Final Report

National Papaya Breeding and Evaluation Program

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PP18000

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Public summary

New red and yellow genetically stable and elite papaya cultivars that meet the agronomic needs of growers and consumer's aesthetic, flavour and sensory preferences have been produced and evaluated. They have been bred for optimal production and quality when grown in either the Tablelands or Coastal regions of northern Queensland. Priority tree agronomic, productivity and fruit quality traits were selected through continuous industry consultation. Field trials conducted in collaboration with major growers showed that the new cultivars produced fruit closer to the ground and of the preferred size range, had thicker trunks and yielded significantly more marketable and thicker fleshed fruit than the industry standard cultivars.

Additional collaboration with the Queensland Department of Agriculture and Fisheries (QDAF) enabled the initial assessment of disease reactions of the new cultivars and other advanced breeding lines to key pathogens. Consumer preference traits were selected by partnering with the Centre for Nutrition and Food Science, Queensland Alliance for Agriculture and Food Innovation (QAAFI), at The University of Queensland. From this, the flavor profiles of the new cultivars were determined and compared with those of the existing red and yellow industry-standard cultivars. The new cultivars possess distinct and consumer preferred flavors. These new cultivars offer potential to the Australian papaya industry for increased marketability and profitability.

Keywords

Papaya, selective breeding, quantitative genetics, varietal development, environmental niches, tree productivity, tree vigour and fruit quality traits

Introduction

Theoverall objective of the project focused on delivering new elite, genetically stable cultivars that meet the needs of growers and the preferences of consumers, and that are adapted to key growing areas in distinct agro-climatic conditions: Tablelands and Coast of Tropical North QLD Queensland.

These varieties will contain multiple consumer and producer-preferred traits and thereby represent a new horizon for expanding the marketability and profitability of the Australian papaya industry.

Previous projects funded by Hort Innovation and delivered by the Griffith University as the collaborative papaya breeding program (PP10005 and PP15000) had developed and validated a suite of highly accurate and robust breeding tools for the selection of a raft of targeted agronomic and fruit quality traits. Subsequently, the team has delivered several red and yellow F3 lines that out-perform the current industry and market standard varieties.

The next step towards the production and delivery of elite cultivars was to stabilise individual lines that are adapted to region-specific environments and farming systems was to be achieved through repetitive single plant reselections from each F3 segregating population (until F6:F7) and at multiple sites within each growing region, at which a level of ~99% genetic stability and a known level of environmental influence will be achieved.

The research partner from the Centre for Nutrition and Food Sciences within the Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, undertook the flavour and sensory work to examine selections with stability of these priority consumer-derived traits; and the partner from the Queensland Department of Primary Industry, Mareeba undertook the disease evaluation to major diseases of the new selections.

Methodology

The parental lines of ‘red’ and ‘yellow’ papaya were assessed and chosen by evaluating the performance of key agronomic and fruit quality traits across several trial sites established in the Tableland and coastal regions of Tropical North Queensland.

The parents of red papaya (Solo × Holland) and yellow papaya (Honey Drew × Starpicked) were crossed to produce F1 hybrids, resulting in a bi-parental mapping population.

The F1 hybrids of each type (red and yellow) were then self-crossed to maximise the segregation of alleles in the F2 generation. A selective breeding method was employed to select individuals in the F2 generation, concentrating on key desirable agronomic, tree productivity, and consumer-driven fruit quality traits.

Desirable plants selected in the F2 were self-pollinated (using controlled self-pollination) over several generations to generate genetically stable advanced-generation breeding lines. A closed hermaphrodite (bisexual) flower was chosen for self-pollination in red papaya, whereas in yellow papaya, female plants were self-pollinated with the male flower of the same genotype. These cycles were repeated over six generations (red papaya) to create genetically stable F7 breeding lines that were subsequently outcrossed to develop F1 papaya hybrids with key traits meeting industry requirements and consumer demands and to create to create genetically stable F6 breeding lines (yellow papaya).

The following key activities were delivered through the project.

PART 1. Stabilise breeding lines of red and yellow papayas to produce SSD F6:F7 seed bulk (Griffith University)

PART 2. Production and assessment of F1 hybrids (Griffith University)

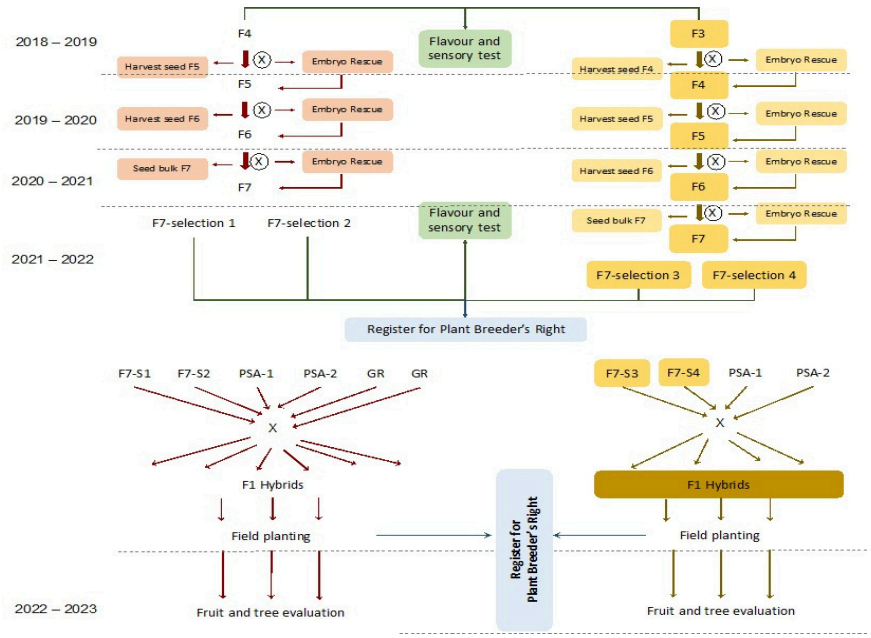
PART 3. Molecular markers for trait selection and genotype profiling for inbred lines and F1 hybrids (Griffith University)

3.1. Development of molecular markers for fruit quality trait selection

3.2. Development of genotype profiles for new cultivars

PART 4. Market-preferred papaya flavours and other sensory types (Heather Smyth, University of Queensland)

Below is a schematic showing the logical flow of the proposed breeding towards final cultivar release within PP18000 by 2022. Where ⊗ = self-pollination, x = cross pollination, PSA-1 and PSA-2 = current parental lines, GR = red papaya from germplasm project (PP15000), red colour indicates breeding for red papayas and yellow colour indicates breeding for yellow papaws, fruit and tree evaluation include tree productivity, fruit quality, field disease incidence and post-harvest disease evaluation.



PART 5. Evaluation of reaction to important papaya disease-causing pathogens (Department of Agriculture and Fisheries; DAF)

PART 6. Registration of the newly developed cultivars with Plant Breeder’s Rights (Griffith Enterprise, Griffith University)

Results and discussion

Semi-commercial trials of elite red and yellow genotypes (F6-F7 generations) along with newly developed F1 red hybrids were conducted in the Coastal (C1; Jo Zappala Farms) and Tablelands [(T1; Lecker farms) and (T2; Rocky Top farms)] regions. Five elite red genotypes F6-F7 generations were produced, including two, bred for the Coastal [(RS1; Sunlight 1) and (RS2; Sunlight 2)] and three for the Tablelands [RS3, (RS4; Sunlight 3) and RS5] regions. Additionally, ten red F1 hybrids (RH1, RH2, RH3, RH4, RH5, RH6, RH7, RH8, RH9 and RH10) and two elite yellow genotypes (Moonlight 1 and Moonlight 2) were selected based on their trait gain percentages compared to the red (RB1) or yellow (1B) industry standards. These were selected through consultation meetings with the papaya industry (05 August 2022 at QDAF Mareeba, 25 August 2022 at Lecker farms and 02 September 2022 at Zappala farms) and included in the semi-commercial trials. The Papaya Breeder (Dr Fawad Ali) developed and formalised semi-commercial trial agreements with the growers at all trial sites. Seed of the most promising breeding and F1 red hybrid lines was provided to the Coastal and Tablelands growers on 2nd September 2022.

Seedlings were grown according to industry standard practices and field transplanted from November to December 2022.

Fruit quality traits, including fruit weight (g), cavity width (cm), flesh thickness (cm) and sweetness (soluble solid contents; oBrix) were assessed following the papaya evaluation handbook (Kanchana-udomkon et al., 2021). Noting, high rainfall and subsequent nursery issues impacted tree evaluation numbers at the Tablelands sites in the 2022-2023 season. A weighted breeding index algorithm was developed and applied to each breeding line, and the trait gain percentages (%) over the standard commercial cultivars (RB1or 1B) were calculated (Table 1).

Table 1. Trait gain percentages (%) achieved for each trait compared to the commercial 'red-RB1' or 'yellow-1B' were calculated for each new line bred for the Coastal (RS1 and RS2) or Tablelands (RS3, RS4, RS5, Moonlight 1 and Moonlight 2) region.

Breeder accession and PBR name	Genotype code, type and locational breeding objective	Generation	Mean agronomic and productivity gains (%) over RB1 or 1B	Mean fruit quality gains (%) over RB1 or 1B	Weighted Breeding Index
C1-6.4.3.12 (Sunlight 1)	RS1 (red for the Coast)	F7	Set fruit 38% closer to the ground than RB1. Trunkcircumference10% more than RB1 10% more marketable fruit than RB1	Medium sized fruit of av. 907g with moderate aroma and improved sweetness (oBrix = 20%) over RB1	1.29
C1-6.4.1.12 (Sunlight 2)	RS2 (red for the Coast)	F7	Set fruit 24% closer to the ground than RB1. Trunkcircumference4% more than RB1 12% more marketable fruit than RB1	Medium size fruit of av. 1022g withmoderatearomaand improved sweetness (oBrix = 24%) over RB1	1.27
T2-6-5.27.30	RS3 (red for the Tablelands)	F6	Set fruit 46% closer to the ground than RB1. Trunkcircumference 16% more than RB1. 1-2% more marketable fruit than RB1	Smaller size fruit of av. 750g with moderate aroma and sweeter fruit (oBrix = 20%) over RB1	1.19
T2-6-3.21.28 (Sunlight 3)	RS4 (red for the Tablelands)	F6	Set fruit 49% closer to the ground than RB1. Trunkcircumference 15% more than RB1 18% more marketable fruit than RB1	Medium size fruit of av. 950g with moderate aroma and sweeter fruit (oBrix = 20%) over RB1	1.28
T2-6-5.27.12	RS5 (red for the Tablelands)	F6	Set fruit 34% closer to the ground than RB1. Trunkcircumference 16% more than RB1 1% more marketable fruit than RB1	Medium size fruit of av. 918g with moderatearomasweeter fruit (oBrix = 17%) over RB1	1.07
ML-2-9-3 (Moonlight 1)	YS6 (yellow for the Tablelands)	F6	Set fruit 27 % closer to the ground than 1B. Trunk circumference 31% more than 1B.	Medium size fruit of av. 1200g with moderate aroma and sweeter fruit (oBrix = 11%) over 1B.	1.18

			12% more marketable fruit than 1B. Set fruit		
ML-2-8-17 (Moonlight 2)	YS7 (yellow for the Tablelands)	F6	12% closer to the ground than 1B. Trunk circumference 26% more than 1B. 16% more marketable fruit than 1B	Medium size fruit of av. 1300g with moderate aroma and sweeter fruit (oBrix = 9 %) over 1B	1.24

Initial grower partner field assessments

Field site evaluations were conducted in 2023 on 17th May at Zappala farm (C1), on 30th June at Lecker farm (T1), and on 6th July at Rocky Top farm (T2). At each site and date, the F6-F7 generation breeding lines, F1 hybrids and commercial standards were visually evaluated by the growers for key desirable agronomic and productivity traits based on tree physical appearance for tree strength and fruit productivity and according to methods in the Papaya Handbook ver. 2 (Kanchana-udomkan *et al*., 2018, see Appendix 1). Fruit quality was also directly visualised (Figure 1).

At each site, lines were ranked based on trunk circumference, height to the first fruit, tree canopy physiology, and number of marketable fruits produced per tree. The trial site-specific ranks were as follows:

1. Lecker Farms (T1) Director Gerard Kath ranked red breeding line RS4 and red F1 hybrids RH6, RH9 and RH10 higher than RB1.
2. Rocky Top Farm (T2) Director Chris Maisel ranked red breeding lines RS3 and RS4 and the red F1 hybrids RH1, RH9 and RH10 higher than RB1.
3. Zappala Farm (C1) Director Joe Zappala ranked red breeding lines RS1 and RS2 and red F1 hybrids RH1, RH9 and RH10 higher than RB1.
4. Moonlight 1 and Moonlight 2 were both ranked well above 1B by the Directors of the T1 and T2 farms.

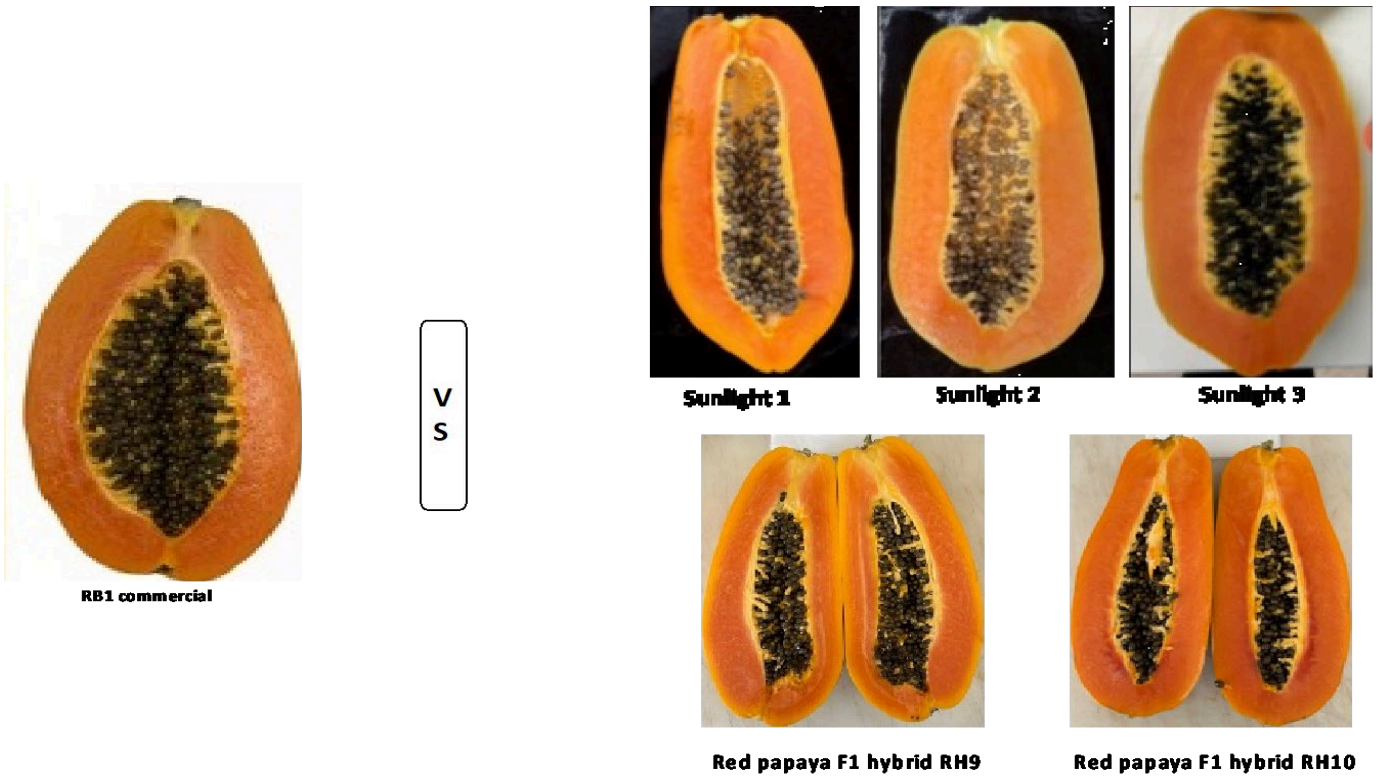


Figure 1. Images (taken by Dr Fawad Ali) depicting desirable fruit quality traits of the three best red papaya breeding lines [(Sunlight 1 (RS1) and Sunlight 2 (RS2) bred for the Coastal) and (Sunlight 3 (RS3) bred for the Tablelands) regions] along with the two best novel F1 hybrids (RH9 and RH10) compared to commercial red 'RB1'.

Comparative performance of red papaya lines in the Tablelands region A total of 1048 seedlings from across the five F6-F7 red advanced and ten F1 red hybrid lines were planted at T1 or T2. All evaluations were undertaken using the methods in the Papaya Handbook ver. 2 (Kanchana-udomkan *et al*., 2018). Nursery issues affected the commercial red cultivar RB1, RS1 (Sunlight 1) and RS2 (Sunlight 2) and the RH5 and RH7 hybrid lines.

F6-F7 red fruit weight ranged from 600g to 1200g, and F1 hybrid fruit weight ranged 600g to 1200g compared to RB1 (~1150g; Figure 2). Significant gains were made in reducing the fruit cavity width for RS3, RS4 and RS5 compared to RB1. At T1, these ranged from -30% to -40% and at T2 these ranged from -31% to -45%. For the F1 hybrids (RH1 to RH10) at T1 these ranged from -20% to -42% and at T2 these ranged from -18% to -49% (Figure 2). Flesh thickness was significantly increased for RS3, RS4 and RS5 compared to RB1, with trait gain percentages at T1 from 2% to 12% and at T2 from 2% to 14% (Figure 2). The F1 hybrids RH1 to RH10 generally had thicker flesh than RB1 with trait gains at T1 from 1% to 16%, and at T2 from 2% to 16%, with the exceptions of RH1, RH4, RH6 and RH7 at T1, and RH4 and RH7 at T2 (Figure 2). All selected F6-F7 lines were sweeter than RB1, ranging at T1 from 10% to 19%, and at T2 from 8% to 20% (Figure 2). Also F1 red hybrid fruit was from 1% to 20% sweeter at T1 and from 2% to 22% sweeter at T2 than RB1, with the exceptions of RH4 and RH7 (Figure 2).

Comparative performance of red papaya lines in the Coastal region

A total of 1276 seedlings from across the five F6-F7 red advanced and ten F1 red hybrid lines were planted at C1 and evaluated using the methods outlined in the Papaya Handbook ver. 2 (Kanchana-udomkan *et al*., 2018).

F6-F7 red fruit weight ranged from 800g to 1100g, compared to RB1 (~1000g). Meanwhile, F1 hybrid fruit weighed from 600g to 1200g (Figure 3). Compared to RB1, trait gains were significant. The fruit cavity widths of RS1, RS2, and RS3 at C1 were reduced from -40 % to -50%, and for the F1 red hybrids they were reduced from -17% to -44% (Figure 3). Compared to RB1, the RS lines at C1 had 20% to 45%, and the F1 hybrids had 8% to 25% thicker fruit flesh (Figure 3). Compared to RB1 at C1, all newly developed germplasm except some F1 Hybrids (RH1, RH2, RH4, RH5, RH7 and RH8) were from 2% to 26% sweeter (Figure 3).

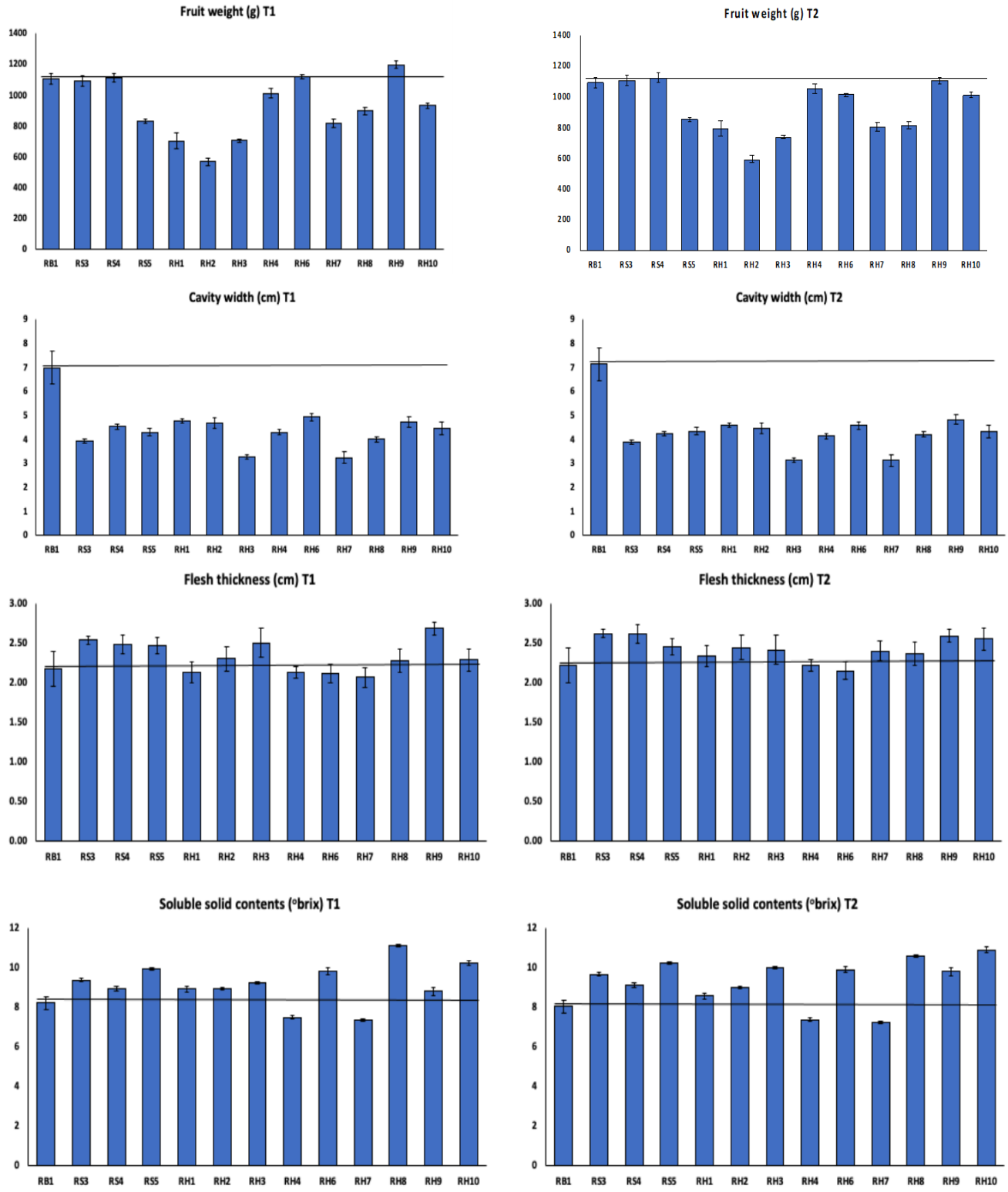


Figure 2. RS3, RS4, RS5, and RH1-RH10 fruit trait variation at T1 and T2 in comparison to RB1.

