, they also initially reduce yield and can cause strong vegetative growth later on. To address this, some tree crop orchardists are using rootstocks and scions that control vigour, allowing for denser plantings, reduced costs, and higher productivity. However, in macadamia farming, options for these vigour-controlling rootstocks and scions are limited.

My research is focused on investigating the genetic aspects of rootstocks and scions for vigour control in macadamia. The following is an overview of this research on rootstocks.

Rootstocks offer a highly cost-effective avenue for orchard efficiency yet remains rarely explored in macadamia. As a part of Hort Innovation-led project "National tree crop intensification program (AS18000)", this study focuses on a rootstock trial planted at Nambour, Queensland. The trial has a single scion 'HAES 741' grafted onto 24 rootstock accessions including a set of cultivars, standard rootstocks, breeding selections and wild germplasm. There are four main rootstock studies that I am working on.

STUDY 1: Effect of rootstocks on early vigour

The study evaluated 24 rootstocks for their impact on early vigour traits (at age 4) including tree height, trunk circumference, canopy area and canopy volume. The analysis confirmed that rootstocks significantly influence vigour traits, with some rootstocks showing notable increases or decreases in vigour compared to standard rootstocks like 'H2' and 'Beaumont'. Several seedling rootstocks such as an accession of *Macadamia jansenii,* 'Mjan1', 'A16' and a potential dwarf selection from the National Macadamia Breeding Program (NMBP) demonstrated reduced vigour. Among cuttings, 'Mjan1', and a high yield efficiency selection from NMBP called 'BHY3' produced the smallest trees. Analysis of correlations showed a positive correlation of canopy traits between grafted trees and ungrafted trees from the same rootstock accession. This suggests a probable trait transmission from rootstock to scion, allowing low vigour selections to be used as rootstocks for canopy management. In future, these rootstocks should be evaluated for their effects at mature age (which is ongoing), in different environments combined with multiple scions.

Pragya Dhakal Poudel measures tree height at the rootstock trial

STUDY 2: DNA markers associated with rootstocks

Releasing a new macadamia cultivar requires more than 20 years spent on crossing different cultivars, years of evaluation and testings in multiple environments and finally selecting the best. Rootstock selection is much slower as it requires evaluation pre and post grafting and also requires a detailed understanding of the rootstock x scion x environment interactions.

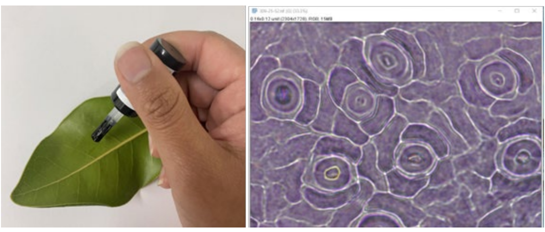
To speed up rootstock development, I am using a method called genome wide association study (GWAS). In GWAS, we compare the DNA of plants with a certain trait to those without it. This helps find specific DNA markers linked to the trait of interest. These markers can then be used for marker-assisted selection where we check cultivars for these markers before evaluating them further. For this study, I collected and analysed genetic information from trunk cambium tissue of the 24 rootstock accessions mentioned earlier. I'm looking for associations between the rootstock genotype and the scion vigour traits to identify markers linked to rootstock-mediated plant vigour. Once we find and confirm these markers, we can use them to choose rootstocks based on their impact on scion vigour.

Collecting trunk cambium tissue for genotyping

STUDY 3: Effect of rootstocks on stomatal traits

Grafting rootstock and scion together allows for communication between soil, roots, and stem. This connection lets rootstocks affect traits of the scion, like the size and density of stomata, which are tiny openings on leaves.

In our study, we looked at 144 grafted trees to study stomatal traits. We found that rootstocks do influence stomatal density but not the size. Stomata regulate gas exchange and transpiration of the leaf which in turn affects water and nutrient uptake, cell expansion and plant growth. Further exploration and interpretation of these findings will provide insights into the intricate relationship between tree vigour and stomatal characteristics. Understanding stomatal behaviour is important because it affects how plants respond to water stress and may lead to plants better adapted to dry conditions.