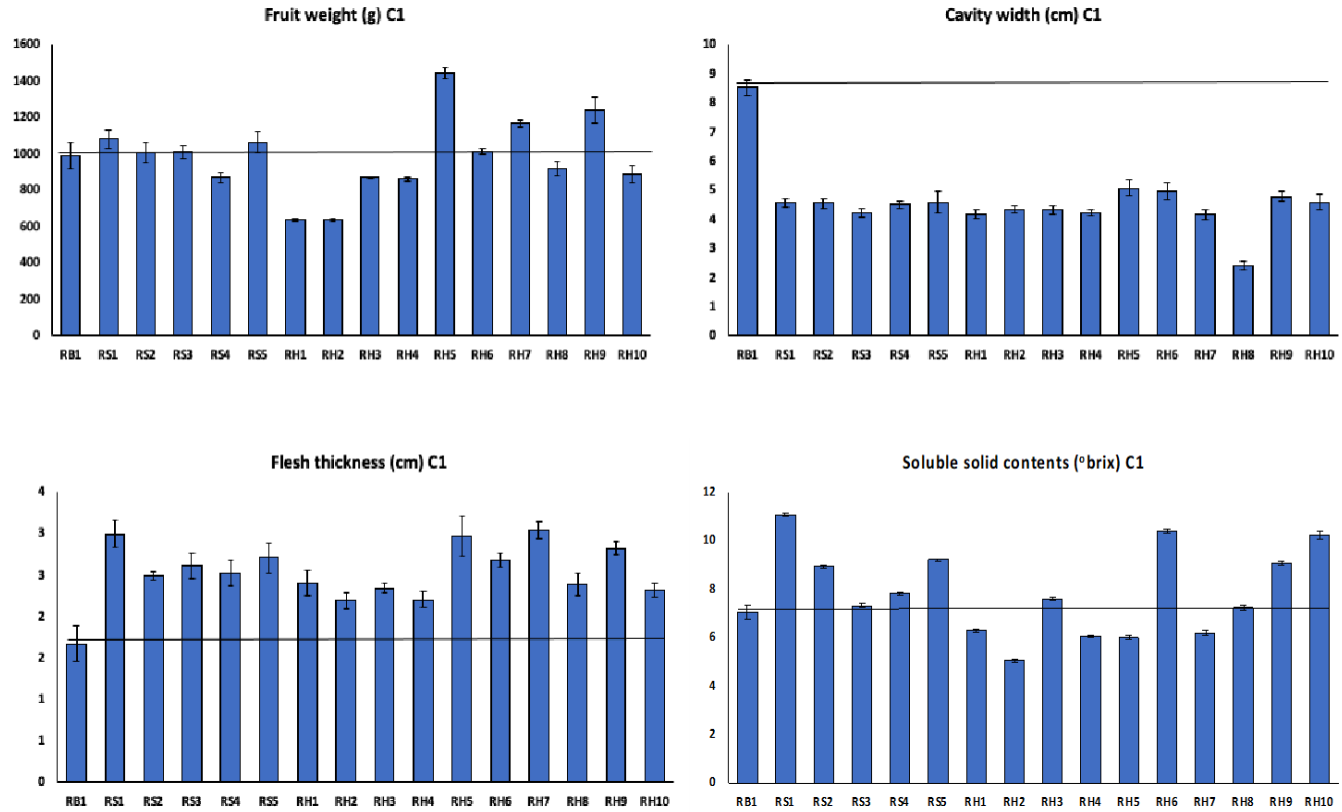


Figure 3. Red F6-F7 and F1 hybrid fruit trait variation compared to RB1 at C1.

Comparative performance of yellow papaya lines in the Tablelands region Atotal of 536 seedlings from two F6-F7 families of yellow elite genotypes and the commercial cultivar '1B' were planted at T1 and T2 (Table 1). Evaluations were conducted according to protocols outlined in the Papaya Handbook ver. 2 (Kanchana- udomkan *et al*., 2018). Overall, the yellow F6 fruit were significantly different from the commercial 1B cultivar (Figure 4). The average fruit weight of the yellow F6 at T1 and T2 ranged from 1200g to 1350g, significantly lighter than 1B (2500g; Figure 4). Compared to 1B, the F6 cavity widths at T1 were from -12% to -28% narrower and at T2 from -14% to -30% narrower (Figure 5). All of the F6 had thicker fruit flesh than 1B, with trait gain percentages at T1 of 21% to 29% and at T2 of 20% to 33% (Figure 5). All F6 fruit were sweeter than 1B, with a gain at T1 of 20% to 27% and at T2 of 21% to 30% (Figure 5).

Figure 4. Images (taken by Dr Fawad Ali) depicting desirable fruit quality traits of the two best yellow papaya breeding lines Moonlight 1 (ML2-9-3) and Moonlight 2 (ML2-9-3) and compared to commercial yellow '1B'.

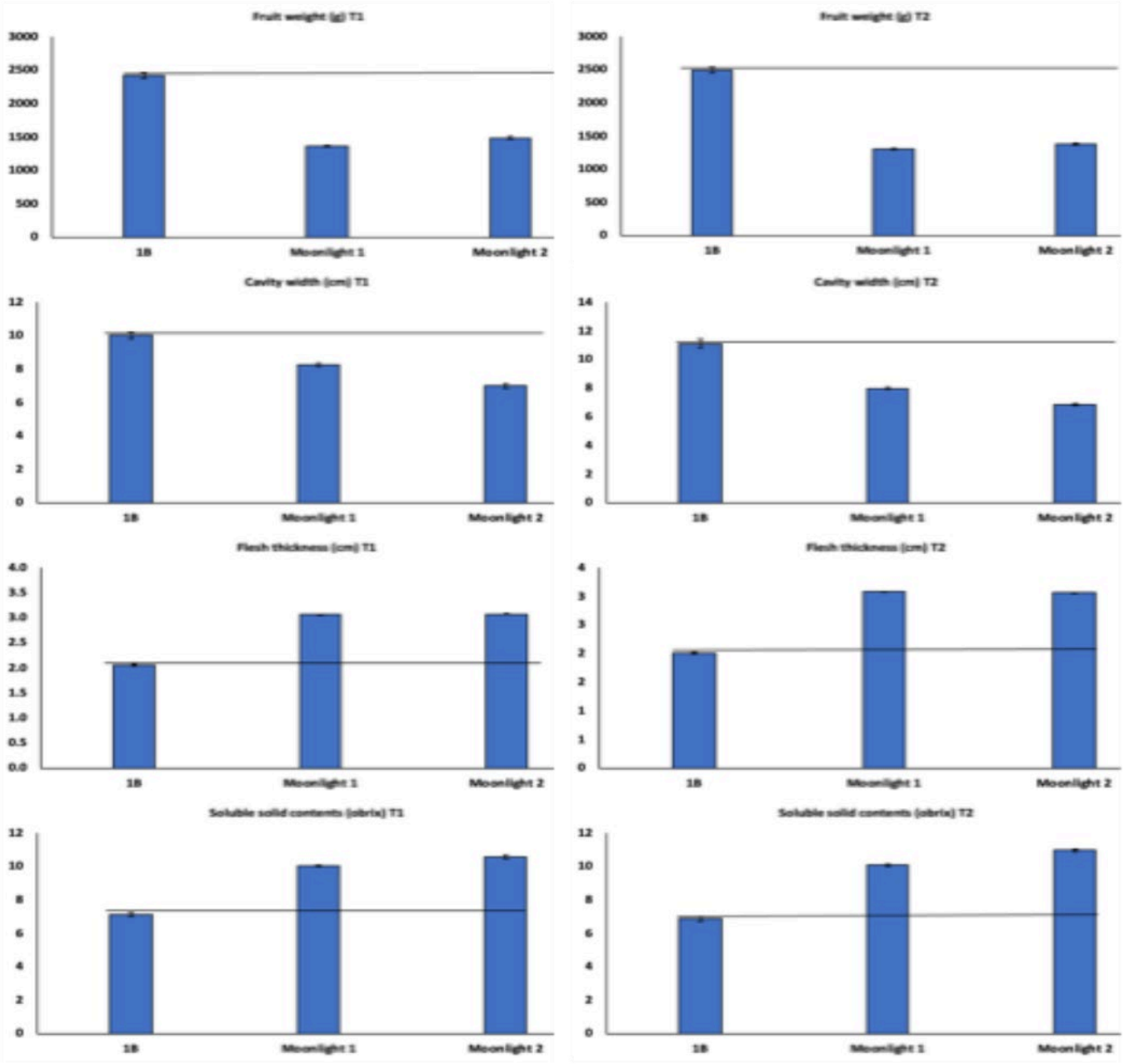


Figure 5. F6 yellow breeding lines ML2-9-3 (Moonlight 1) and ML-2-8-17 (Moonlight 2) evaluated at T1 or T2, compared to 1B

Genotyping and genetic relationships determined among PP18000 germplasm. Genotyping of 37 genotypes, included advanced generation PP18000 red and yellow breeding lines (F6-F7 generations), F1 red papaya hybrids, commercial cultivars (red-RB1 and yellow-1B), and international germplasm collection, was performed using Diversity Array Technology Sequencing (DArTSeq; https://www.diversityarrays.com) from which single nucleotide polymorphism (SNP) loci were identified and compared among genotypes. Phylogenetic analysis was carried out using IQ- TREE v. 2.1.2 (Nguyen et al., 2015) under maximum likelihood as the optimality criterion, using ModelFinder (Kalyaanamoorthy et al., 2017) for model selection and measuring bootstrap resampling frequencies and SH-aLRT (Guindon et al., 2010) from 1,000 pseudoreplicates. Two main genetic clades were identified that separated the genotypes into red or yellow phenotypes (Figure 7). Recombinant inbred lines were most closely related to each other. The red papaya breeding lines bred for the Tablelands and Coastal regions appeared in a single clade, indicating that they were derived from 'Solo' and 'Holland' backgrounds. The SNP 'fingerprints' for individual genotypes will be useful in molecular identification, particularly those that discriminate the industry standard and newly developed cultivars (Figure 7).

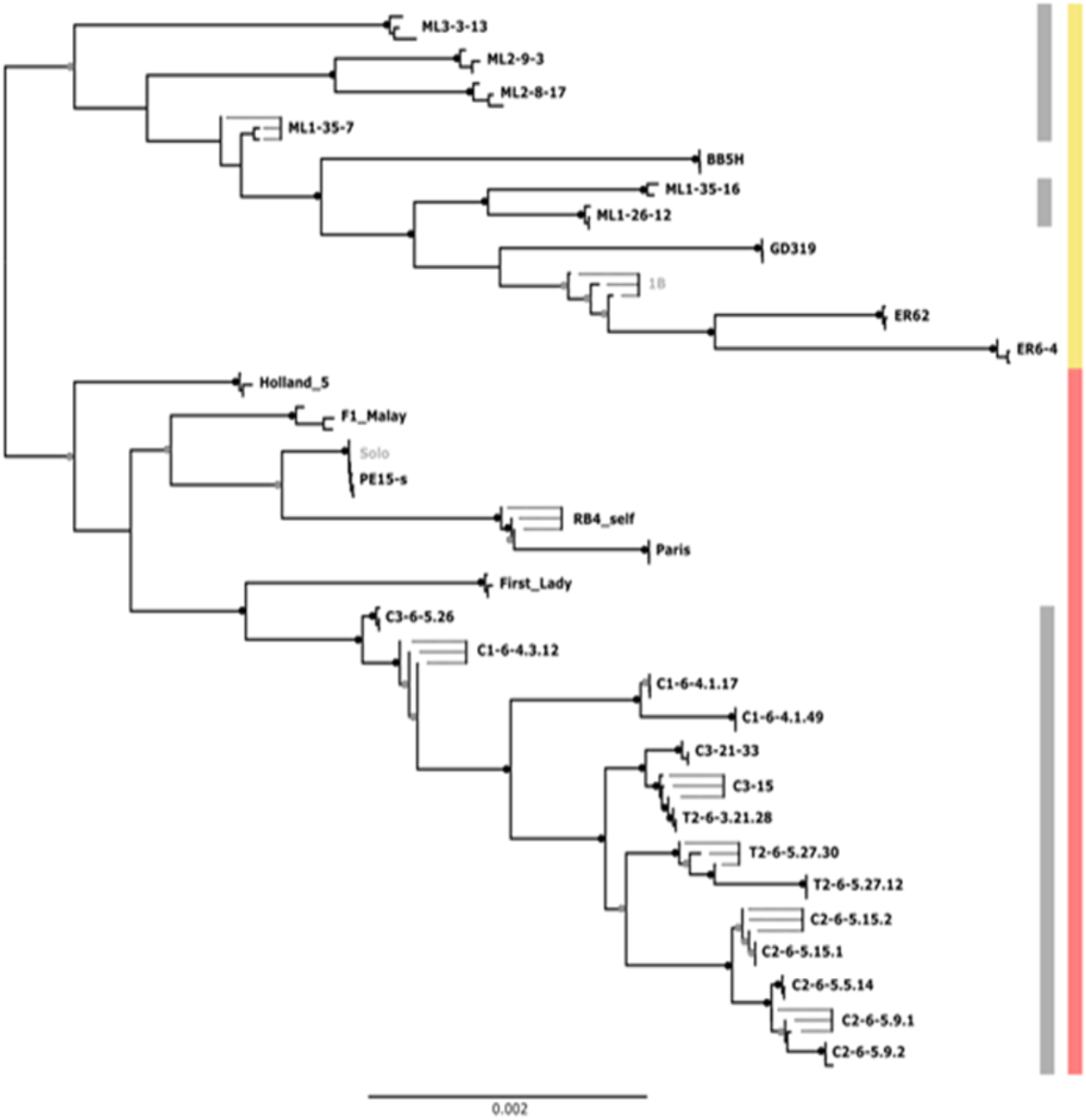


Figure 7. Phylogenetic tree representing SNP relatedness among the papaya germplasm. The scale bar represents the number of nucleotides substitutions per site, red bar = red genotypes, yellow bar = yellow genotypes and grey bar = RILs.

Outputs

Table 2. Output summary

Output	Description	Detail
Trial/location-specific trait data of red papaya breeding lines and comparison with RB1.	The trial/location-specific trait data from semi-commercial trials of five elite red papaya genotypes (F6-F7 generation), including two new cultivars that were bred and selected for the Coastal [(RS1: Sunlight 1), (RS2: Sunlight 2)] and three cultivars bred and selected for the Tablelands [(RS3), (RS4: Sunlight 3), (RS5)] regions. Trials were conducted at one Coastal trial site (C1: Zappala farm) and two Tablelands trial sites (T1: Lecker farm and T2: Rocky Top farm).	The trial/location-specific environmental impacts data on the key desirable traits assessed across the new cultivars. The significant genetic gains achieved ranged from 2% to 60% percent for all selected traits within the new cultivars and F1 hybrids over the industry standard cultivars RB1 and 1B. At site T1 red F1 hybrids RH6, RH9 and RH10 were ranked higher than RB. At site T2 red F1 hybrids RH1, RH9 and RH10 were ranked higher than RB1. At site C1 red F1 hybrids RH1, RH9 and RH10 were ranked higher than RB1.
Trial/location-specific trait data of novel red papaya hybrids and comparison with RB1.	The trial/location-specific trait data from semi-commercial trials of ten red F1 hybrids (RH1 to RH10). Trials were conducted at T1, T2 and C1.	
Trial/location-specific trait data of yellow papaya breeding lines/cultivars and comparison with 1B.	The trial/location-specific trait data for two elite yellow genotypes/cultivars (F6 generation; Moonlight 1 and Moonlight 2). Trials were conducted at T1 and T2.	
Genetic relationships revealed among the parental genotypes, advanced lines, new cultivars and broader germplasm.	DArTSeq generated SNPs set. Revealed relationships among germplasm and confirmed heterogeneity produced among the F1 hybrids.	The SNP 'fingerprints' for individual genotypes will be useful in molecular identification, particularly those that discriminate the industry standard and newly developed cultivars.
Plant Breeder Rights (PBR) for papaya cultivars	PBR applications including Stage I and Stage II	Varieties protected through PBR (Sunlight 1, Sunlight 2 and Sunlight 3)

Outcomes

Table 3. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Genetically stable advanced generation breeding lines, new hybrids and cultivars of red and yellow papaya with significant trait gain percentages over the current commercial standard cultivars RB2 and 1B.	Aligns to the project outcome and strategy – to developed new cultivars that are aligned with grower and consumer guided requirements and that will provide a marketing edge for increased productivity and salability.	Genetically stable new cultivars with significant trait gain percentages over existing cultivars.	**Sunlight 1** sets fruit 38% lower to the ground, with a 10% thicker trunk circumference and 10% more marketable fruit than RB1. The fruit is medium-sized (~900g) with a moderate aroma and is 20% sweeter than RB1. **Sunlight 2** sets fruit 24% lower to the ground, with a 4% thicker trunk, and produces 12% more marketable fruit than RB1. The fruit is medium-sized (~1000g) with a moderate aroma and is 24% sweeter than RB1. **Sunlight 3** sets fruit 49% lower to the ground, with a 15% thicker trunk, and produces 18% more marketable fruit than RB1. The fruit is medium-sized (~950g) with a moderate aroma and is 20% sweeter than RB1. **Moonlight 1** sets fruit 27% lower to the ground, with a 31% thicker trunk and produces 12% more marketable fruit than 1B. The fruit is ~1200g with a moderate aroma and is 11% sweeter than 1B. **Moonlight 2** sets fruit 12% lower to the ground, with a 26% thicker trunk, and produces 16% more marketable fruit than 1B. The fruit is ~1300g with moderate aroma and is 9% sweeter than 1B.
A set of SNP markers	Aligns to the investment requirement to ensure protection of the new cultivars	High throughput DNA sequencing technology (DaRTseq array) was performed to produce SNP markers that can be applied to uniquely	Observed heterozygosity (*Ho*) was calculated from single nucleotide polymorphism (SNP) data and unique SNP markers for new cultivars

		identify new cultivars.	identified.

Monitoring and evaluation

Table 4. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous opportunities improvement The continual improvement by
Report information and data on progenies generated.	F7 generations of red and F6 generations of yellow papaya progenies were generated from which several lines were proposed as new cultivars. Further, novel F1 red hybrids (RH11, RH12, RH13, RH14 and RH15) were produced and seed was provided to the growers for propagation and transplantation into the field in 2024.	identifying appropriate grower partners who would be able to participate in commercial trials and to provide further feedback through field evaluation and gain exposure to new varieties.
Comparative performance data from evaluation trials	Tree performance data of the advanced generation breeding lines and F1 red hybrids was collected in October 2023 and reported.	Tree performance data would be generated by working closely with the growers to design the experimental trials and collected uniform data using papaya evaluation handbook ver. 2
International germplasm collection information.	Select broad germplasm was re-evaluated at Rocky Top farms, and data was collected in October 2023. Global collaboration was established to broaden the Australian papaya germplasm collection and to seek germplasm that may benefit the Australian papaya industry to improve key traits.	To broaden the genetic bottleneck of Australian papaya gene pool, to introduce new traits and select those that are commercially important and provide valuable genetic stocks for developing climate resilient cultivars

The Papaya Reference Group (PRG) meeting was held at Cairns (Bolands Centre Offices) on Tuesday, the 21st of November 2023; Dr Fawad Ali presented (1 hour and 30 minutes) and compiled the information in a simple layout with bar graphs.

Meeting minutes were prepared, and discussion points and outcomes were noted below:

Name of the participant	Position/organization
Gerard Kath	Chairman Papaya Seeds Australia; Lecker Farms
Jo Zappala	Director Zappala Farms
Chris Maisel	Director Rocky Top Farms
Dr Ben Callaghan	& R&D Manager and Breeding Investments Hort Innovation Australia
Sarah Strutt	Industry Services and Delivery Manager, Hort Innovation
Jane Wightman	Head of Industry Services and Delivery, Hort Innovation Papaya Breeding and Genetics Griffith University
Dr Fawad Ali	
Tom McCue (online)	R&D Manager Hort Innovation

Recommendations

Possible future directions for follow on R&D activities of Papaya Industry delivered by Dr Fawad Ali (21st November 2023 at Boland Centre Offices, Cairns, Queensland, Australia).

Proposed title: Seed multiplication and commercial assessment of elite papaya genotypes

Key points discussed with industry partners and stakeholders.

- Assess the stability of traits in F1 hybrid in fully commercial trials.
- Optimising farming practices, e.g. Nitrogen use, pests and diseases, etc.
- Deeper investigation for more accurate future selection of the genetic components of the top preferred traits. Implementing the bio-economic quantitative genetic model will rank traits in elite papaya genotypes.
- Development of novel mapping populations
- Development of novel F1 hybrids (red-and-yellow papaya types)
- Out-crossing with targeted (that favourite types)
- Linking to Frontiers funded projects guided by industry/breeder needs.
- A further collaboration with the potential to introduce new international germplasm.
- Based on the relationship developed through PP18000, germplasm from South America (focusing on Brazil and Mexico) and Indo-Pacific regions (Malaysia, the Philippines, and Thailand) could be used.

Recent developments for the future of the papaya industry will assist with further research for the papaya industry.

- F7 generation red papaya breeding lines were developed for Tablelands.
- Five (5) novel F1 hybrids were developed for the Tablelands and Coast.
- Seed bulking crosses were done for RH9 and RH10 for commercial trials.
- Three new mapping populations were developed using RH1, RH9 and RH10 based on the flavour profile, fruit sweetness, tree agronomy and productivity traits.
- The F7 generation of yellow papaya breeding lines
- Sex-determination assay
- Practical application of the project findings
- Possibilities of future RD&E that directly flow from the work undertaken and its results

Refereed scientific publications

Journal article

1. Ali, F.; Kanchana-udomkan, C.; Ford, R. The Inheritance Pattern of Key Desirable Agronomic and Fruit Quality Traits in Elite Red Papaya Genotypes. Horticulturae 2022, 8, 845. https://doi.org/10.3390/horticulturae8090845
2. Fawad Ali and Rebecca Ford. (2024) The use of seedling root and shoot traits to predict the saleable yield of elite red papaya genotypes (In press *Euphytica; Springer; Research Article*).
3. Nantawan, U., Kanchana-udomkan, C., Bar, I. and Ford, R. (2019) Linkage mapping and quantitative trait loci analysis of sweetness and other fruit quality traits in papaya. BMC Plant Biol 19, 449 (2019). https://doi.org/10.1186/s12870-019-2043-0

Chapter in a book or paper in conference proceedings

Conference Full-length Oral talk (-Presentations)

1. Fawad Ali and Rebecca Ford. Genetic diversity underpinning papaya improvement. Plant and Animal Genome Conference (PAG), 19-22 September 2023: Westin Perth-Western Australia.
2. Fawad Ali, Chutchamas Kanchana-udomkan, Ido Bar and Rebecca Ford. Papaya Breeding program-PP18000 Genetic diversity underpinning key fruit quality traits. Sensory Genetics Workshop (19th of October 2023), Griffith University Nathan campus, Brisbane, Queensland-Australia
3. Fawad Ali, Chutchamas Kanchana-udomkan, Ziwei Zhou, Vino Rajandran and Rebecca Ford.
4. Rounding the curve towards registration of new papaya varieties. Australasian Plant Breeding Conference, Gold Coast (8-12 May 2022), QLD-Australia.
5. Fawad Ali, Chutchamas Kanchana-udomkan, Ido Bar and Rebecca Ford. Breeding Update PP18000, current activities and future targets. Sensory Genetics workshop held at the University of Queensland, Long Pocket Campus, Indooroopilly (20 Oct 2022).
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Intellectual property

ProgresstowardsPlant Breeder’s Rights protection of new cultivars

Three novel cultivars of red papaya varieties (Sunlight 1, Sunlight 2 and Sunlight 3) have been protected by applying plant breeder’s rights (PBR) through Intellectual Property (IP) Australia.

Appendices

CONFIDENTIAL - PBR details for papaya varieties and associated information provided separately to Hort Innovation to assist with PBR and future commercialisation of papaya varieties.

Appendix 1 - Papaya Evaluation Handbook 2nd Edition

Appendix 2 – Final Presentation to Papaya SIAP

Appendix 1 - Papaya Evaluation Handbook 2nd Edition

Papaya Evaluation Handbook

Productivity and fruit quality traits

Second edition/August2021

Project Number: PP18000

Authors: ChutchamasKanchana-udomkan,UsanaNantawanand Rebecca Ford

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Introduction

Productivity traits and visual appearance are the first quality determinants made by growers, wholesalers, retailers or consumers. After consulting with a reference group of papaya growers, breeders and marketers from Queensland, important traits which should be considered in applied papaya breeding were identified. They include those relating to a tree's productivity: total saleable yield, consistent fruit set over time (i.e. no yield gap), consistent fruit size, ease of harvest and disease resistance, and to fruit quality traits: appearance, fruit shape, flesh colour, sweetness and flavour. These traits are varied and quantitativelyexpressed inpapaya germplasm.

The first edition of Papaya Handbook was published in July 2017. It was referred to IBPGR (1988) and Kanchana-udomkan(2015). The aim was to provide a standard evaluation procedure for routine selection. The information in the handbook was provided by experienced growers, researchers, breeders and marketers.

The second edition is developed to publish the updated version that applied to PP18000: National Papaya Breeding and Evaluation Program. This edition includes weighting index for each trait, the formula to calculate breeding index for the crop, the colour scale was compared to the Royal Horticultural Society colour chart and given the colour code, and table for recording data is provided.

Objectives

The main objective of this handbook is to develop a standard protocol to evaluate papaya tree productivity and fruit quality for robust, reliable and practical evaluation.

Overview

Evaluations of papaya trees are to be performed three times over the tree development. The list of traits to be assessed in each evaluation is presented in Table 1.

Table 1: Listoftraits for evaluation for papaya fruit quality and tree productivity.

No.	Tree age	Productivity traits	Fruit quality traits
1	5 months	Sex type Height to first fruit Trunk circumference Number of side shoots Peduncle length	
2	10-12 months	Peduncle length Saleable yield Yield gap Number of non-marketable fruit Fruit size (fruit length, width and weight) Cavity size (cavity length and cavity width)	Skin gloss Skin freckle Skin colour Fruit firmness Fruit shape Teat shape Stalk insertion Cavity shape Flesh colour Flesh colour consistency Flesh thickness Flesh texture Flesh sweetness (°Brix)
3	15 months	Same as the 2nd evaluation	Same as the 2nd evaluation

Fruit and Tree Handling Procedure

Tree Evaluation:

Papaya trees are labelled using a permanent marker to write downonthe tree trunk for an individual line number.

The trees are recorded for sex type, measured for trunk circumferences and height to first fruit in centimetre using a measuring tape, counted for number of side shoots for large (more than 10 cm), medium (between 5-10 cm) and small (less than 5cm), and recorded for peduncle length. A table to record this dataset is presented on page 28. The detail methods to evaluate each traitare presentedinProductivitytraits section(Page 9-16).

Fruit Evaluation:

Atatrialsite:threepapayafruitpertreearelabelledusingapermanentmarkertowrite downonthe skin. The fruitare thento harvestand place ina cardboard box.

Inalaboratory:Papayafruitarecleanedusingadampcloth.Thefruitarethenevaluated for external appearance including skin characteristic, shape, fruit firmness, fruit weight. After that the fruit are then longitudinally section to assess for fruit size, cavity and flesh characteristic. A table to record this dataset is presented on page 28. The detail methods to evaluate each trait are presented in Productivity traits section (Page 17-27).

Clean fruit using a damp cloth

Evaluate for external appearances: skin characteristics and shape

Hand pressure for fruit firmness and weight the fruit on a digital scale.

Fruit is longitudinal sectioned and evaluated for internal appearances including fruit width and length, and flesh characteristics. Then, the fruit is cross sectioned to assess cavity characteristics.

Fruit is cut in in 3 x 3 cm cube to be assessed for flesh texture and °Brix

Figure:Summary of fruit evaluation procedure in a laboratory

Weighted breeding index

Followingconsultationwith thebreedersandmarketers,a rakedlist of breeding priority traits was produced. This was then used to develop a weighted selection index. This was also achieved by incorporating the method of Herrington et al. (2012), who suggested to include cost of production and profitability of the crop and then weight the traits according to investment.

Step 1: Identify traits and their industry/consumer-derived preferred value or range. Traits and details are presented on Table 1.

Table 2: Traits and their industry/consumer-derived preferredvalue or range

Traits and their industry/consumer-derived preferredvalueor range	
Fruit weight:	Yellow: 0.9 – 1.5 kg
	Red:0.9–1.2kg
Peduncle length:	Medium or long
Fruit shape:	Yellow:Roundshape
	Red:Elongate shape
Stalk insertion:	Flattened (#2)
Teat shape:	Flat (#3)
Cavityshape:	Round (#1)
Flesh colourconsistency:	100% flesh colour consistency

Weighted breeding index

Step 2: Comparing traits with the value of industry standard varieties either red or yellow papaya and calculate weighted selection indices using the formula on Table 2.

Table 3: Traits and calculation of breeding index for tree productivity and fruit quality traits

Traits expected to have a higher value than in the industry standard		***Traits expected to have a lower value than in the industry standard***	
Weighting score (A)	***List of traits:***	***Weighting score (B)***	***List of traits:***
1	Saleable yield	0.75	Height to first fruit
0.25	Trunk circumference	0.5	Number of side shoots
1	Flesh ratio	0.75	Skin freckle
0.25	Skin gloss	1	Number of non-saleable fruit
0.25	Skin colour		
0.75	Fruit firmness		
0.75	Flesh colour		
1	Flesh thickness		
0.5	Flesh texture		
1	Flesh sweetness		
Calculation of weighted selection index:		***Calculation of weighted selection index:***	
$\text{Index} = A \times \frac{\text{Value of breeding line}}{\text{Value of standard variety}}$		$\text{Index} = B \times \left(2 - \frac{\text{Value of breeding line}}{\text{Value of standard variety}}\right)$	

Step 3: All the weighted selection indices will be summed up, and then divided by the total score to achieve one breeding index for each variety. The index more than 1.0 = better than industry standard varieties

Productivity traits

Sex type

Papayatreeswere classed as either dioecious (male and female flowers onseparate trees) or gynodioecious (hermaphrodite flower onthe same tree)

Figure 1: Female flower

Figure 2: Hermaphrodite flower

Figure 3: Male flower

Productivity traits

Height to first fruit

Height to the first fruit is measured in centimetre (cm) from the ground to the first marketable fruit

Figure 4: Measurement of height to the first marketable fruit

Productivity traits

Trunksize

Trunk circumference is measured in centimetre from 15 cm-height above the ground using a measuring tape.

Figure 5: Measurement of trunksize

Productivity traits

Number of side shoots

Sideshoots arecounted afterthe tree reaches its maturity (at 5 to 6 months after field planting). Itis categorised inthree groups bylength of the side shoots.

Small=length of the side shoots is less than5centimetres

Medium =length of the side shoots is between5and 20centimetres

Large =length of the side shoots is more than20centimetres

Figure 6:Side shoot of papaya

Figure 7:Three categories of side shoot, left: a small side shoot, middle: a medium side shoot, right: a large side shoot

Productivity traits

Peduncle length

Peduncleorstemlengthis scored using the 1, 3and 5rating scale;where

1=shortpeduncle (less than3cm),

3=medium peduncle (between3and 5cm)and

5=long peduncle (greater than5cm).

Figure 8: Measurement of peduncle length

Figure 9: Three categories of peduncle length, left: short peduncle; middle: medium peduncle; right: long peduncle

Productivity traits

Saleable yield

Saleable yield is estimated in kilograms (kg) by counting the total number of marketable fruit per tree within 45 cm column starting from the peduncle of the lowest fruit. Fruit weight of each tree is obtained from average fruit weight of three to five mature marketable fruits. Saleable yield for each tree is calculated using formula

Saleable yield (kg) = number of fruits per tree x Average fruit weight (kg)

Figure 10: Yield on papaya trees. The white bars indicate the length of fruit column at 45 centimetrefor counting number of saleable fruit

Productivity traits

Number of non-marketable fruit

Numberofnon-marketablefruitarecountedfor carpelliod and wasted fruit within 45 cm columnatthe same time whenevaluating saleable yield.

Figure 11:non-marketable fruit*l,e ft*:Carpelloidfruits, *right*: wasted fruit

Productivity traits

Yield gap

Yield gapis estimated by the pattern of fruit set of each tree and scored between 1 and 5; where there is

1=no space observedbetweentwo harvesting times

3=less than50% of a fruitspace betweentwo harvesting times

5=greater than50% of a fruitspace betweentwo harvesting times

Figure 12: Gap of yield on papaya trees. Left: rating at 1 as there is no space on the fruit column; middle: rating at 3 for the gap on the fruit column less than 50%; right: rating at 5 for the gap on the fruit column less than 50%.

Fruit quality traits

Maturity and ripening stages

Maturityandripeningisratedthenumericsystem detailed below;

1=mature green

2= 25% colour 3

=50% colour 4

=75% colour

5=fullripe

Figure 13: Five fruit maturity and ripening stages

Fruit quality traits

Skin Characteristic

Skin colour

Skincolourwasvisually observed and recorded using a numeric rating system as detailed:

1 = light green: equivalent to the colour code 139D of the Royal Horticultural Society (RHS)

2 = green: equivalent to the colour code 137C of RHS

3 = dark green: equivalent to the colour code 137A of RHS

4 = green/slight yellow: equivalent to the colour code 151A of RHS

5 = yellow/slight green: equivalent to the colour code 153D of RHS

6 = yellow-orange: equivalent to the colour code 22A of RHS

7 = orange: equivalent to the colour code 26A of RHS

8 = orange-red: equivalent to the colour code 34B of RHS

9 = red: equivalent to the colour code N34B of RHS

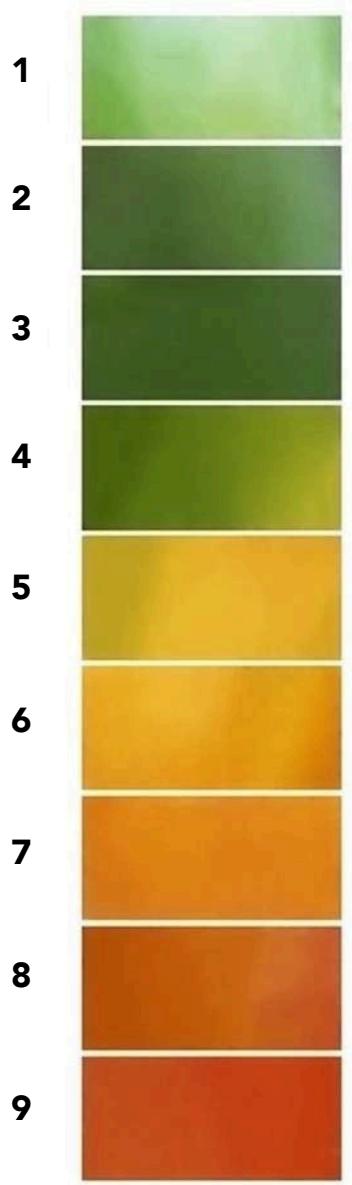


Figure 14:Rating for skin colour

Fruit quality traits

Skin Characteristic

Skin gloss

Skingrossisvisually observed and scored using a rating system; where

1 = dull

2 = average

3 = glossy

4 = very glossy/ excellent skin quality

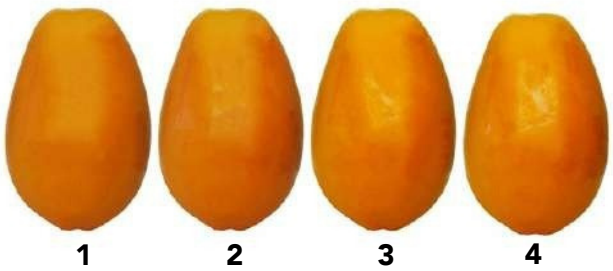


Figure 15: Rating for skin gloss

Skin freckle

Skin freckle or winter spots are observed on mature fruits at the ripe full colour stage. The severity of freckle is recorded using a rating system of 1 to 5 where

1 = winter spots cover less than 1% of the surface 2 = winter spots cover 1% to 25% 3 = winter spots cover 26% to 50% 4 = winter spots cover 51% to 75% 5 = winter spots cover more than 75%

Figure 16: Rating for skin freckle

Fruit quality traits

Shape

Fruit shape

Papayafruit isgrouped into irregular shape, round shape and elongate shape. Each fruit is scored in numeric system as detail below:

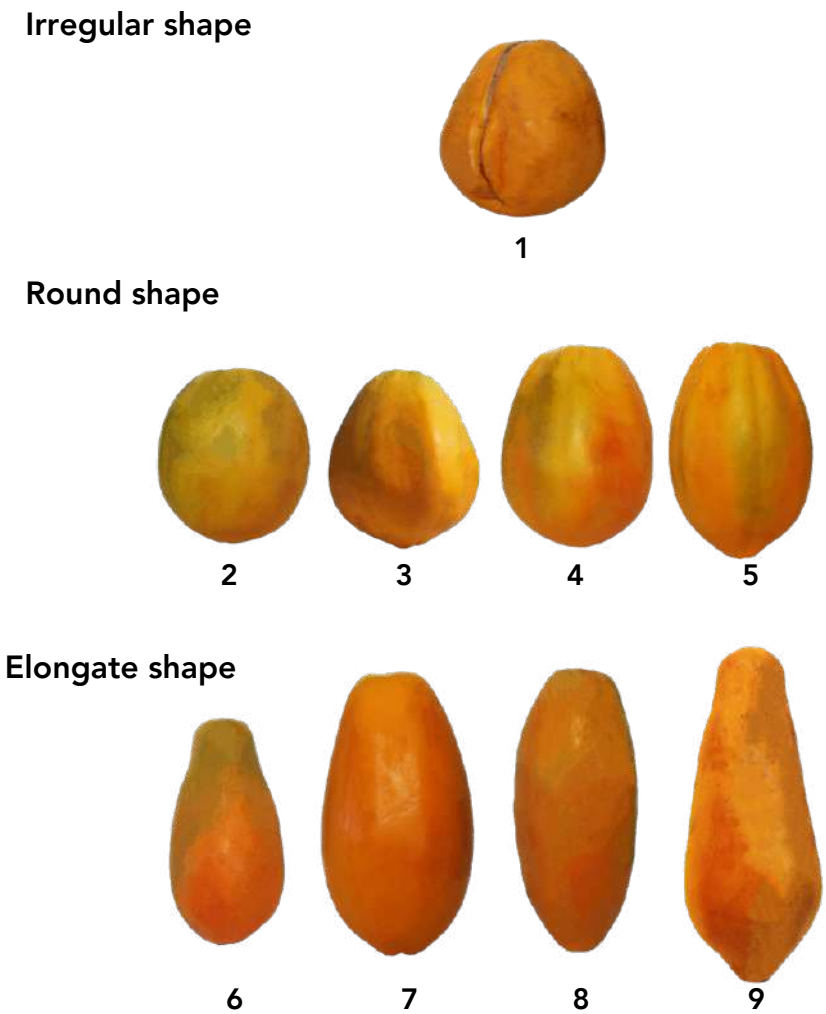


Figure 17: Rating for papaya fruit shape

Fruit quality traits

Shape

Teatshape

Teatofeachfruitis scored in numeric system as below

1 = blossom end defect

2 = sunken

3 = flat

4 = pronounced

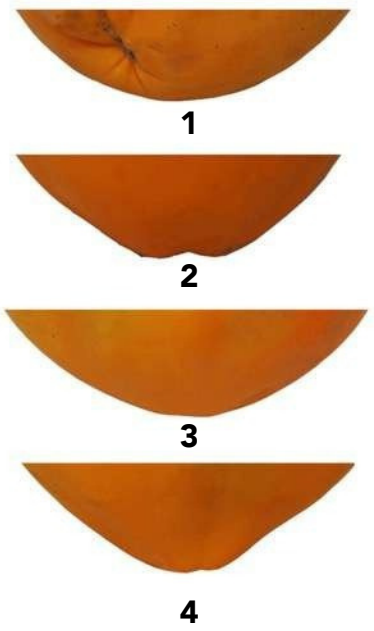


Figure 18:Rating for teat shape

Stalk insertion point

Theinsertionpointofthestalkof each fruit is scored using the numeric system detailed below;

1 = depressed

2 = flattened

3 = inflated

4= pointed

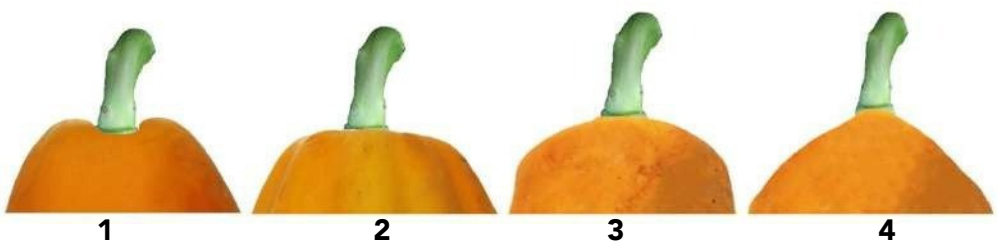


Figure 19: Rating for stalk insertion point

Fruit quality traits

Fruit firmness

Fruitfirmness is assessed at ready to eat stage by using a firmness tester and by hand pressure at the centre of the fruit.

Fruit is assessed by holding a fruit with both hands and pressing centre fruit with the thumps and rating the firmness as

1 = soft

2 = intermediate/ rubbery

3 = firm/hard

Figure 20: Evaluation of fruit firmness, rating by using thumb pressure.

Fruit size

Fruit weight

Fruitweightisrecorded in gram using a measurement from a digital scale

Figure 21: Fruit weight on a digital scale

Fruit quality traits

Fruit size

Fruit width

Fruit width is measured by measuring the diameter (cm) at the middle part of the papaya fruit using a ruler.

Fruit length

Fruit length ismeasured by measuring the length (cm) at the middle part of the papaya fruit using a ruler.

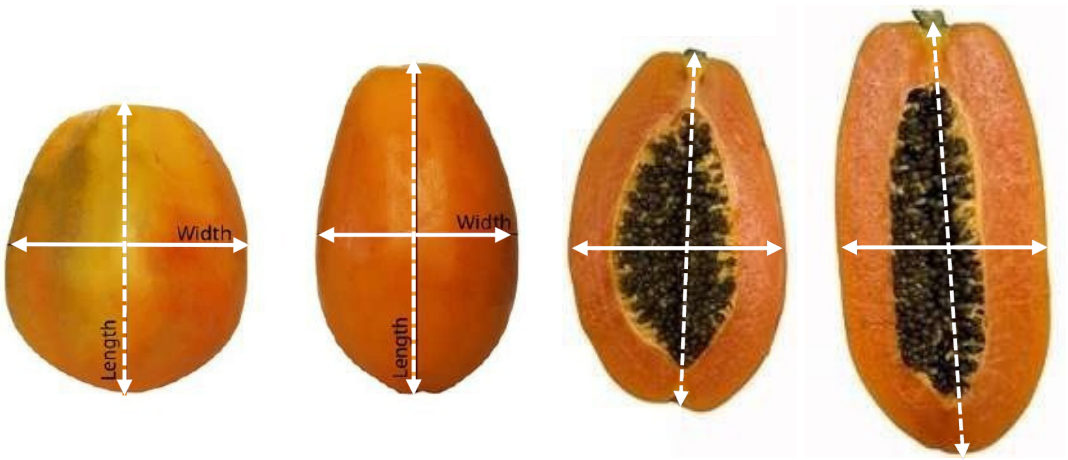


Figure 22: Determination of fruit width (solid white arrow) and length (dot white arrow)

Fruit quality traits

Cavity

Cavity width

Cavity widthis measured between one flesh wall to another at the central cavity of each fruit using a ruler.

Cavity length

Cavity length is measured between one flesh wall to another at the central cavity of each fruit using a ruler.

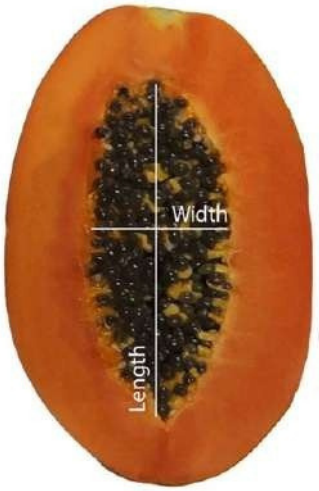


Figure 23: Measurement of cavity size

Cavity shape

Eachfruitiscross-sectioned laterally. The central cavity of each fruit is scored as detailed below;

1 = round

2 = angular or pentagon

3 = slightly star or star

4 = flower

Figure 24: Rating for cavity shape

Fruit quality traits

Flesh characteristic

Flesh ratio

Fleshrationiscalculated using fruit and cavity with and length using the formula below:

$$\text{Flesh ratio} = 1 - \left(\frac{\text{CavitywidthxCavityLength}}{\text{Fruit width x Fruit Length}}\right)$$

Flesh colour

Eachfruitiscross-sectioned in half laterally, and scored for flesh colour as

1 = white: equivalent to the colour code 11D of RHS

2 = creamy white: equivalent to the colour code 1D of RHS

3 = slight pink: equivalent to the colour code 27C of RHS

4 = pale yellow: equivalent to the colour code 6B of RHS

5 = yellow: equivalent to the colour code 153D of RHS

6 = yellow-orange: equivalent to the colour code 22A of RHS

7 = orange: equivalent to the colour code 26A of RHS

8 = orange-red: equivalent to the colour code 34B of RHS

9 = red: equivalent to the colour code N34A of N34A RHS

Figure 25: Rating for flesh colour

Fruit quality traits

Flesh characteristic

Flesh colour consistency

Eachfruit is cross-sectionedin half laterally and scored for consistency of flesh colour as detailed in the numeric rating scale for consistency of flesh colour; where

1 = less than 50% colour consistency

2 = colour inconsistency is between 50-75%

3 = flesh colour is more than 75% consistency

1 2 3

Figure 26: Rating for consistency in flesh colour

Flesh thickness

Flesh thickness is measured in centimetre from the skin to the seed cavity using a ruler

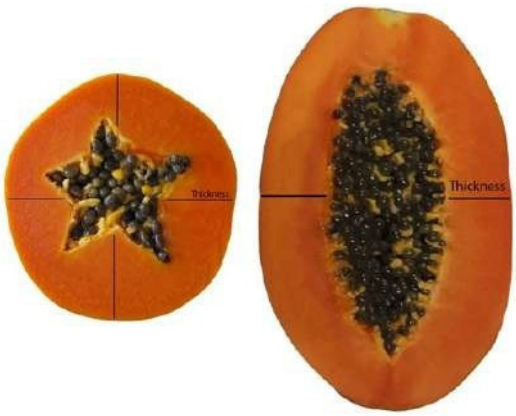


Figure 27: Measurement for flesh thickness

Fruit quality traits

Flesh characteristic

Flesh texture

Fleshtextureisassessed at ready to eat stage by using a firmness tester and mouth feel.

Mouth feel. Fruit sample is cut into a small cube and the flesh texture is assessed by mouth sensory to score as

1 = soft texture

2 = intermediate/ rubbery

3 =firm

Flesh sweetness (°Brix)

Totalsoluble solid(SS)is measured on ripe fruits using a digital refractometer. The measurement is recorded in a °Brix scale.

Figure 28: Measurement for flesh sweetness using a digital refractometer

Table to record tree and fruit data

Evaluation 1: Tree evaluation

Date			***Location***						
Line	Tree#	Sex	Height (cm)	Trunk (cm)	# Side shoot			Peduncle length	Note
					S	M	L		
	1								
	2								
	3								

Evaluation 2 and 3:

Tree evaluation

Date			***Location***			
Line	Tree#	Peduncle length	# Total fruit	# non-marketable fruit	Yield gap	Note
	1					
	2					
	3					

Fruit evaluation

Date			***Location***						
Line	***Tree #***	***Fruit #***	***Skin Characteristic***				***Shape***		
			Colour	***Gloss***	***Freckle***	***Disease***	***Fruit***	***Teat***	***Stalk***
	1	1							
	1	2							
	1	3							

Line	***Tree #***	***Fruit #***	***Fruit Firmness***	***Weight (g)***	***Measurement (cm)***				
					Fruit width	***Flesh thickness***	***Cavity width***	***Fruit length***	***Cavity length***
	1	1							
	1	2							
	1	3							

Line	***Tree #***	***Fruit #***	***Cavity Shape***	***Flesh***					***Note***
				Colour	***Consistency***	***Texture***	***Brix***	***Flavour***	
	1	1							
	1	2							
	1	3							

Papaya Evaluation Handbook

Productivity and fruit quality traits

Secondedition/August2021

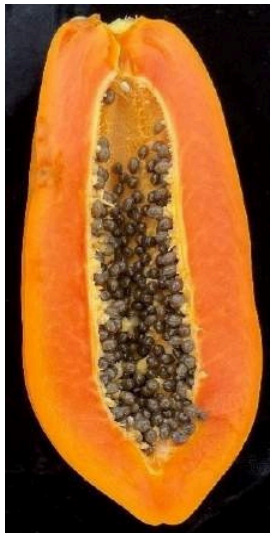
Project Number: PP18000

Appendix 2 - Final Presentation to Papaya SIAP

Papaya Breeding Program-PP18000 Genetic diversity underpins desirable agronomic, productivity and fruit quality traits.