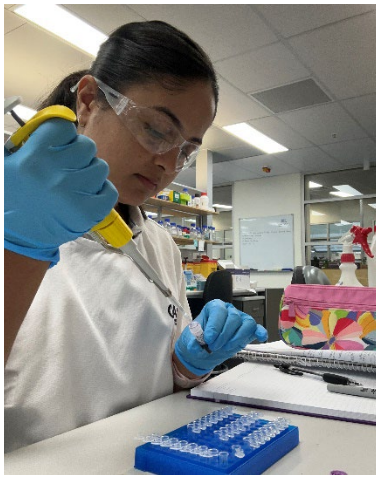


Using nail polish imprints to obtain stomatal images

STUDY 4: Aquaporin genes in vigour-control mechanism

While knowing which scion traits are affected by rootstock is important, understanding how rootstock influences scion vigour is crucial. Identifying associated traits or genes is key to pinpointing the most advantageous rootstock via targeted breeding.

To understand the molecular basis of vigour-control in macadamia, this study explores the role of Aquaporin (AQP) genes. AQPs are key water transport proteins that play a significant role in regulating plant size by controlling the movement of water across cell membranes. Using bioinformatic analyses, we identified 37 AQP genes in macadamia. From this set, we selected 11 genes for further study to assess their varying levels of activity in rootstocks exhibiting low and high vigour. This examination allows us to determine gene activity under different vigour conditions. We compare the level of activity, known as differential expression, in RNA samples extracted from leaves, stems, and roots collected during the study.



Pragya Dhakal Poudel prepares RNA samples to perform a RT-qPCR for differential gene expression analysis

In conclusion, this project adopts a comprehensive approach including field trials, genetic analyses, bioinformatics, and molecular studies to better understand rootstocks and the mechanisms underlying vigour control. Rootstock screening from Study 1 has provided insights into the degree of rootstock effects on early vigour. However, potential drawbacks of reduced early vigour should be acknowledged. It is equally crucial to continue evaluating these rootstocks for their effects on yield and growth until maturity. Additionally, our research into DNA markers associated with rootstocks holds promise for accelerating rootstocks selection. The outcome from this project provides an important guide for the development of rootstocks, thereby supporting vigour management for efficient and productive orchards.

Acknowledgements

This work has been supported by the National Tree Crop Intensification in Horticulture Program (AS18000), funded by the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Queensland's Department of Agriculture and Fisheries and the Queensland Alliance for Agriculture and Food Innovation – The University of Queensland, Plant and contributions from the Australian Government; and Queensland Government funded project "Advance Queensland Industry Research fellowship: Integrating technologies for rapid domestication in macadamia (AQIRF073-2022RD5)".

I acknowledge the University of Queensland for providing financial support through scholarship and stipend provided. I thank my advisors Dr Mobashwer Alam, Prof Bruce Topp, Dr Lindsay Shaw and Dr Joanne De-Faveri for their consistent support and guidance. A special thanks to Dr Max Cowan for his assistance and support throughout the project.

Biosecurity, Research & Policy

Managing early fruit drop in mango

Every season, a considerable amount of fruit is lost prematurely as result of fruit dropping before reaching maturity. Studies show that early fruit drop occurs because of the complex interplay among dynamic changes in molecular signalling, plant hormones, sugars, nutrients, and plant stresses. Little is known about the molecular regulation of early fruit drop in mango, and it is hoped that this project could shine a light on some of the important processes involved.

Sophie Jones harvesting mangoes at the DAF Walkamin Research Station.

The University of Queensland PhD student, Sophie Jones, is investigating this as part of the Hort Innovation funded project '*Investigating the control of fruit drop in mango to support innovative solutions for Australian growers (MG21004)*'. This project is in collaboration with Dr Lindsay Shaw and Professor Christine Beveridge at The University of Queensland, Dr Ryan Orr and Dr Gerhard Rossouw from the Department of Agriculture and Fisheries (DAF, Queensland), and Dr Harley Smith from (CSIRO, South Australia). Sophie is trying to develop targeted management strategies to minimise early fruit drop by understanding the molecular pathways that regulate fruit drop in mango. She is particularly focusing on the relationship between plant hormone pathways, and carbohydrate reserves, and the effects of externally applied plant growth regulators (PGRs) on these pathways.