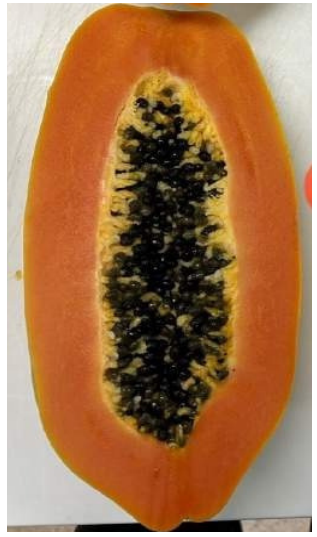
Moonlight 1

Moonlight 2



Sunlight 1

Sunlight 2



Sunlight 3

Red papaya F1 hybrid RH9

Red papaya F1 hybrid RH10

Dr Fawad Ali

Papaya Breeding and Genetics

Project Aim (PP18000)

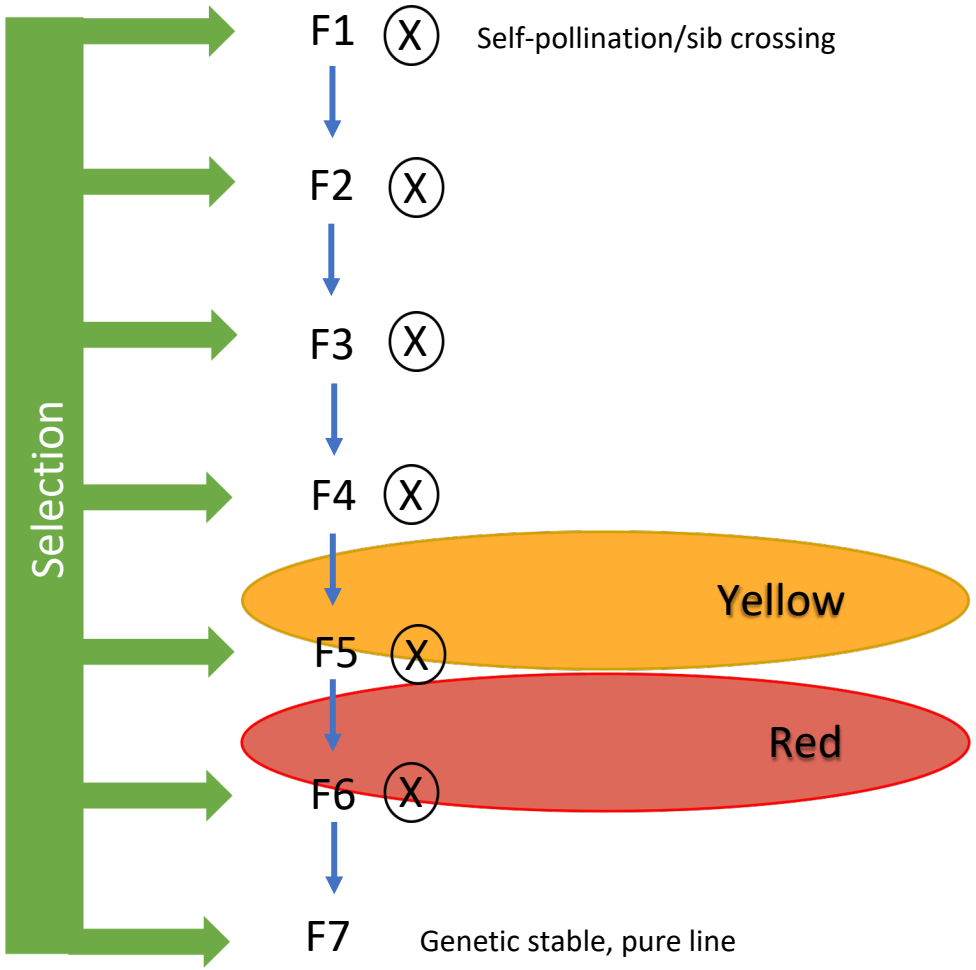
Dr Chat Kanchana-udomkan

Deliver new papaya varieties:

- Genetically stable
- Propagate by seed
- Preferences of consumers
- Needs of growers
- Adaptation to key growing areas of Tropical North Queensland that contributes 85% of papaya production in Australia

Breeding Strategies

Female Parent X Male Parent



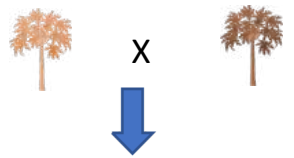
Dr Chat Kanchana-udomkan

Year 2011-2014

Germplasm evaluation

Year 2012:

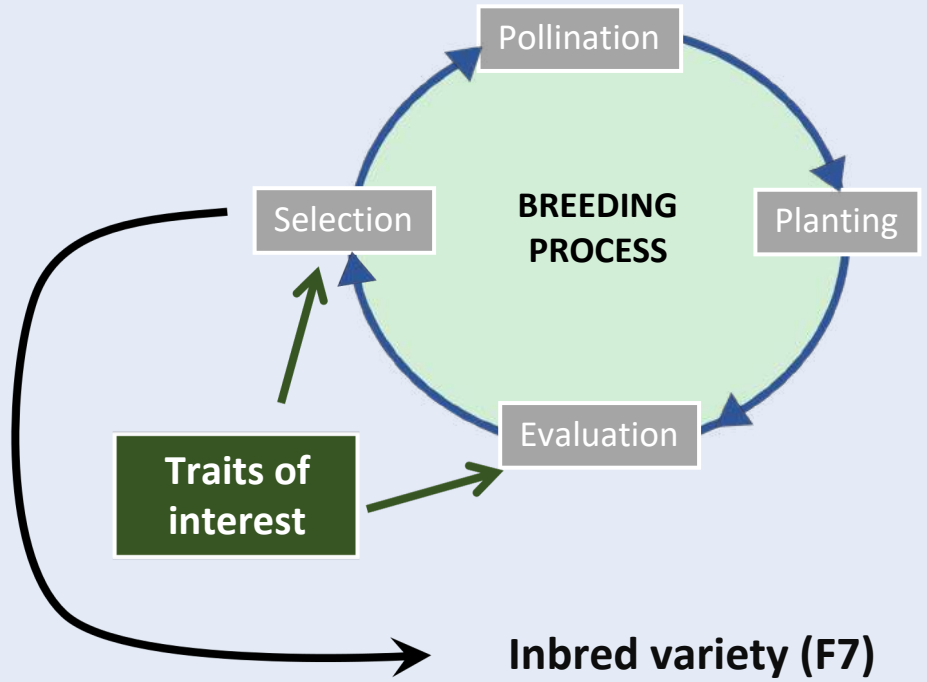
ProduceF1breeding lines



F1 breeding lines
16 crosses

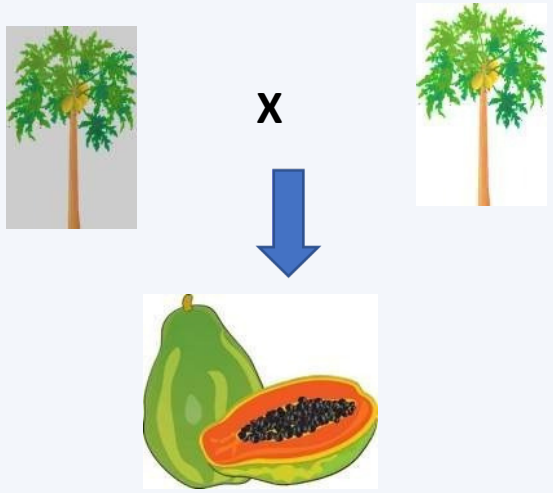
Breeding strategies
Propagation through seed

- Production of pure lines/inbred lines: i.e. Sunrise Solo, Holland



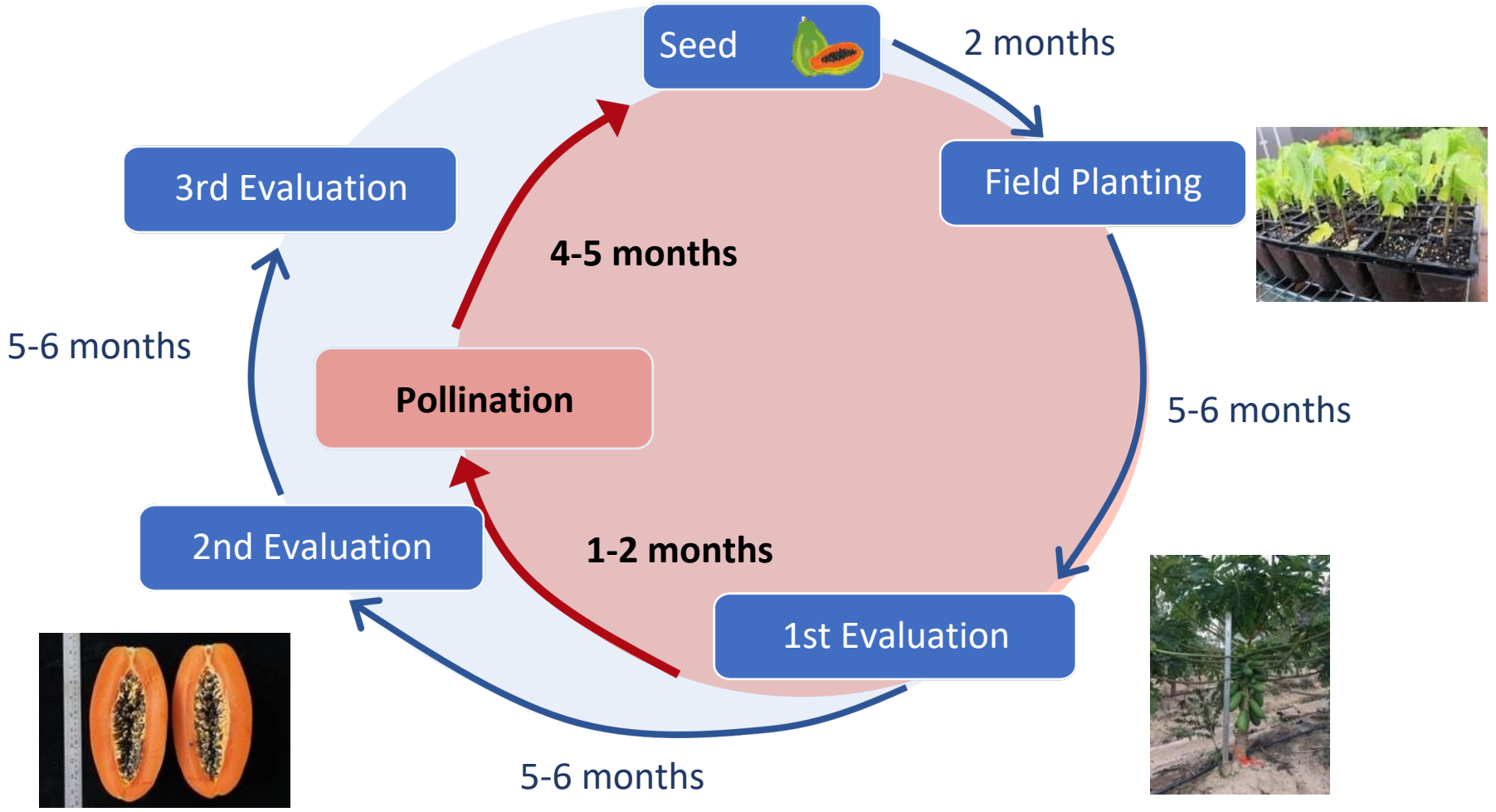
- Production of F1 hybrid varieties: i.e. RB1, Eksotika

Genetic Stable materials



Papaya Breeding Process

- Evaluation: 17-20 months
- Production of new generation: 12-15 months



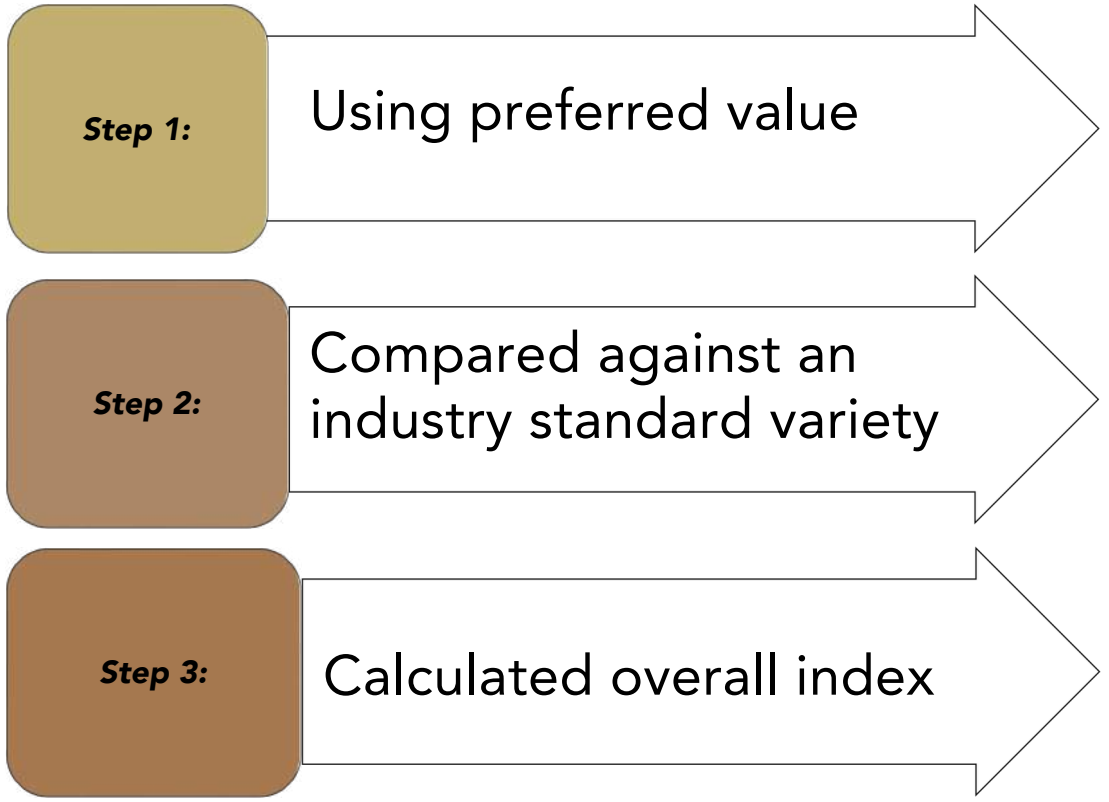
Trait's selection in consultation with the papaya industry

Traits of interest

Table 1: List of traits for evaluation for papaya fruit quality and tree productivity.

No.	Tree age	Productivity traits	Fruit quality traits
1	5 months	Sex type Height to first fruit Trunk circumference Number of side shoots Peduncle length	
2	10-12 months	Peduncle length Saleable yield Yield gap Number of non-marketable fruit Fruit size (fruit length, width and weight) Cavity size (cavity length and cavity width)	Skin gloss Skin freckle Skin colour Fruit firmness Fruit shape Teat shape Stalk insertion Cavity shape Flesh colour Flesh colour consistency Flesh thickness Flesh texture Flesh sweetness (°Brix)
3	15 months	Same as the 2nd evaluation	Same as the 2nd evaluation

Weighted breeding index



Weighted breeding index

Step 1: Identify traits and their industry/consumer-derived preferred value or range. Traits and details are presented on Table 2

Table 2: Traits and their industry/consumer-derived preferred value or range

Traits and their industry/consumer-derived preferred value or range	
Fruit weight:	Yellow: 0.9 – 1.5 kg
	Red: 0.9 – 1.2 kg
Peduncle length:	Medium or long
Fruit shape:	Yellow: Round shape
	Red: Elongate shape
Stalk insertion:	Flattened (#2)
Teat shape:	Flat (#3)
Cavity shape:	Round (#1)
Flesh colour consistency:	100% flesh colour consistency

Step 2: Comparing traits with the value of industry standard varieties either red or yellow papaya and calculate weighted selection indices using the formula on Table 3

Table 3: Traits and calculation of breeding index for tree productivity and fruit quality traits

Traits expected to have a higher value than in the industry standard		Traits expected to have a lower value than in the industry standard	
Weighting score (A)	**List of traits:**	**Weighting score (B)**	**List of traits:**
1	Saleable yield	0.75	Height to first fruit
0.25	Trunk circumference	0.5	Number of side shoots
1	Flesh ratio	0.75	Skin freckle
0.25	Skin gloss	1	Number of non-saleable fruit
0.25	Skin colour		
0.75	Fruit firmness		
0.75	Flesh colour		
1	Flesh thickness		
0.5	Flesh texture		
1	Flesh sweetness		
Calculation of weighted selection index:		**Calculation of weighted selection index:**	
$\text{Index} = A \times \frac{\text{Value of breeding line}}{\text{Value of standard variety}}$		$\text{Index} = B \times \left(2 - \frac{\text{Value of breeding line}}{\text{Value of standard variety}}\right)$	

Step 3: All the weighted selection indices will be summed up, and then divided by the total score to achieve one breeding index for each variety. The index more than 1.0 = better than industry standard varieties

Priority traits agreed with industry and growers

Productivity Traits: Yield related traits

Figure 2: Sex type; a) female, b) Hermaphrodite, c) male

Figure 4: Rating for side shoot;
1) small, 2) medium, 3) large

Figure 3: Measurement of height to the first marketable fruit

Figure 5: Rating for peduncle length;
1) short, 2) medium, 3) long

Figure 6: Rating for gap of yield on papaya trees; →
1) no space on the fruit column, 3) < 50% gap on the fruit column, 5) > 50% gap on the fruit column

Fruit Quality Traits

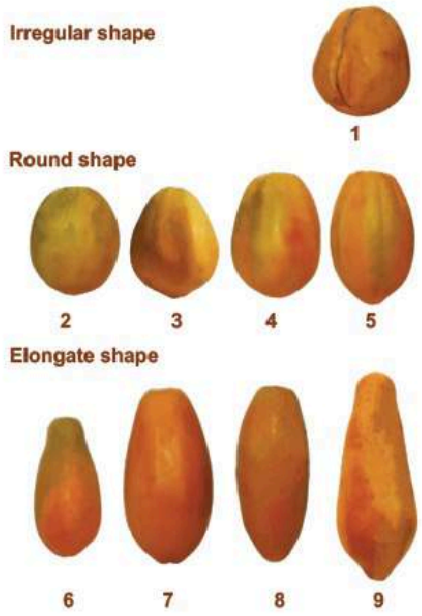


Figure 7: Rating for papaya fruit shape

Figure 14: Rating for consistency in flesh colour

Figure 12: Rating for skin colour

Light green	Green	Dark green	Green/ slight yellow	Yellow/ slight green	Yellow orange	Orange	Orange red	Red
1	2	3	4	5	6	7	8	9

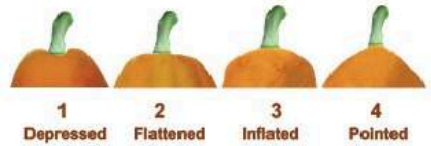


Figure 8: Rating for stalk insertion point

Figure 9: Rating for teat shape

Figure 10: Rating for cavity shape

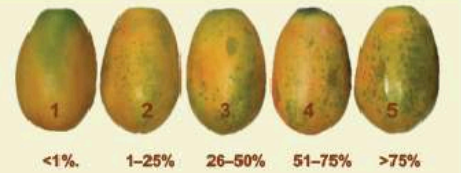


Figure 11: Rating for skin freckle or winter spots

Figure 13: Rating for flesh colour

White	Creamy white	Slight pink	Pale yellow	Yellow	Yellow orange	Orange	Orange red	Red
1	2	3	4	5	6	7	8	9

Desirable traits –grower vs consumer

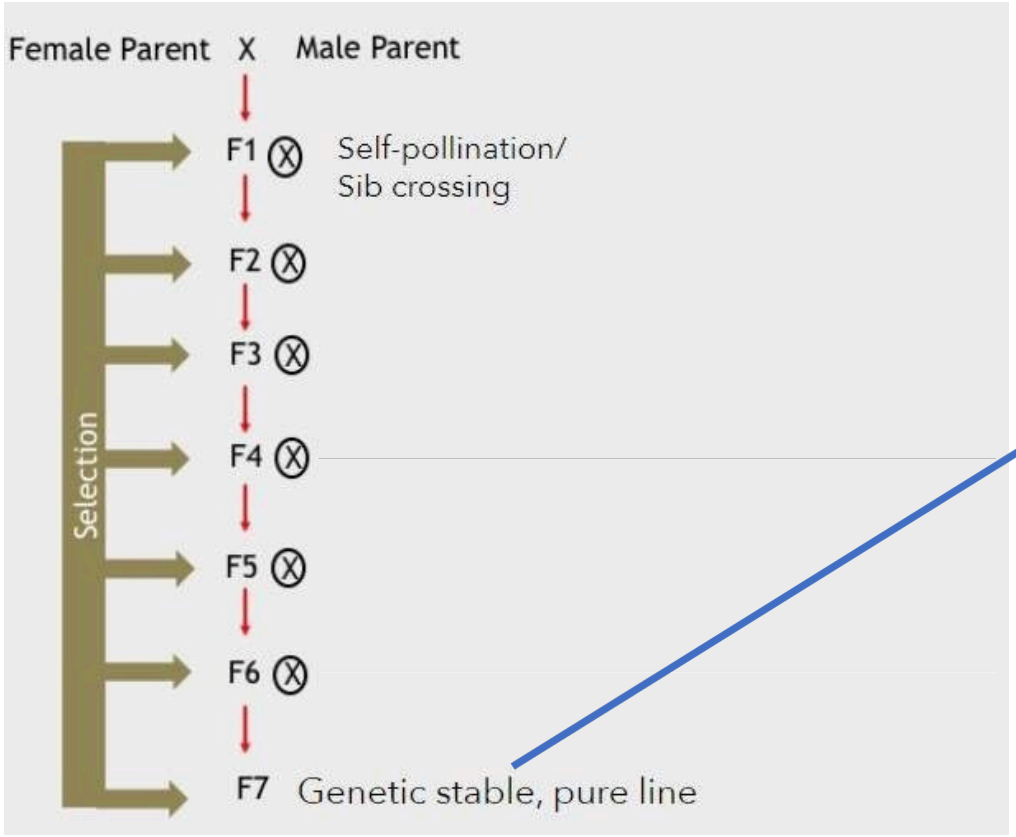
Grower	Consumer
Height to fruit set	Fruit cavity width
Trunk circumference	Sweetness
Number of marketable fruit	Aroma
Fruit weight	Flavour

….. there are many others

Selective breeding considerations:

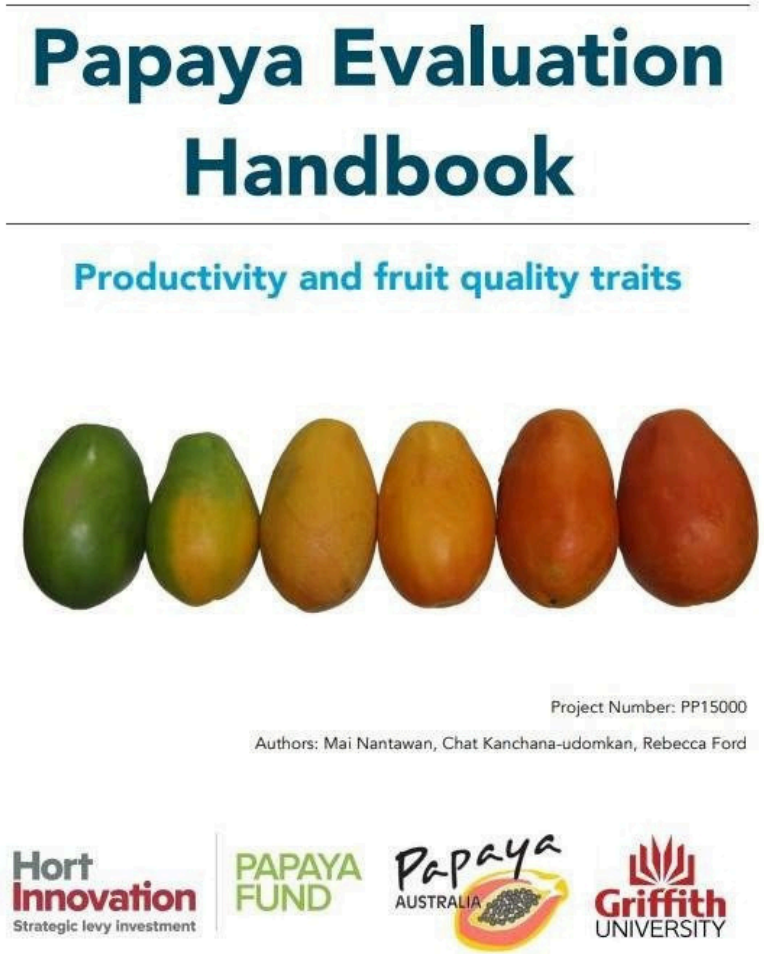
- Genetic inheritance
- Genetic gain
- Stability of trait selections
- Heterosis breeding

Papaya Breeding Program-PP18000
Current Scenario



F1 Hybrids are currently being field trialed

Standardising trait evaluation



SCAN ME

Summary of success so far……

- Fruit shape (oblong)
- Width of central fruit cavity (narrow)
- Firmness (very much firm)
- Sweetness (very sweet)
- Height of attachment of the first flower (very low)
- Aroma (moderate)

Semi-commercial trials

Advanced Red and Yellow papaya breeding lines (F6:F7 generations)

Plus, F1 red papaya hybrids

So far selected, the advanced generation breeding lines for the Coast and Tablelands region

Breeder's Name at F6 or F7 generation	Type of papaya	Proposed name under PBR registration
C1-7-1	Red Papaya for the Coast	Sunlight 1
C1-7-2	Red Papaya for the Coast	Sunlight 2
T2-6-1	Red Papaya for the Tablelands	Sunlight 3
ML-2-9-3	Yellow Papaya for the Tablelands	Moonlight 1
ML-2-8-17	Yellow Papaya for the Tablelands	Moonlight 2

F1 red papaya hybrids developed for the Coast and Tablelands regions

F1 red papaya hybrids	Female parent	Male parent
RH1 (Red) F1 Hybrid	C2-6-5.9.2-10 (Line 2; female)	Solo (Male)
RH2 (Red) F1 Hybrid	C2-6-5.9.2-10 (Line 2; female)	C2-6-5.9.1.2 (Line 1; Male)
RH3 (Red) F1 Hybrid	C2-6-5.15.2-11 (Line 5; female)	Solo (Male)
RH4 (Red) F1 Hybrid	C2-6-5.15.2-11 (Line 5; female)	C2-6-5.5.14-5 (Line 3; Male)
RH5 (Red) F1 Hybrid	C2-6-5.15.2-10 (Line 5; female)	Paris (Male)
RH6 (Red) F1 Hybrid	C2-6-5.15.2-10 (Line 5; female)	Solo (Male)
RH7 (Red) F1 Hybrid	C1-6.4.3.12 (Line 8; female)	Paris (Male)
RH8 (Red) F1 Hybrid	C3-6-5.26-11 (Line 7; female)	Solo (Male)
RH9 (Red) F1 Hybrid	C3-6-5.26-11 (Line 7; female)	C2-6-5.5.14-5 (Line 3; Male)
RH10 (Red) F1 Hybrid	C2-6-5.5.14 (Line 3; female)	C1-6.4.3.12 (Line 8; Male)

Three (3) **trial sites,** including **two (2)** at the **Tablelands and one (1)** at the **Coast**

Five (5) RIL (F6:F7 generations) **red papaya**

Ten (10) F1 novel red papaya hybrids

Two (2) advanced generation (F6:F7 generations) breeding lines of **yellow papaya**

Five (5) elite red and two (2) elite yellow papaya breeding lines selected for *PBR protections* and further proceeding with *commercialization* pathways

Type of papaya	Agronomic and productivity gains (%) over RB1 (reds) or 1B (yellows)	Fruit quality gains (%) over RB1 (reds) or 1B (yellows)	Weighted Breeding Index
RS1 (red for the Coast)	Set fruit 38% closer to the ground than RB1. Trunk circumference10% more than RB1 10% more marketable fruit than RB1	Medium size fruit of 907g with moderate aroma and improved sweetness (obrix= 20%) than RB1	1.29
RS2 (red for the Coast)	Set fruit 24% closer to the ground than RB1. Trunk circumference 4% more than RB1 12% more marketable fruit than RB1	Medium size fruit of 1022g with moderate aroma and improved sweetness (obrix= 24%) than RB1	1.27
RS3 (red for Tablelands)	Set fruit 46% closer to the ground than RB1. Trunk circumference 16% more than RB1. 1-2% more marketable fruit than RB1	Smaller size fruit of 750g with moderate aroma and sweeter fruit (obrix= 20%) than RB1	1.19
RS4 (red for Tablelands)	Set fruit 49% closer to the ground than RB1. Trunk circumference 15% more than RB1 18% more marketable fruit than RB1	Medium size fruit of 950g with moderate aroma and sweeter fruit (obrix= 20%) than RB1	1.28
RS5 (red for Tablelands)	Set fruit 34% closer to the ground than RB1. Trunk circumference 16% more than RB1 1% more marketable fruit than RB1	Medium size fruit of 918g with moderate aroma sweeter fruit (obrix= 17%) than RB1	1.07
ML1 (yellow for Tablelands)	Set fruit 27 % closer to the ground than 1B. Trunk circumference 31% more than 1B. 12% more marketable fruit than 1B.	Medium size fruit of 1200g with moderate aroma and sweeter fruit (obrix=11%) than 1B.	1.18
ML2 (yellow for Tablelands)	Set fruit 12% closer to the ground than 1B. Trunk circumference 26% more than 1B. 16% more marketable fruit than 1B	Medium size fruit of 1300g with moderate aroma and sweeter fruit (obrix= 9 %) than 1B	1.24

Agronomic and Productivity traits-Papaya breeding lines plus F1 hybrids at Tablelands (T1 and T2)

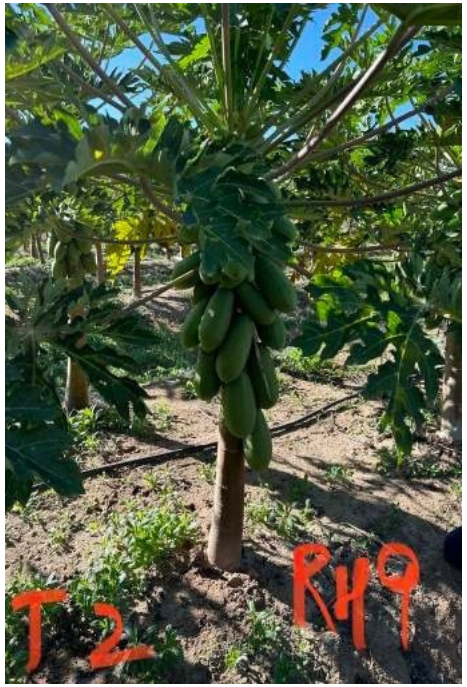
Vs

RB1 commercial

RB1-commercial

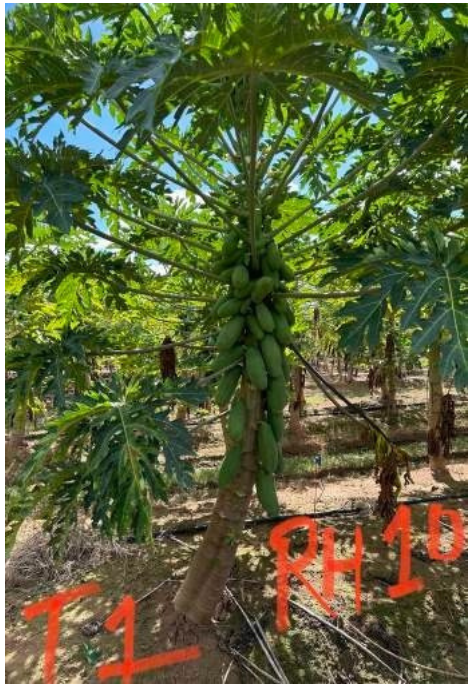
V
S

Sunlight 3



Red Papaya F1 hybrid RH9-T2

Red Papaya F1 hybrid RH9-T1



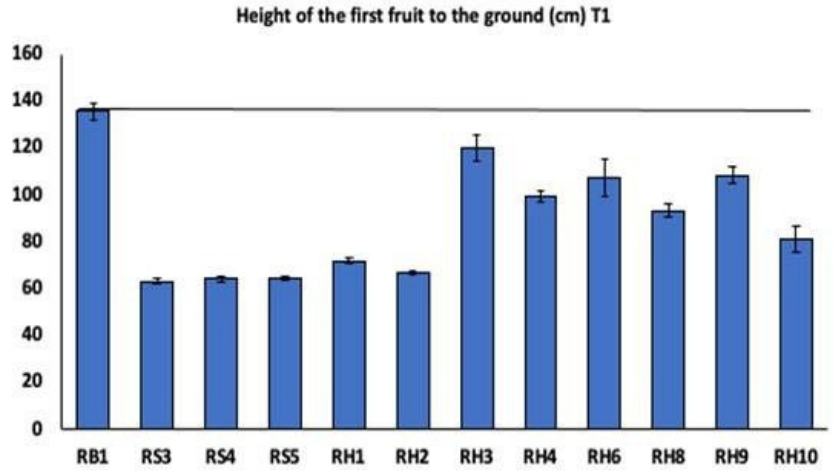
Red Papaya F1 hybrid RH10-T1

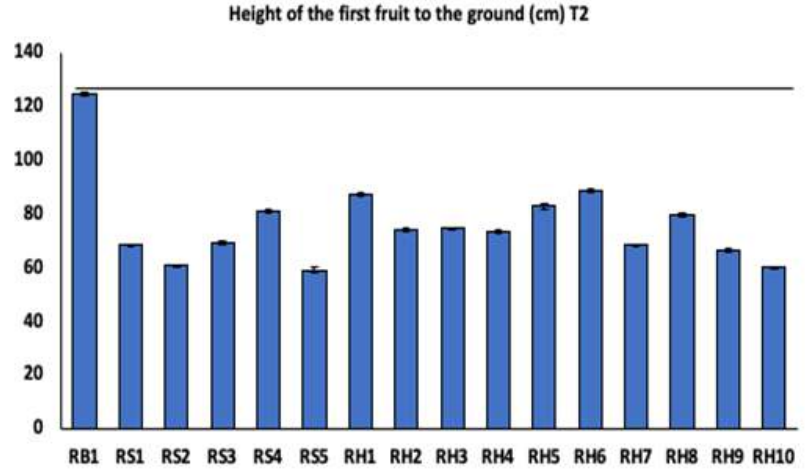
RIL Gains compared to RB1 = -35% to -48% lower to the ground

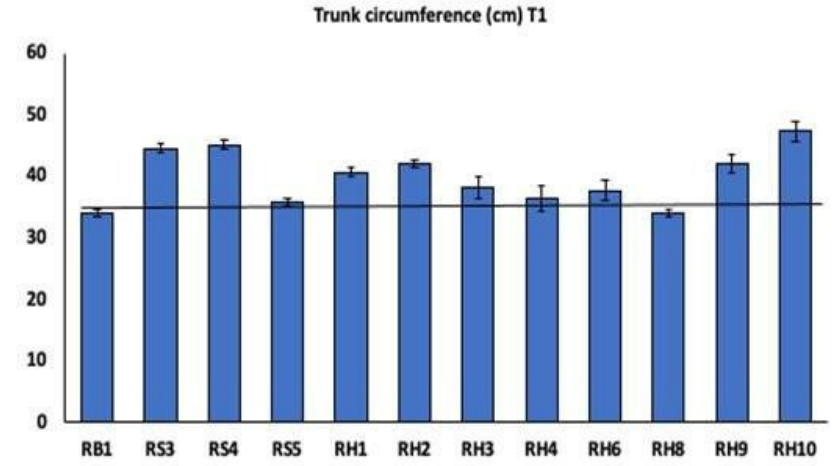
RIL Gains compared to RB1 = 5% to 32% thicker trunk

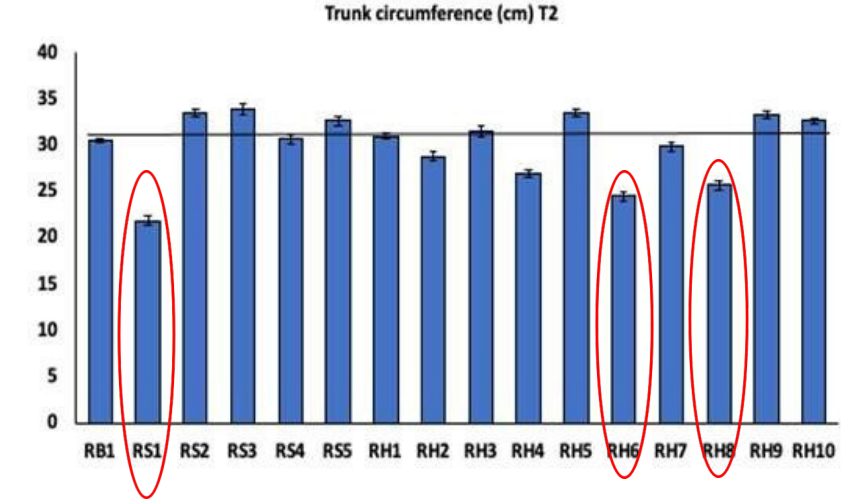
F1 Gains compared to RB1 = -11% to -51% lower to the ground

F1 Gains compared to RB1 = 1% to 39% thicker trunk









Red Papaya breeding lines plus F1 hybrids at Tablelands (T1 and T2)

RIL Gains compared to RB1 = 30%

to 60% more marketable fruit

RIL Gains compared to RB1 = -20%

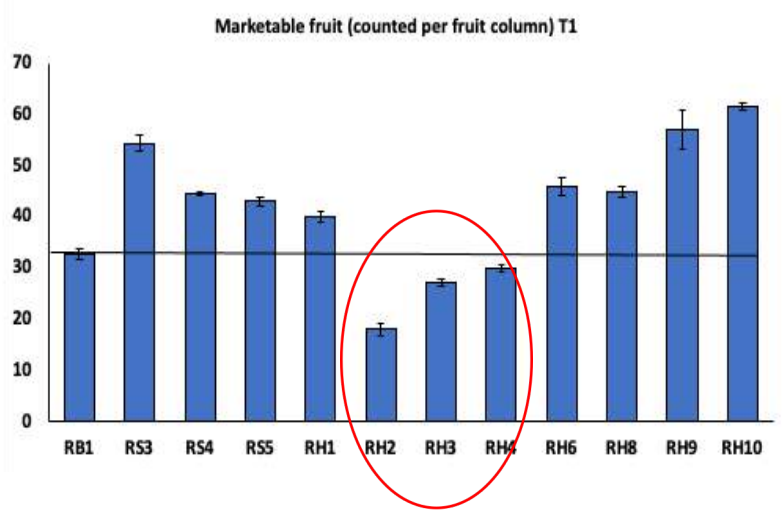
to -50% less wasted fruit

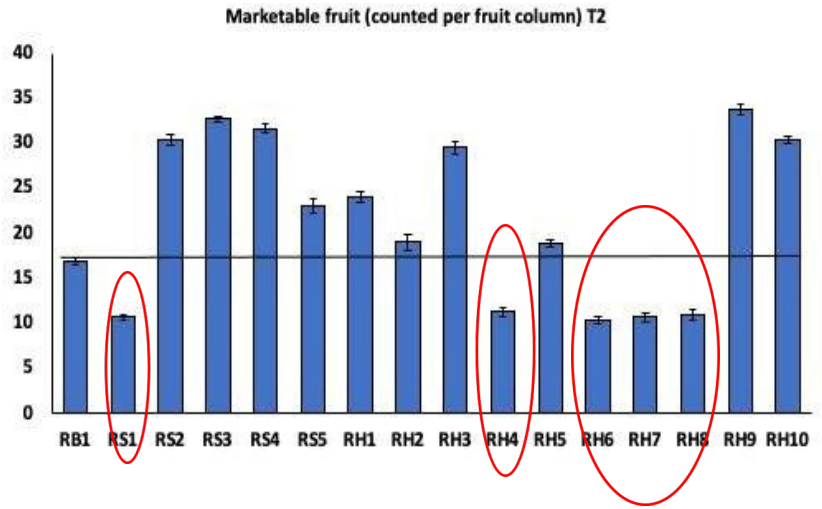
F1 Gains compared to RB1 = 31%

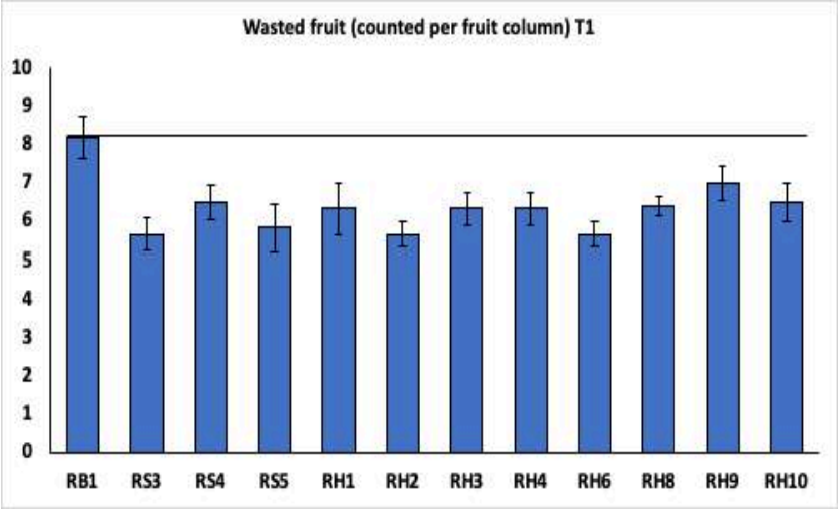
to 90% more marketable fruit

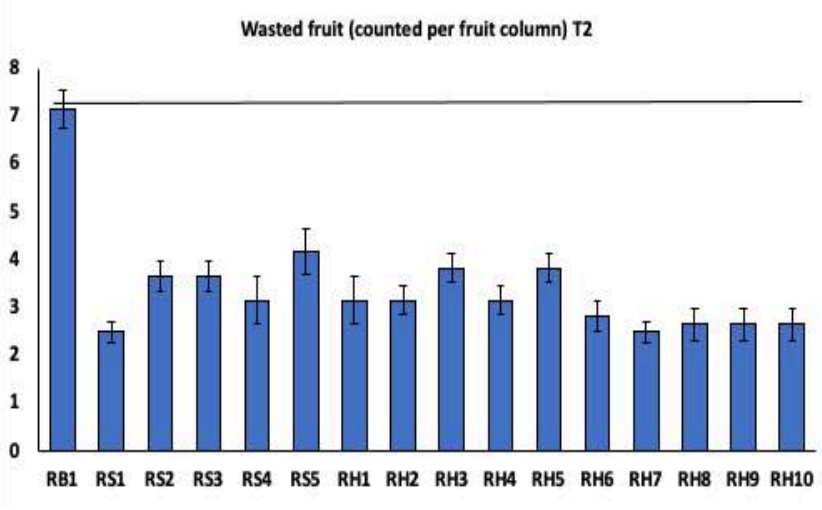
F1 Gains compared to RB1 = -14%

to -60% less wasted fruit









Fruit Quality-Red Papaya breeding lines plus F1 hybrids at Tablelands (T1 and T2)

Sunlight 3

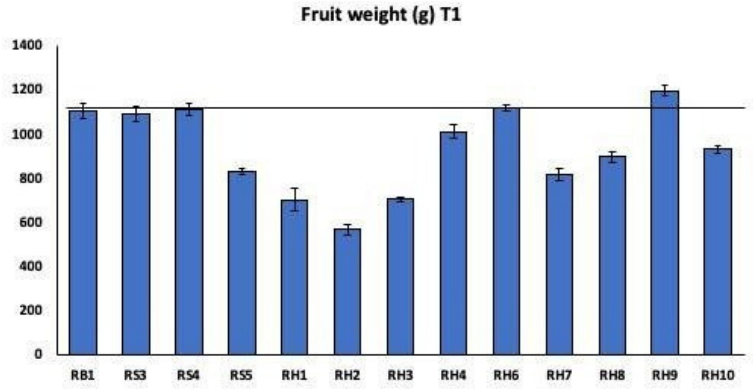
Red papaya F1 hybrid RH9

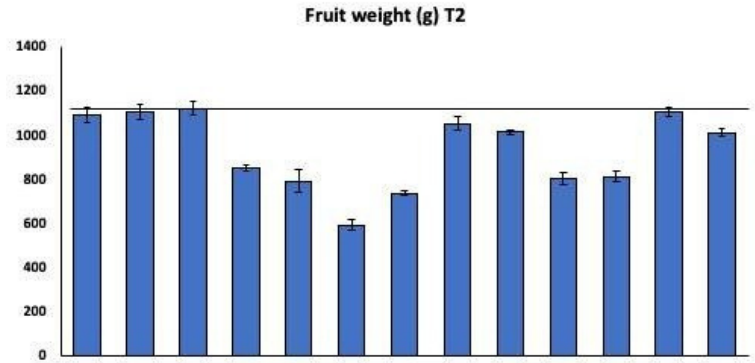
Red papaya F1 hybrid RH10

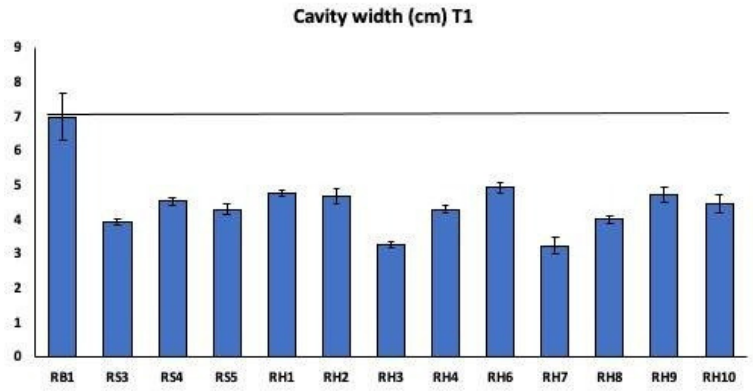
RIL Gains compared to RB1 = +1% to -20% fruit weight RIL compared to RB1 = -30% to -45% reduced cavity

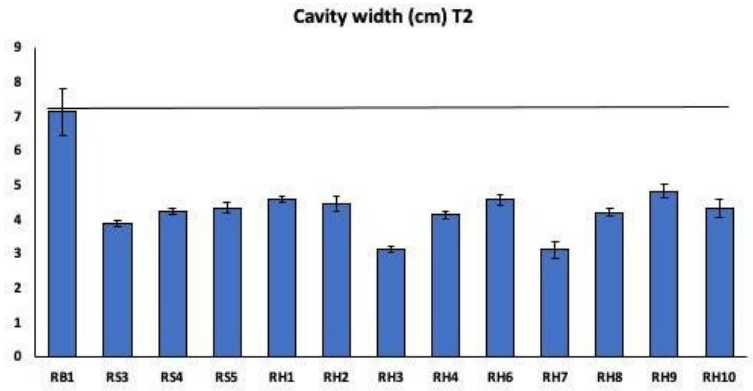
F1 Gains compared to RB1 = +10% to -30% fruit weight