What hormones regulate fruit abscission?

The signalling molecules that play a crucial role in the growth and development of plants are known as phytohormones (plant-derived hormones or commercial PGRs). There are five main hormones that control the development and ripening of fruit. There are plant growth promoting hormones, auxin, cytokinin and gibberellins. These hormones are more abundant during the early season. They promote cell division and enlargement which plays a critical role in the regulation of fruit development and size. These promoter hormones are also involved in regulating floral induction and seed germination. Additionally, there are plant growth inhibiting hormones, ethylene and abscisic acid. They are more abundant towards the end of the season, where they both play a significant role in fruit ripening. They do this largely by stimulating the degradation of the cell wall, which causes the fruit to ripen. In many fruit crops, high levels of these inhibiting hormones combined with a reduction of growth promoting hormones stops fruit growth and causes the early fruit drop process. However this has not been investigated in mango.

How can early fruit drop be controlled?

PGRs have been widely used in horticulture as an attempt to control early fruit drop, as well as improve flowering and fruit quality. They do this by modifying hormone levels or interacting with their synthesis and signalling. The four main PGRs that have been used to control fruit retention are: gibberellic acid (GA3), 1-naphthaleneacetic acid (NAA, a synthetic auxin), N-(2-Chloro-4-pyridyl)-N'-phenylurea (CPPU, a synthetic cytokinin), and paclobutrazol (PBZ, gibberellin biosynthesis inhibitor). Some of these products are not currently registered for use in mango, and can only be used for experimental purposes.

Previous experimental trials using these PGRs in mango have had variable results. For example, pre-harvest application of CPPU at low concentrations has shown to improve fruit retention, as well as promote fruit enlargement and increase fruit firmness, but other studies have shown no effect. The effectiveness of NAA application on fruit retention appears dependent on time of application and the concentration used. Some studies report up to three-fold increases in fruit retention using applications of 25 and 50ppm, while many studies have not found an effect on fruit retention. A similar trend is seen with application of GA3, particularly when applied early in the season. Combinations of PGRs may be more effective than single treatments because of the multiple phytohormones that are targeted through their application.

The variability of these results appears dependent on PGR concentration, timing of application, and cultivar. As the majority of PGR studies have been conducted outside of Australia, it is essential to investigate the effects of these PGRs on Australian cultivars grown under Australian conditions.

What role do carbohydrates play in this?

Plants rely on carbohydrates- such as sugars and starches for growth and reproduction. Plants acquire sugars from mature leaves and green stems via the process of photosynthesis. In tree crops, these sugars and starches are essential for fruit development, and can be used directly or are initially stored as reserves until required. These reserves further contribute to fruit development. However, if insufficient sugars are available to support fruit development,

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this can lead to early fruit drop as the plant tries to balance carbohydrate availability against demand. It has been suggested that carbohydrates and phytohormones work together to regulate fruit abscission through a complex signal transduction system, however very little is known about the molecular signals involved in this interaction in mango.

Exploring the regulation of fruit drop in mango

This project aims to identify and measure changes in molecular signals associated with early fruit drop. Using a recently developed method, we'll be able to simultaneously measure phytohormone levels and gene expression from a piece of tissue as small as a grain of rice. Using the available mango genome sequences, we are identifying and monitoring genes potentially associated with hormone biosynthesis and signalling, sugar signalling and fruit ripening. We are also able to measure levels of starch and complex sugars, including sucrose, fructose, and glucose, in a variety of mango tissues. With these tools, we'll be able to observe molecular changes occurring throughout mango fruit development and relate these changes to fruit drop. We can then assess how application of PGRs influence these molecular interactions and identify an optimal timepoint for application of PGR treatments to improve fruit retention.

Acknowledgements

The project 'Investigating the control of fruit drop in mango to support innovative solutions for Australian growers (MG21004)' is a strategic levy investment under the Hort Innovation Mango Fund. This project has been funded by Hort Innovation, using the mango research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture. Sophie Jones is a recipient of an Earmarked scholarship from The University of Queensland.

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Unlocking the role of carbohydrates in mango trees: enhancing productivity

Carbohydrates are a key factor controlling fruit bearing capacity of mango trees. An insufficient supply of carbohydrates can impede tree growth, disrupt flowering, fruit development and retention, and lead to irregular bearing. Dr Gerhard Rossouw, from the Queensland Department of Agriculture and Fisheries at Mareeba, is investigating how mango trees use carbohydrates under the five-year National Tree Crop Intensification in Horticulture program (AS18000). This research will help develop management strategies to improve yields and reduce year-to-year fruit bearing variability.

Why is it important to investigate carbohydrates in orchard intensification research?

Intensified orchards, characterised by increased planting density and smaller, highly productive trees, require careful management of carbohydrates for optimal fruit development. The objective of the research being conducted through the National Tree Crop Intensification in Horticulture program is to examine the impact of orchard intensification on carbohydrate use. The findings will guide development of management approaches that promote sustainable and productive orchard systems.

To achieve this, two experiments are underway at the Walkamin Research Station based trial (Mareeba, Far North Queensland). The first aims to investigate the impact of variety and planting density on carbohydrate reserves and their correlation with fruit yields.

The second takes an innovative approach by investigating the impact of extreme cropping on carbohydrate status within the tree and the implications for future productivity. By pushing the boundaries of cropping intensity, the project aims to shed light on the thresholds and limits of carbohydrate reserve use, as well as the potential long-term impacts on orchard productivity.

Carbohydrates, mainly starch and sugars, play pivotal roles in regulating both vegetative growth and crop development in mango trees. During leaf photosynthesis, light and carbon dioxide from the atmosphere are used to generate sugars. These sugars are transported to growing organs like flowers and fruits, providing energy and the building blocks for growth. Excess sugars are stored as starch in various tissues, including the roots, trunk, branches, and shoots. When growing tissues require more sugars than are available from the leaves, starch reserves are converted back into sugars to meet the demand.

A tree with an ample supply of carbohydrates can bear larger and more fruit, producing a higher yield. However, an excessive availability of carbohydrates can lead to disproportionate vegetative growth, disrupting the balance within the tree's canopy. This imbalance can negatively affect productivity and fruit quality. On the other hand, insufficient carbohydrate supply can also have detrimental effects.

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Mango fruit drop: How research is looking for solutions

Ryan Orr, Lindsay Shaw, Sophie Jones, Gerhard Rossouw, Harley Smith, and Geoff Dickinson

"The fruit drop is so bad some people may not even bother to pick."

To hear this after a strong flowering was surprising, and heartbreaking. Flowering, as the first determinant of the year's crop, is often seen as the pivotal step in fruit production. Achieving strong flowering only to be undone by extensive immature fruit drop is devastating.

Each stage in mango fruit development is susceptible to stresses, such as temperature extremes, water constraints or flooding, pest damage, overcropping, or excessive vegetative flush. Often a combination of these stresses determines how much, or how little, crop the tree will retain to harvest.

This year, unfortunately, there are many reports of excessive fruit drop. Here, we will highlight some of the possible causes of fruit drop, how several causes may apply this year, and research underway at the Queensland Department of Agriculture and Fisheries (QDAF), University of Queensland (UQ), Queensland University of Technology (QUT) and CSIRO to support the Australian mango industry.

WHAT CAUSES MANGO FRUIT DROP?

In mango and other fruit tree crops, the dropping, or abscission, of immature fruit is a natural process used to adjust crop load in response to resource availability, but it can be increased due to stress and damage to the tree or fruit. During the growing season, the tree balances the drain on resources from leaves and storage compartments (stems, branches, trunks and roots) to support the growth of flowers, fruits and vegetative shoots.

The first wave of immature fruit drop occurs just after flowering. Typically, flowering occurs in excess of what the tree can support, presumably to compensate for incomplete flower fertilisation, and damage to flowers and fruitlets. QDAF research has demonstrated that only when the majority of flower panicles are removed is harvestable yield is reduced (Orr et al., 2023). Similarly, when immature fruit were slightly thinned in early development, yields were relatively unchanged.

This is because the tree compensates by dropping fewer fruit during late stage development, and also by producing larger fruit (Yeshitela et al., 2004). Unfortunately, it is possible for events outside of natural attrition to cause extreme immature fruit drop and the tree can no longer compensate to restore maximum yield.