F1 Gains compared to RB1 = -18% to -49% reduced cavity

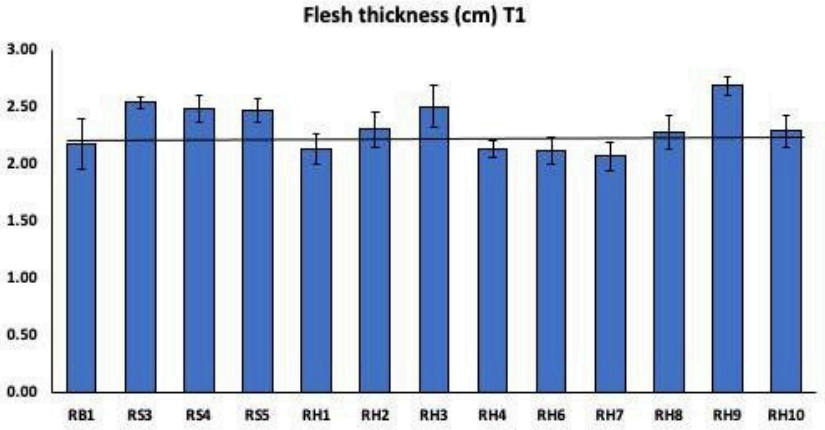


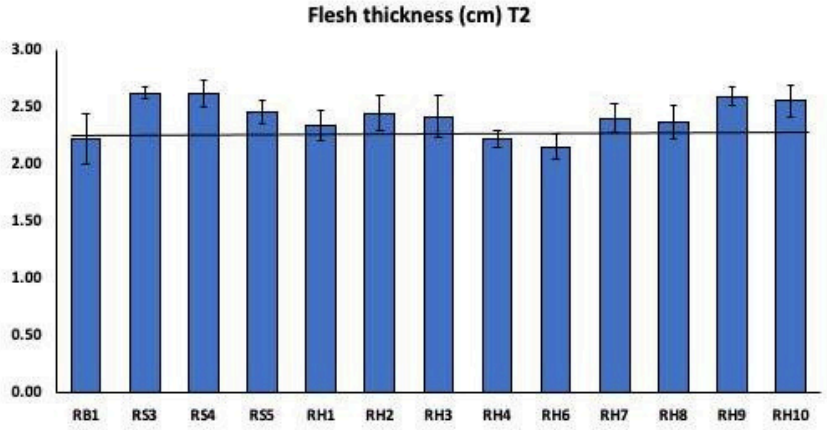


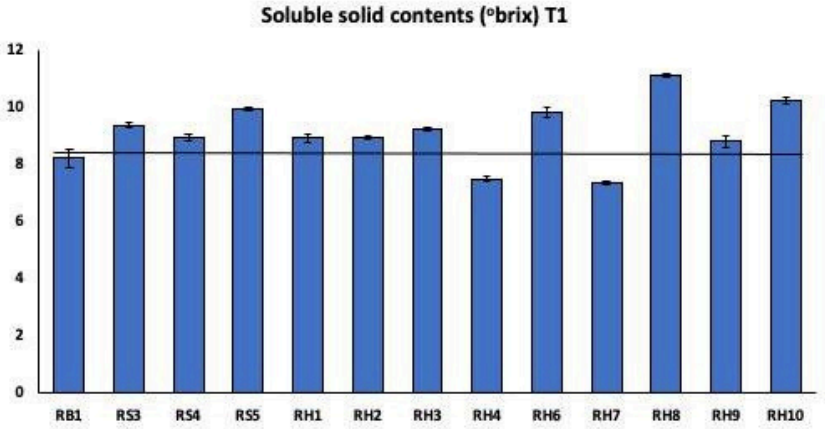


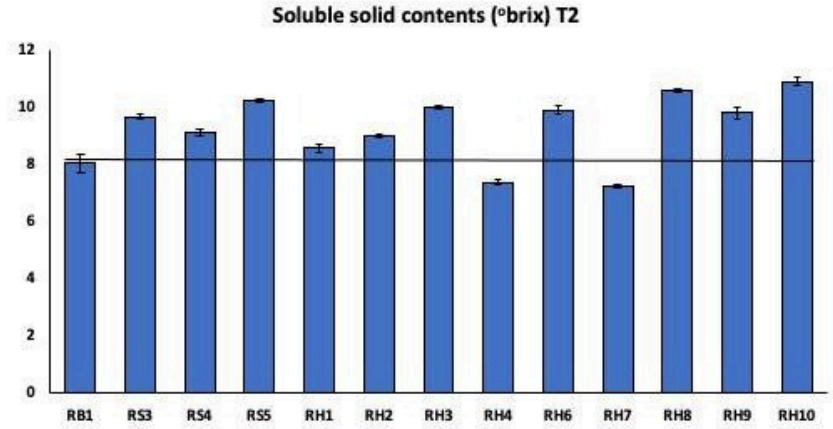


RIL Gains compared to RB1 = 2% to 14% thicker flesh
RIL Gains compared to RB1 = 8% to 20% sweeter fruit
F1 Gains compared to RB1 = 1% to 16% thicker flesh
F1 Gains compared to RB1 = -10% to +22% sweeter fruit

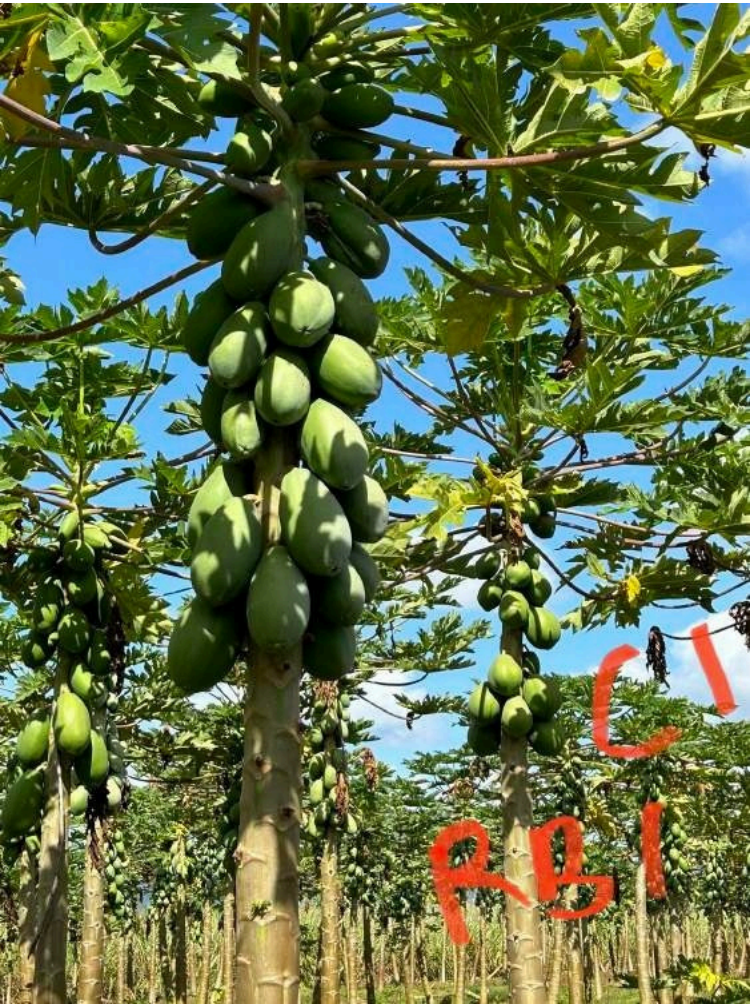






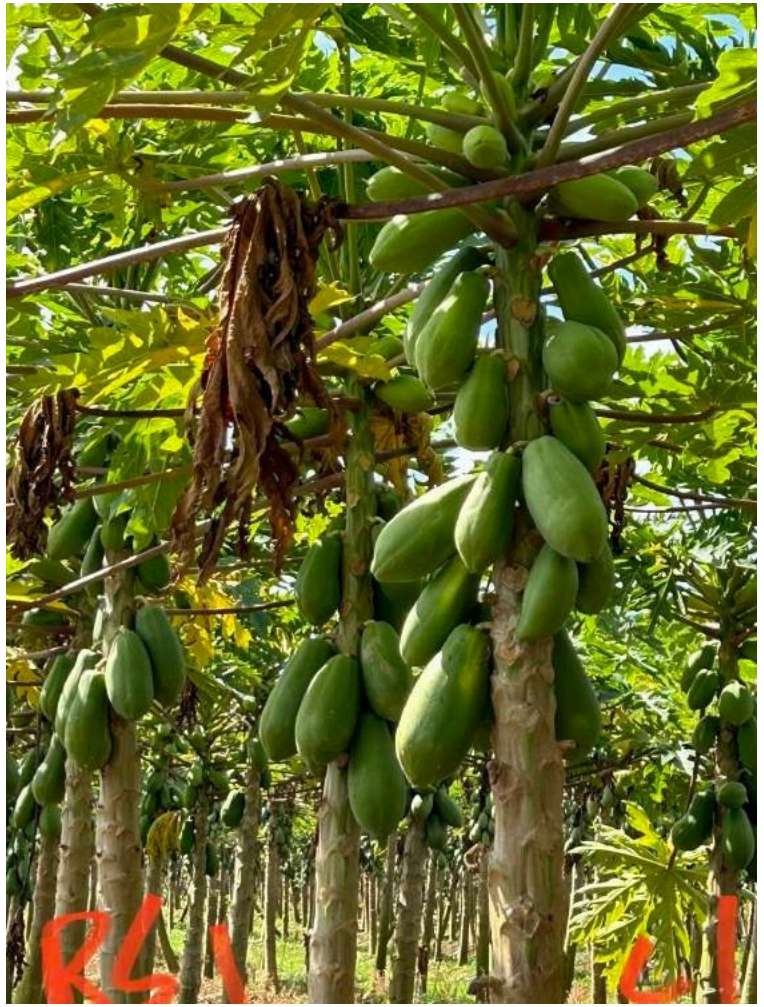


Red Papaya breeding lines at Coast (C1) VS RB1 commercial



RB1-commercial

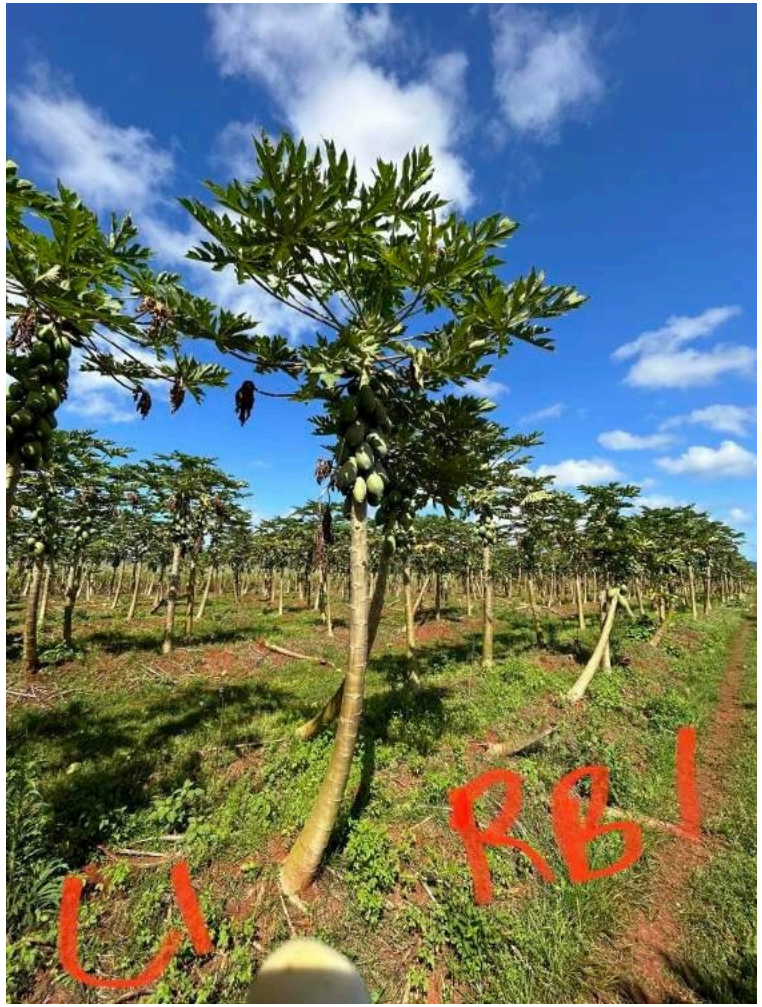
V
S



Sunlight 1

Sunlight 2

Red Papaya F1 hybrids at Coast (C1) Vs commercial RB1



RB1-commercial

V
S

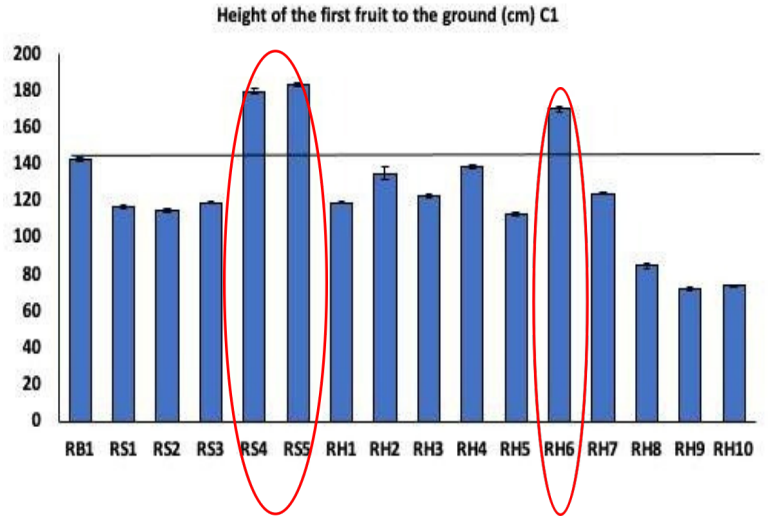
Red Papaya F1 hybrid RH9-C1

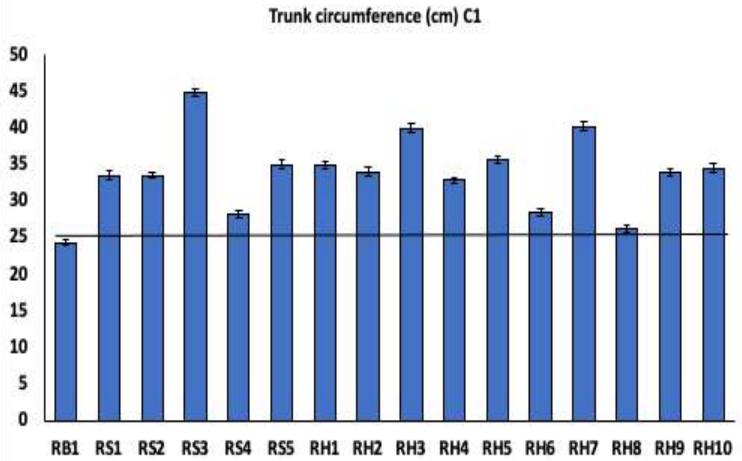
Red Papaya F1 hybrid RH10-C1

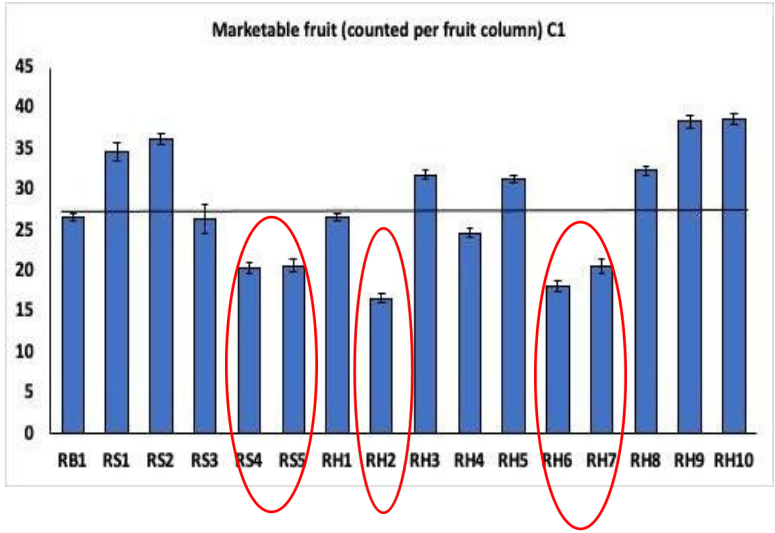
Red Papaya breeding lines plus F1 hybrids at Coast (C1) Vs RB1 commercial

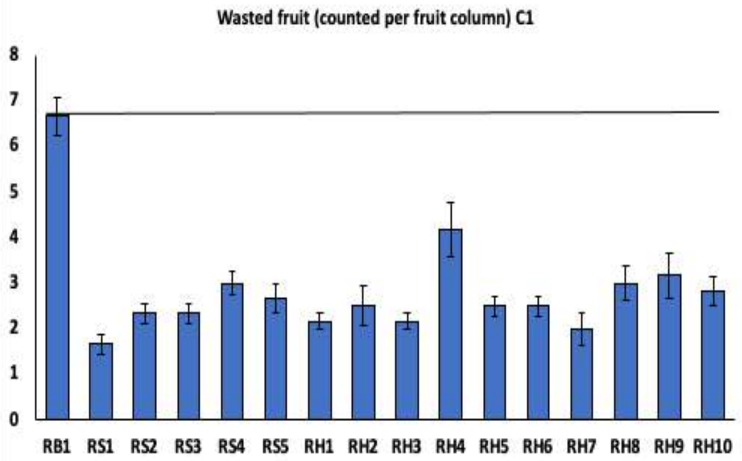
RIL Gains compared to RB1 = -16% to -19% lower to the ground
RIL Gains compared to RB1 = 15% to 80% thicker trunk
RIL Gains compared to RB1 = 28% to 35% more marketable fruit
RIL Gains compared to RB1 = -50% to -70% less wasted fruit

F1 Gains compared to RB1 = -2% to -49% lower to the ground
F1 Gains compared to RB1 = 17% to 40% thicker trunk
F1 Gains compared to RB1 = 1% to 60% more marketable fruit
F1 Gains compared to RB1 = -37% to -65% less wasted fruit



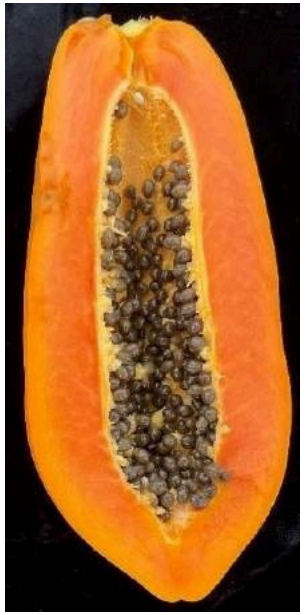






RB1 commercial

V
S



Sunlight 1

Sunlight 2

Red papaya F1 hybrid RH9

Red papaya F1 hybrid RH10

Fruit Quality-Red Papaya breeding lines plus F1 hybrids at Coast (C1)

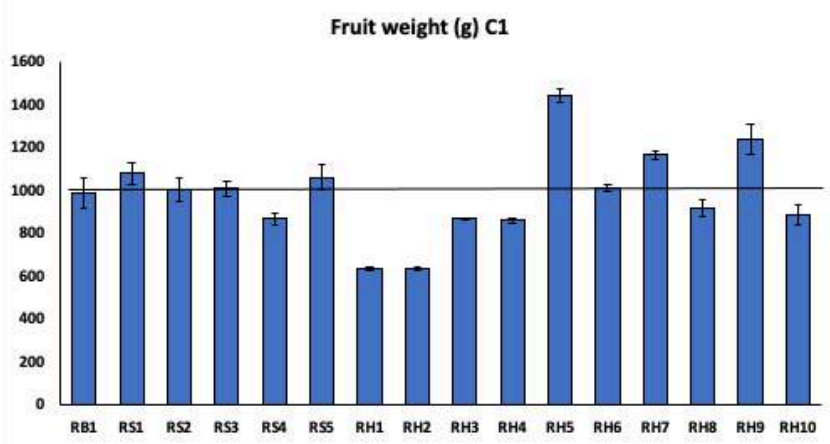
- RIL Gains compared to RB1 = +1% to +8% fruit weight
- RIL Gains compared to RB1 = -40% to -70% reduced cavity
- RIL Gains compared to RB1 = +20% to 45% thicker flesh
- RIL Gains compared to RB1 = 8% to 25% sweeter fruit

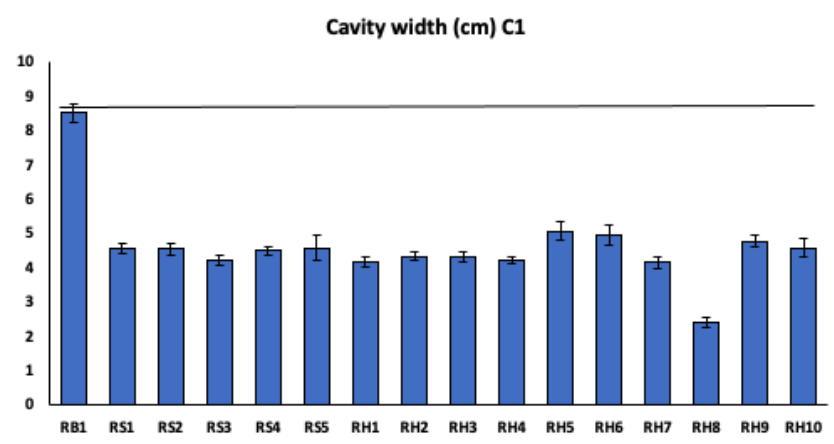
Ø F1 Gains compared to RB1 = -35% to +30% fruit weight

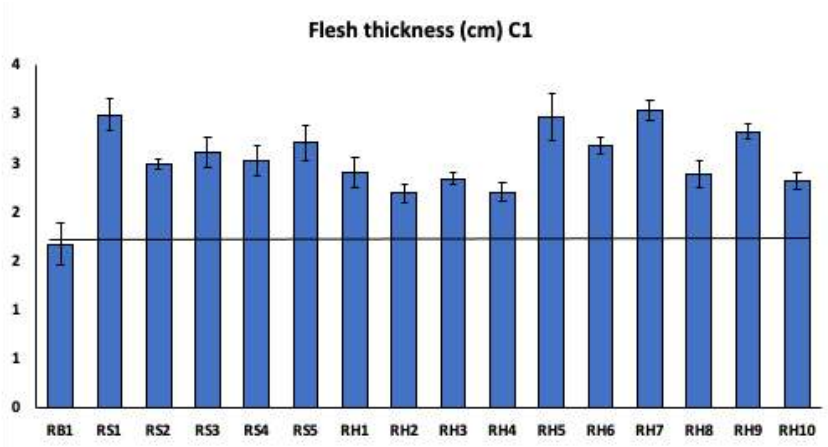
Ø F1 Gains compared to RB1 = -17% to -40% reduced cavity

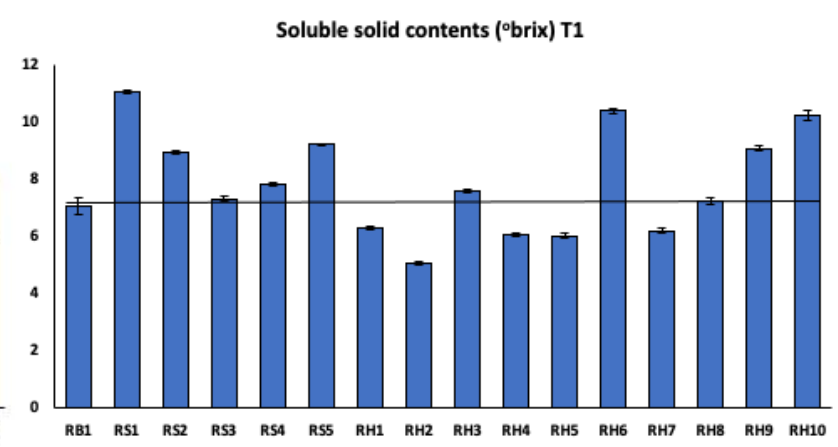
Ø F1 Gains compared to RB1 = 10% to 42% thicker flesh

Ø F1 Gains compared to RB1 = -15% to +26% sweeter fruit









Broad sense Heritability and Genetic Advances –Red Papaya

Heritability is the genetic makeup effect and is influenced by the environment.