Immature fruit drop in excess of natural shedding occurs when a tree is experiencing stress. These stress events can take many forms and work at various geographical scales as shown in Table 1.

Figure 1: Early fruit drop in Walkamin, Queensland.

Table 1: Causes of tree stress and the geographical stage at which they may cause fruit drop.

STRESSOR	SCALE OF EFFECT
Drought	National / Regional
Flood, Cold / Frost, Heat, Wind	Regional
Physical damage, Irrigation failure	Tree / Orchard
Pest / Disease	Fruit / Tree / Orchard
Natural crop shedding	Global

The tree responds to stress in a similar way to humans. Just as our bodies release hormones, such as adrenaline, to manage difficult situations, a mango tree produces specific plant hormones that activate gene networks to cause abscission of immature fruit. As a result, immature fruit drop helps the tree conserve energy, dedicating resources to tree survival and to ensure the remaining fruit reach maturity. While there are many plant hormones that are involved in fruit development, ethylene, abscisic acid and jasmonic acid are the main hormones that control how plants respond to stress (Patharkar and Walker, 2019). These hormones are associated with the abscission of immature fruits and have been shown to increase during fruit ripening (Fenn and Giovannoni, 2021; Patharkar and Walker, 2019; Sawicki et al., 2015).

Given reports of severe fruit drop from across Queensland growing regions in 2024, it is likely the current heavy immature fruit drop is due to weather-related factors. However, isolating a single weather component is challenging, given many aspects of weather change together such as temperature with rainfall or humidity. It is also likely, at least in part, that remarkably heavy flowering and associated natural shedding of excess crop load has played a role.

WHAT RESEARCH IS BEING DONE ON THIS TOPIC?

Understanding the regulation of crop load and developing strategies to minimise immature fruit drop are the objectives of the Hort Innovation mango research and development levy funded project, Investigating the control of fruit drop in mango to support innovative solutions for Australian growers (MG21004).

This project, led by UQ, is focused on exploring molecular signals associated with immature fruit drop, such as changes in hormone activity, carbohydrate levels, and gene signalling. This knowledge is being used to develop tools to mitigate fruit drop, such as plant growth regulator treatments (PGRs). PGRs combat signals associated with natural fruit shedding during development but many are not currently registered for mango.

Two seasons of PGR trials in mango have demonstrated promise at reducing early fruit drop, with a third trial underway this season. This research is not targeted at providing resilience against environmental stresses but seeking improved understanding of the natural process of immature fruit drop, to help manage its impact. For more information on this project see the article in the winter 2023 edition of Mango Matters (Jones et al., 2023).

Genetics for next generation orchards (AS23003), funded by Frontiers developed by Hort Innovation, is undertaking five years of research led by QUT and UQ into the molecular mechanisms behind flowering, fruiting, and vegetative growth to improve breeding, productivity, and climate resilience. This project has a strong relationship with the National Tree Crop Intensification in Horticulture Program (AS18000), also funded by Frontiers developed by Hort Innovation.

The QDAF team has been developing intensive production systems since 2014, with the aim of optimising profitability, resource use efficiency and improving climate resilience through more intensified production systems. The research has increased the understanding of how mango cultivars produce, store, and use energy reserves, a key aspect of buffering impacts of plant stress.

Through the combined outcomes of these research projects, and better prediction of adverse weather events, the mango industry will be better placed to use pre-emptive tools to minimise fruit drop and consequential yield losses.

ACKNOWLEDGEMENTS

Investigating the control of fruit drop in mango to support innovative solutions for Australian growers (MG21004) has been funded by Hort Innovation using the mango research and development levy and funds from the Australian Government.

The National Tree Crop Intensification in Horticulture program (AS18000) and Genetics for next generation orchards (AS23003) are funded through Hort Innovation Frontiers, and contributions from the Australian Government.

Figure 2: Researchers removing the leaves from a Keitt mango tree in Walkamin, Queensland to better understand how energy storage and leaf function regulate fruit drop and retention.

FURTHER INFORMATION

For further information on this topic please contact Dr. Ryan Orr (Ryan.orr@daf.qld.gov.au) or Dr. Lindsay Shaw (Lindsay.shaw@uq.edu.au).

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