Genetic advance is the estimation of genetics but is influenced by the environment.

Traits	$H^2_{b.s}$ at T1	$H^2_{b.s}$ at T2	$H^2_{b.s}$ at C1
Height to the first fruit (cm)	0.88	0.75	0.82
Trunk circumference (cm)	0.40	0.76	0.65
Number of marketable fruit (counted)	0.78	0.84	0.89
Number of wasted fruit (counted)	0.76	0.65	0.66

Traits	$H^2_{b.s}$ at T1	$H^2_{b.s}$ at T2	$H^2_{b.s}$ at C1
Fruit weight (g)	0.68	0.78	0.79
Cavity width (cm)	0.34	0.66	0.59
Flesh thickness (cm)	0.71	0.82	0.79
Soluble solid contents (obrix)	0.81	0.82	0.77

Traits	GA at T1 (%)	GA at T2 (%)	GA at C1 (%)
Height to the first fruit (cm)	11.83	15.99	17.37
Trunk circumference (cm)	11.22	14.38	11.58
Number of marketable fruit (counted)	12.15	10.11	10.32
Number of wasted fruit (counted)	13.15	12.31	10.15

Traits	GA at T1 (%)	GA at T2 (%)	GA at C1 (%)
Fruit weight (g)	2.83	5.79	11.37
Cavity width (cm)	9.22	10.38	5.58
Flesh thickness (cm)	6.15	5.11	6.32
Soluble solid contents (obrix)	11.15	15.31	16.15

Genotypic correlation (Measure of association among the traits)-Red Papaya

Ability to select multiple traits at the same time
Some traits have Positive while others have negative associations.

Traits	Height to the first fruit (cm)	Trunk circumference (cm)	Number of marketable fruit (counted)	Number of wasted fruit (counted)
Height to the first fruit (cm)	1	-0.54	-0.34	0.13
Trunk circumference (cm)		1	0.59	-0.32
Number of marketable fruit (counted)			1	-0.13
Number of wasted fruit (counted)				1

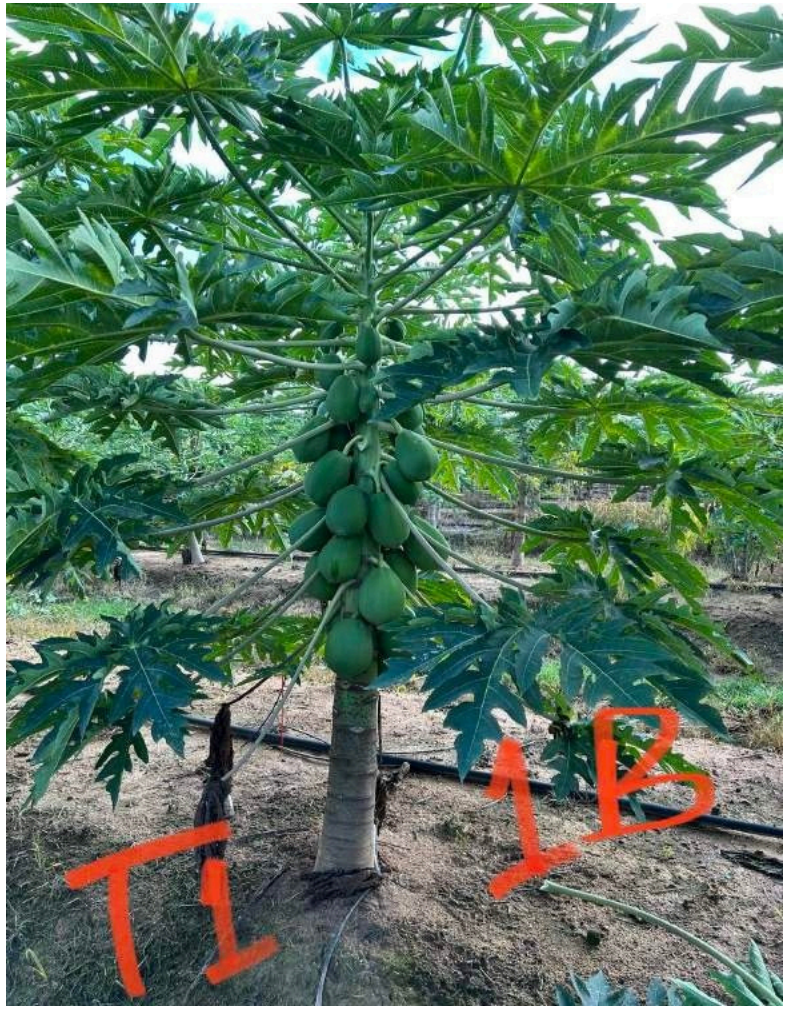
Traits	Fruit weight (g)	Cavity width (cm)	Flesh thickness (cm)	Soluble soli contents (ob
Fruit weight (g)	1	٠.١٢	٠.٥٤	0.20
Cavity width (cm)		1	0.36	٠.٠٩
Flesh thickness (cm)			1	٠.١٤
Soluble solid contents (obrix)				1

Fawad Ali* & Rebecca Ford (2022). The Inheritance Pattern of Key Desirable Agronomic and Fruit Quality Traits in Elite Red Papaya Genotypes.Horticulturae,8(9), 845. https://doi.org/10.3390/horticulturae8090845

Fawad Ali* and Rebecca Ford. (2023) Allele Mining in Tropical Fruit Crop Carica papaya L; Current scenario and future perspective. In Press: CRC Taylor & Francis 2023.

Fawad Ali* and Rebecca Ford. (2023). Saleable yield prediction using seedling root and shoot traits in red papaya elite genotypes. Under Review, Scientifica horticulturae

Yellow Papaya breeding lines



1B commercial

V
S

Moonlight 1

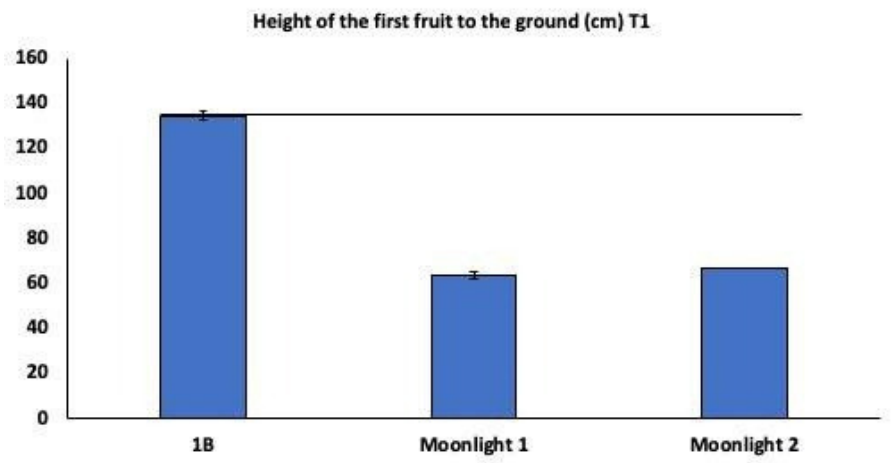
Moonlight 2

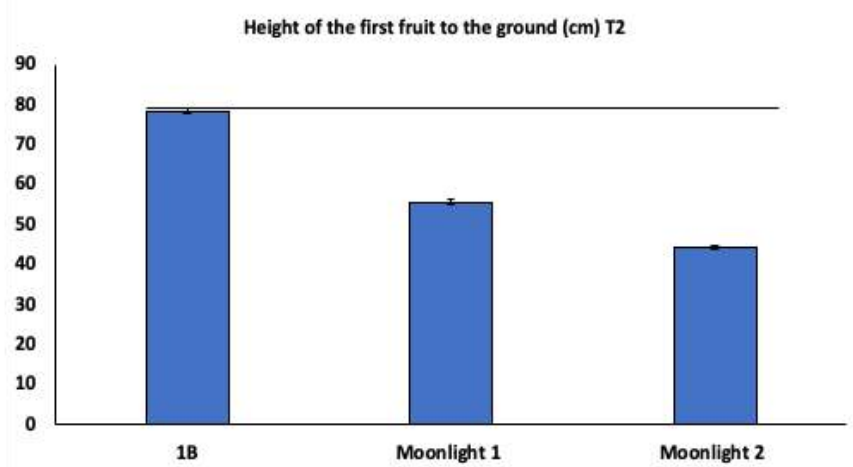
Agronomic and productivity traits-Yellow Papaya breeding lines

RIL Gains compared to 1B = -29% to -52% lower to the ground
RIL Gains compared to 1B = 12% to 30% thicker trunk

Broad sense heritability ($H^2_{b.s}$)-Yellow papaya

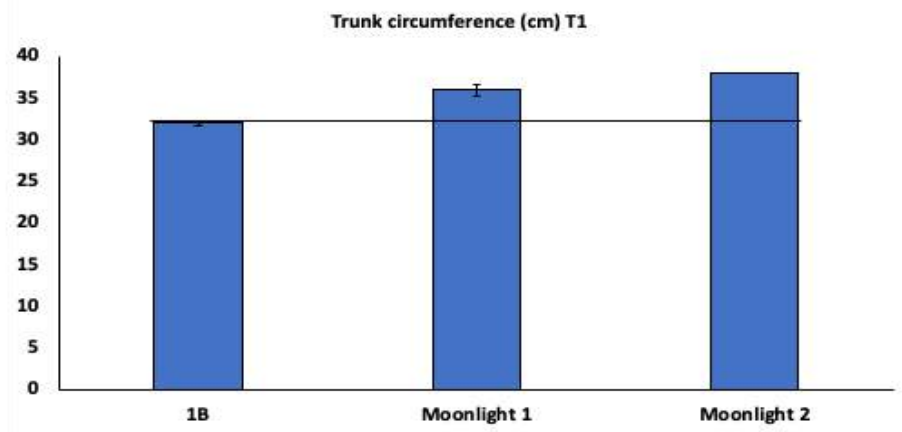
Traits	$H^2_{b.s}$ at T1	$H^2_{b.s}$ at T2
Height to the first fruit (cm)	0.65	0.82
Trunk circumference (cm)	0.85	0.66
Number of marketable fruit (counted)	0.77	0.81
Number of wasted fruit (counted)	0.82	0.88

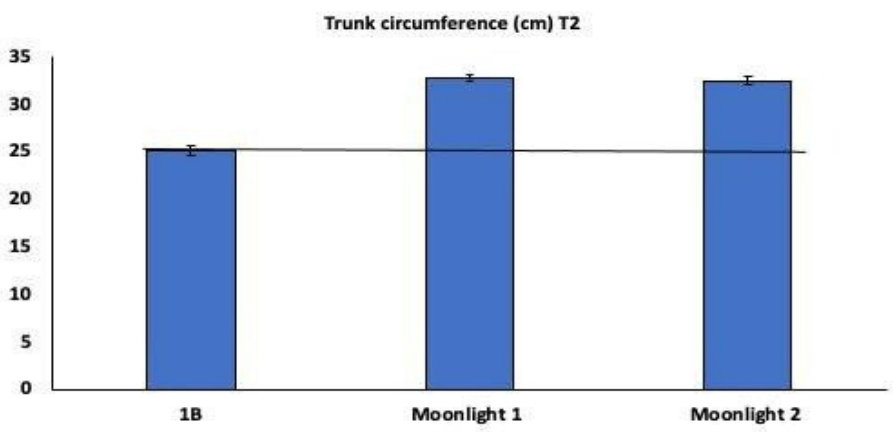




Genetic Advances (GA)-Yellow papaya

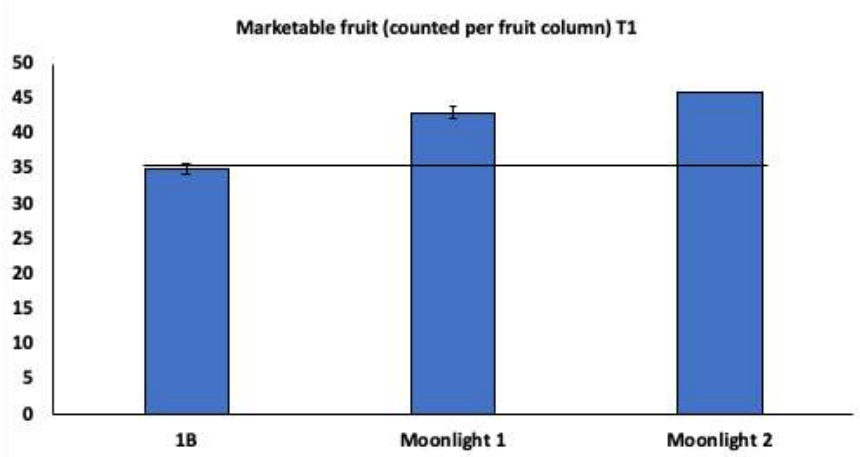
Traits	GA at T1 (%)	GA at T2 (%)
Height to the first fruit (cm)	7.88	١٦.٤٦
Trunk circumference (cm)	10.88	15.55
Number of marketable fruit (counted)	٨.٥٦	١٢.٢٢
Number of wasted fruit (counted)	١٥.١١	١٤.٨٨

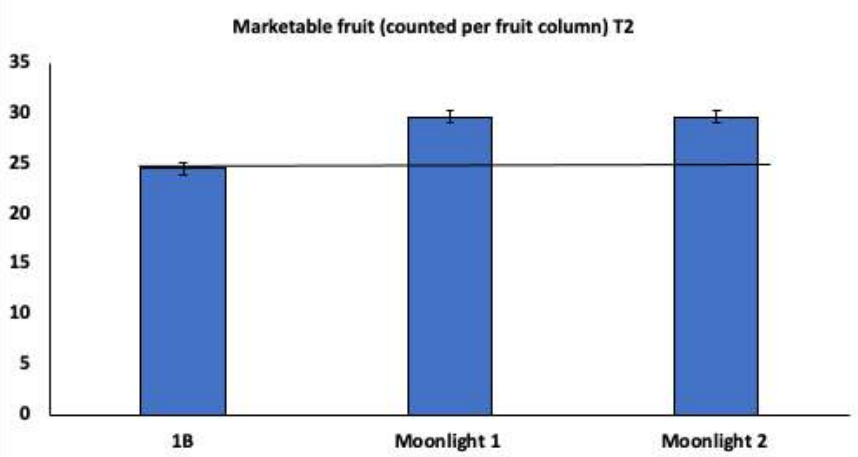


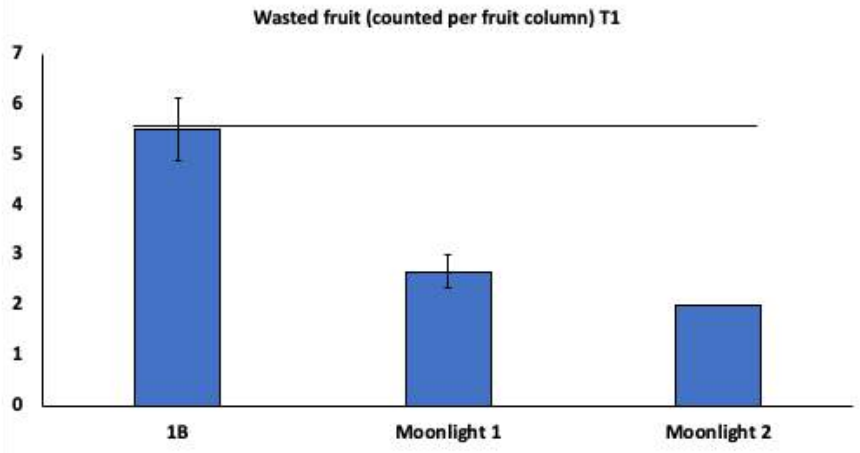


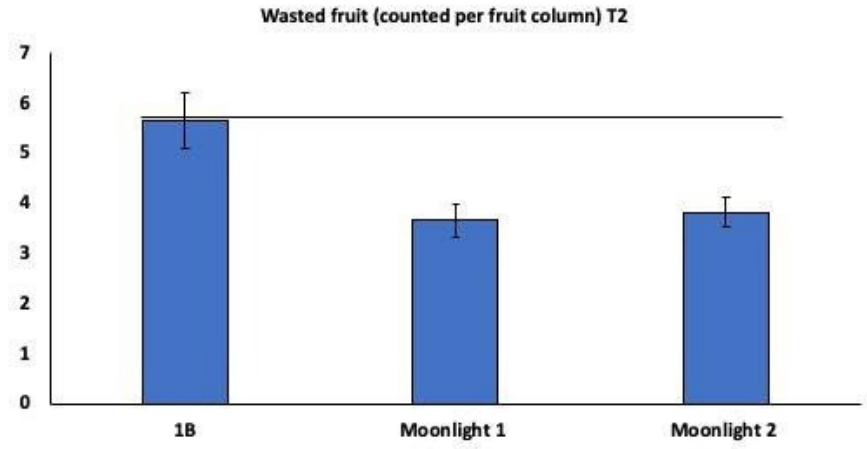
Yellow Papaya breeding lines

RIL Gains compared to 1B = 20% to 31% more marketable fruit
RIL Gains compared to 1B = -32% to -63% less wasted fruit

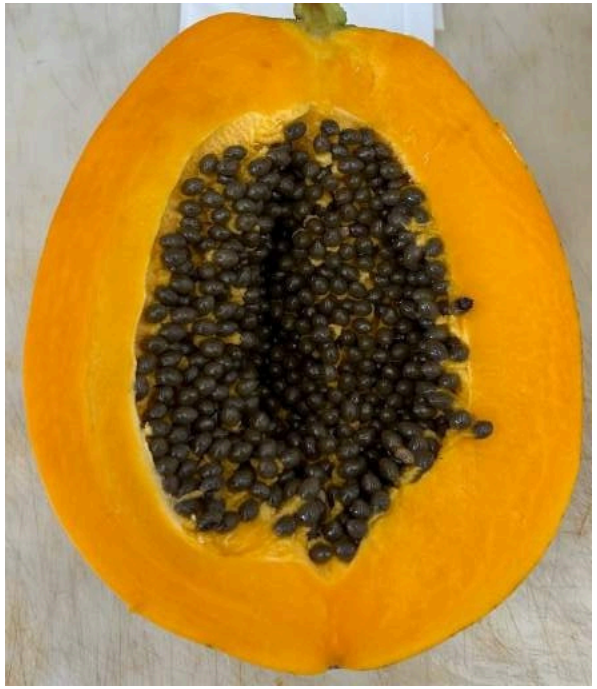








Moonlight 1 and Moonlight 2
Vs
1B commercial



1B commercial

V
S

Moonlight 1

Moonlight 2

Fruit quality-Yellow Papaya breeding lines

RIL Gains compared to 1B = -40% to -51% fruitweight

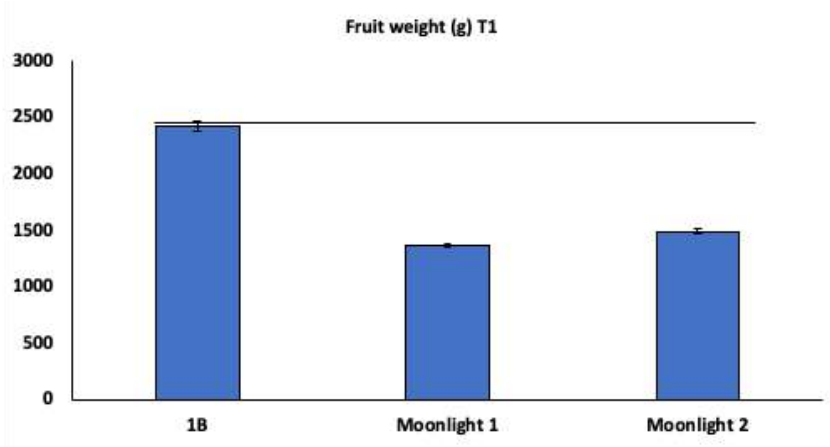
RIL Gains compared to 1B = -12% to -30% reduced cavity

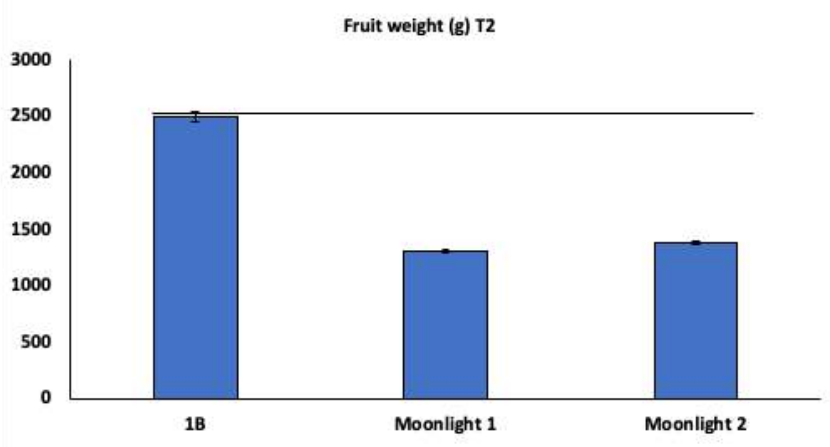
Broad sense heritability ($h^2_{b.s}$)-Yellow papaya

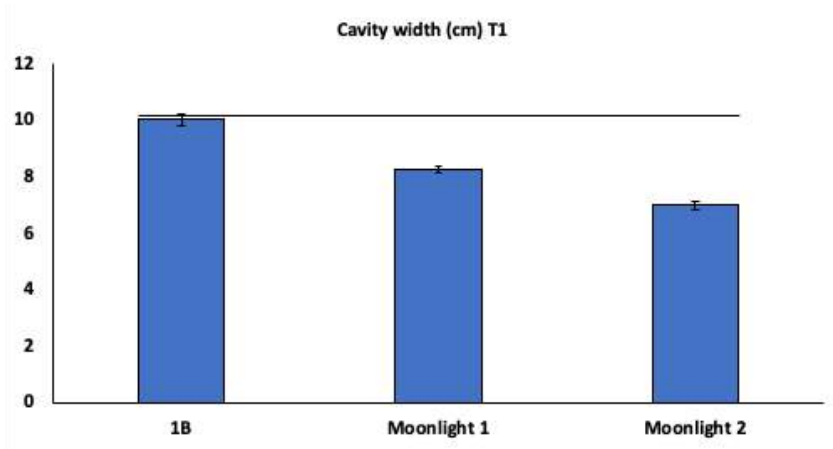
Traits	$H^2_{b.s}$ at T1	$H^2_{b.s}$ at T2
Fruit weight (g)	0.75	0.80
Cavity width (cm)	0.78	0.61
Flesh thickness (cm)	0.69	0.85
Soluble solid contents (obrix)	0.79	0.77

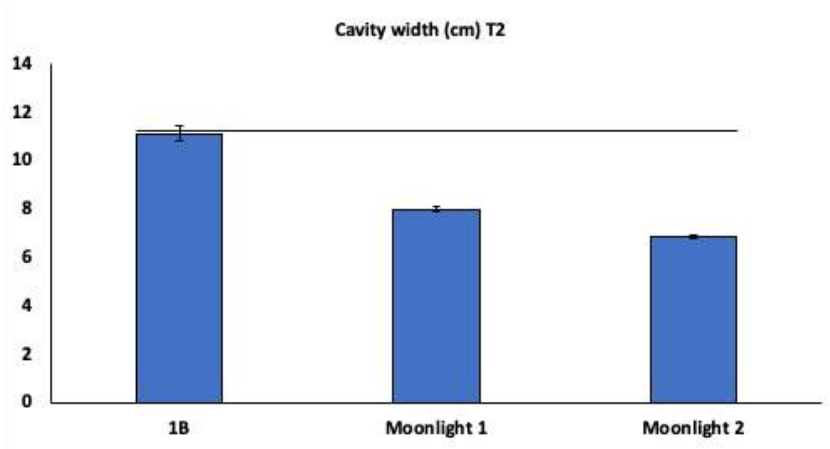
Genetic Advances (GA)-Yellow papaya

Traits	GA at T1 (%)	GA at T2 (%)
Fruit weight (g)	5.56	١٢.٣٣
Cavity width (cm)	6.88	6.45
Flesh thickness (cm)	٤.٥٦	٦.٢٦
Soluble solid contents (obrix)	١٠.٩٩	٩.١٢



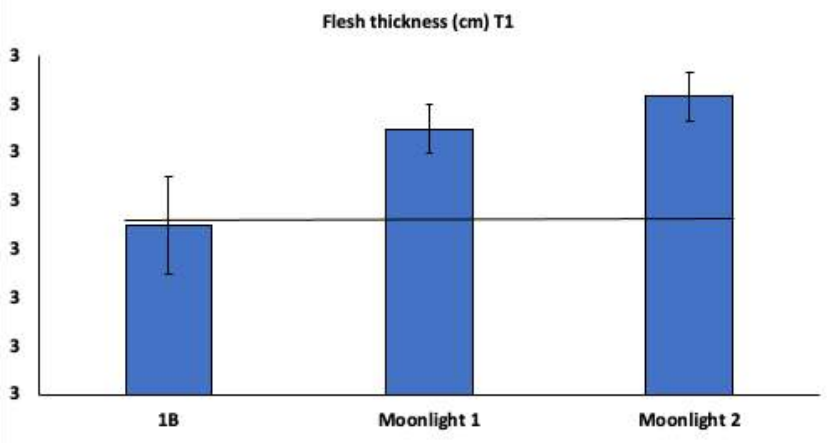


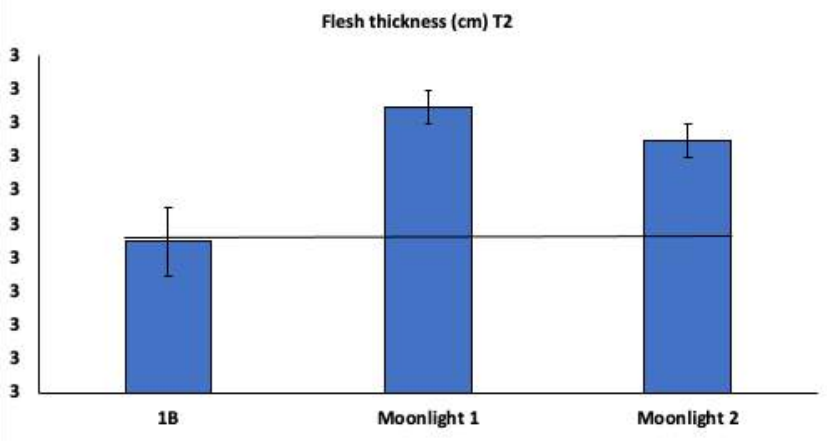


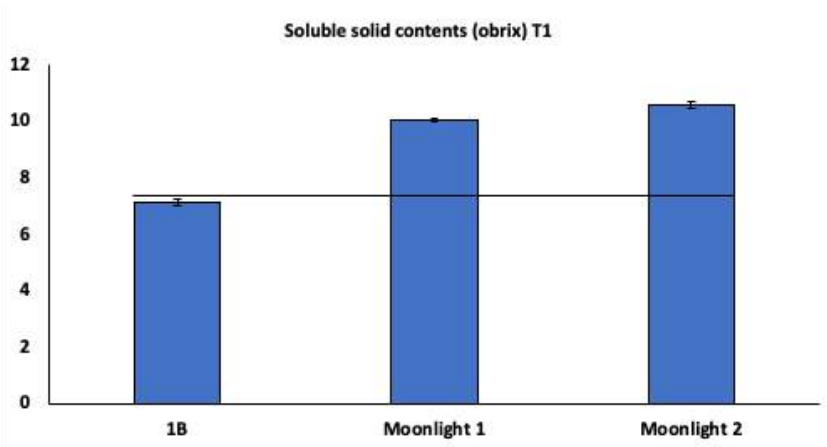


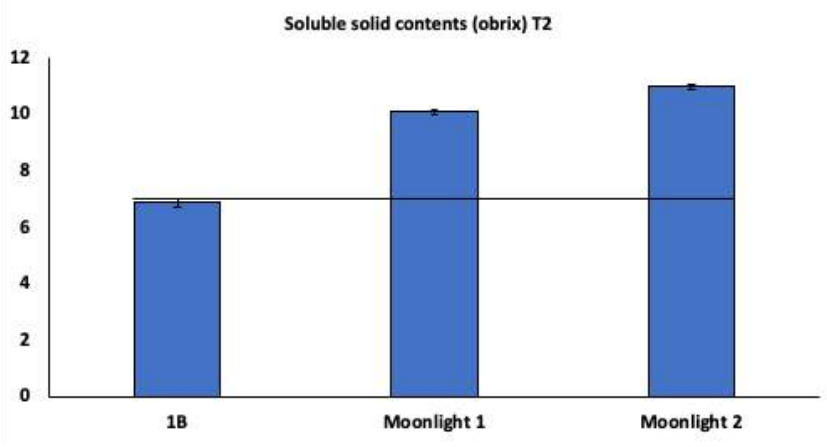
Yellow Papaya breeding lines

RIL Gains compared to 1B = +20% to 33% thicker flesh
RIL Gains compared to 1B = 20% to 30% sweeter fruit



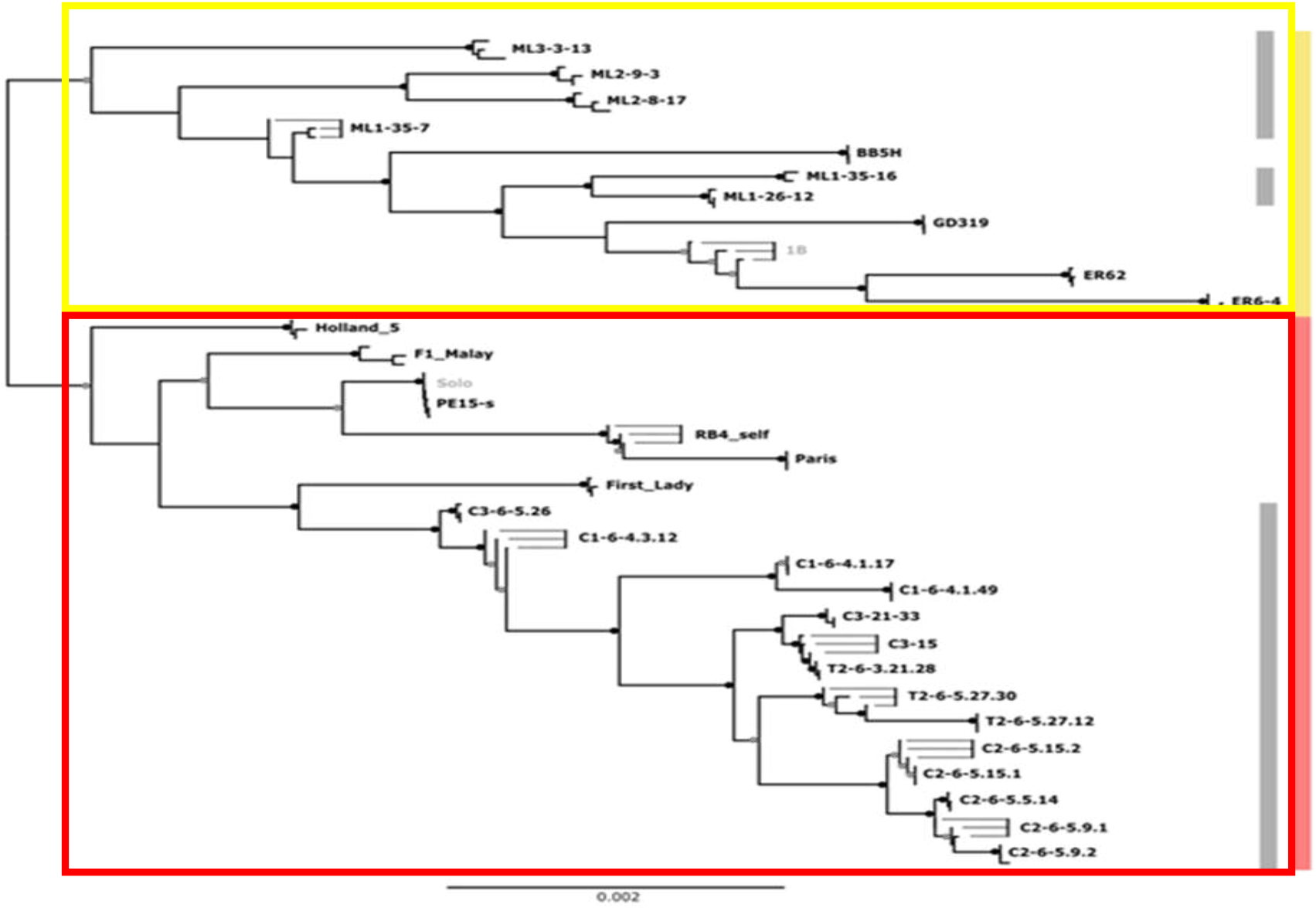




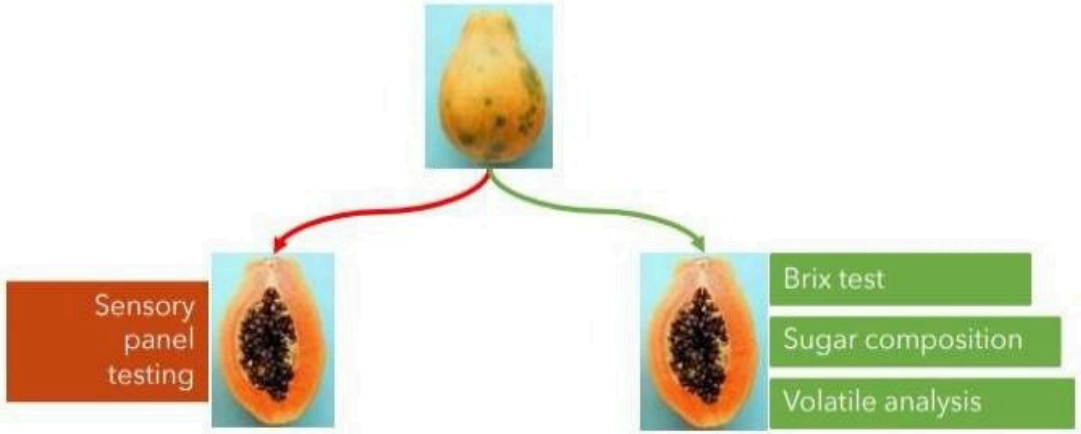


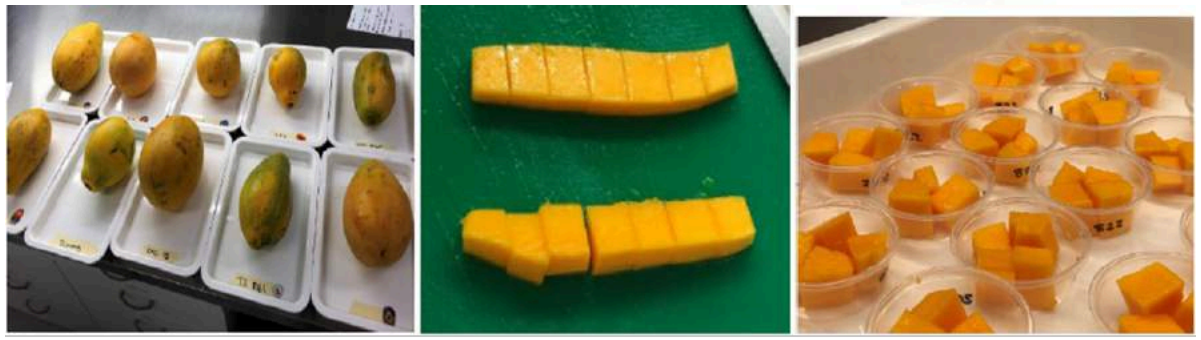
Available and sought genetic diversity among papaya (red-and-yellow) gene pool-PP18000

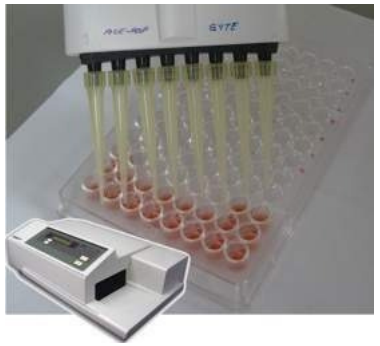
- oSeflected improvement traits based on existing gene pool-PP18000
- Sufficient genetic diversity in the current PP18000 gene pool
- The heterozygosity (Ho) of the **F1 red papaya hybrids (0.15 to 0.32) > parents (0.01 to 0.09)**
- Considerable effect of **heterosis breeding**.
- However, the future might look for new traits of interest for the Australian market.

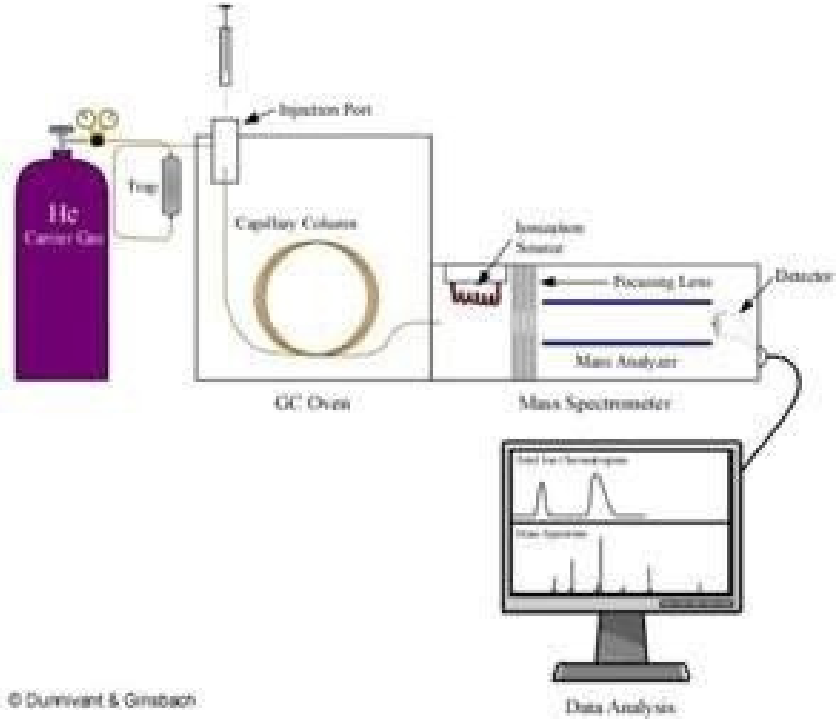


Sensory Tasting at UQ









Sensory characteristics of papaya samples
PCA (Attributed variation observation)

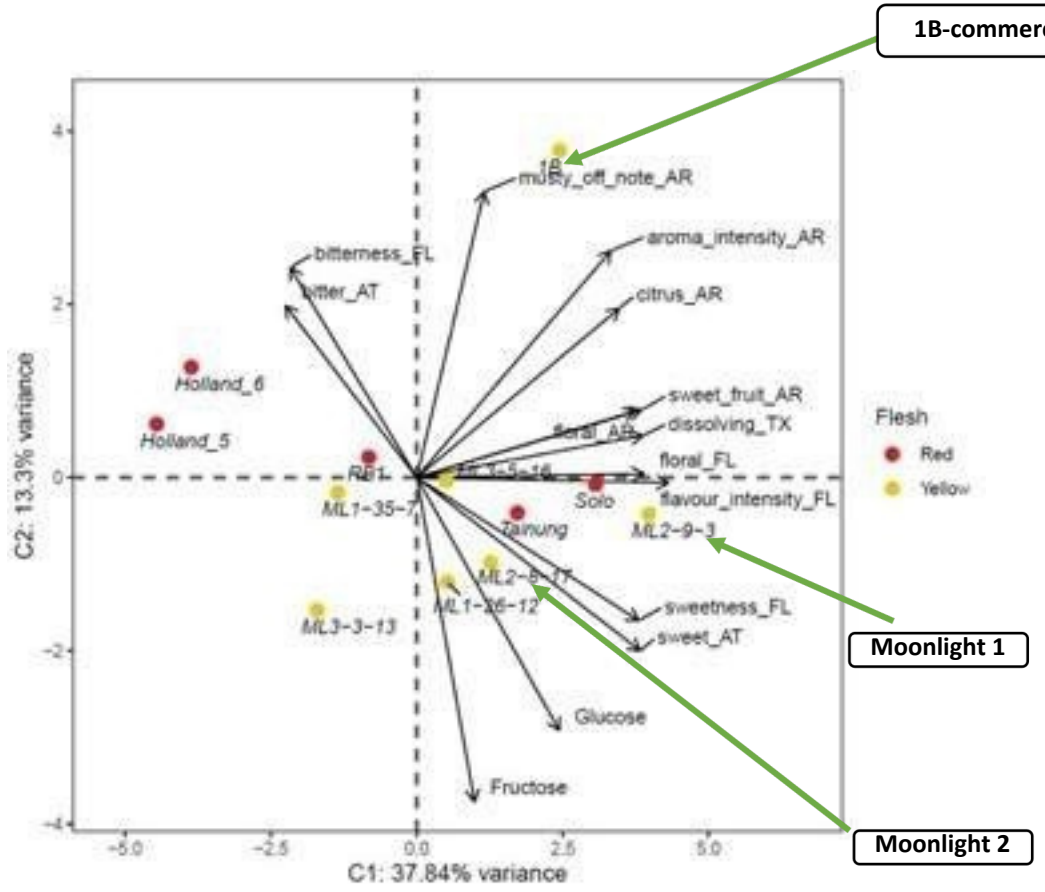


Figure 1: **Sensory characteristics** of papaya samples from the **August 2022** sensory panel.

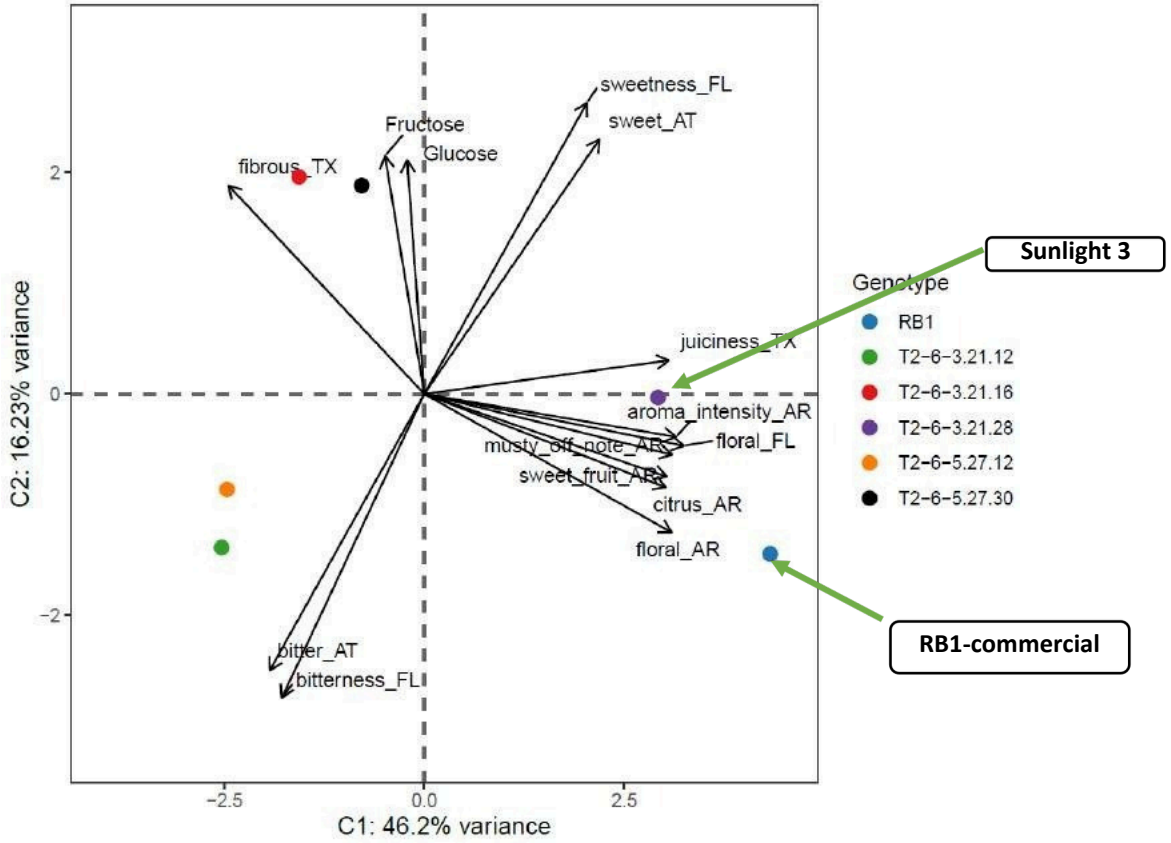
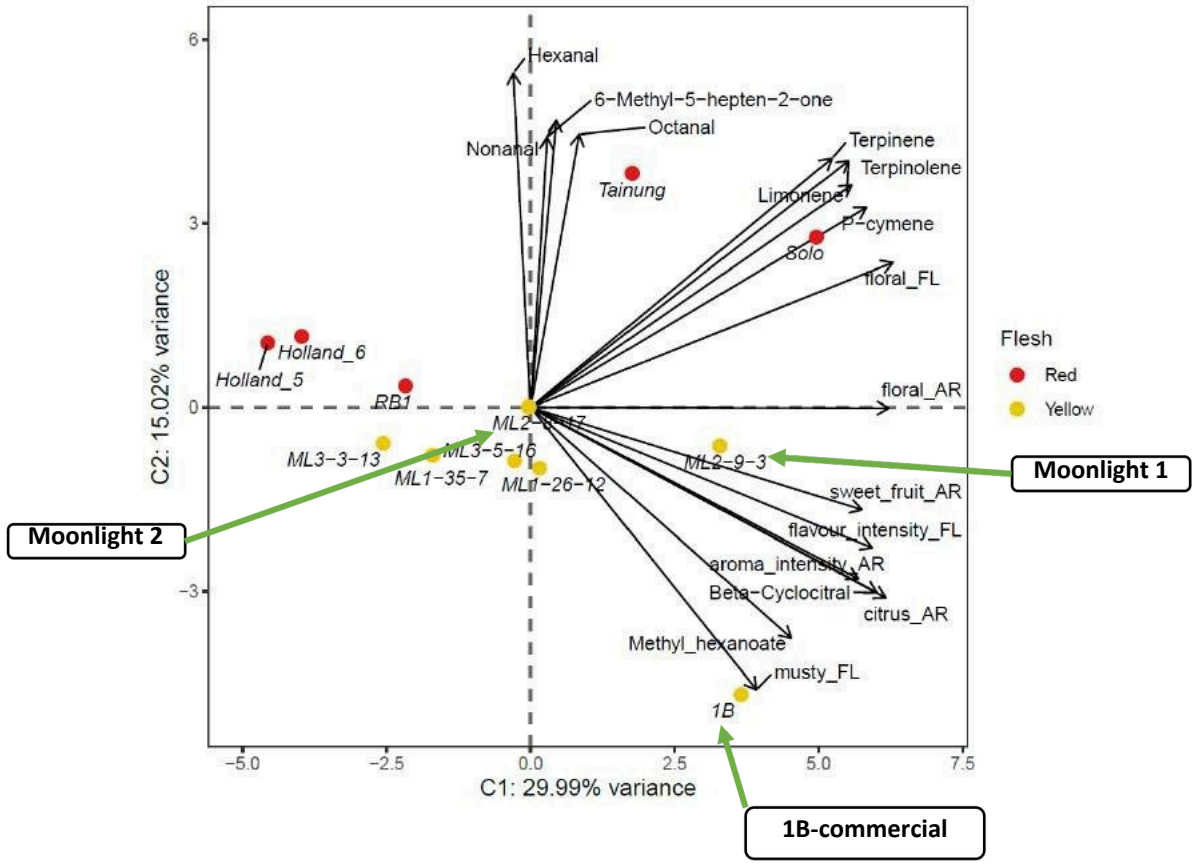
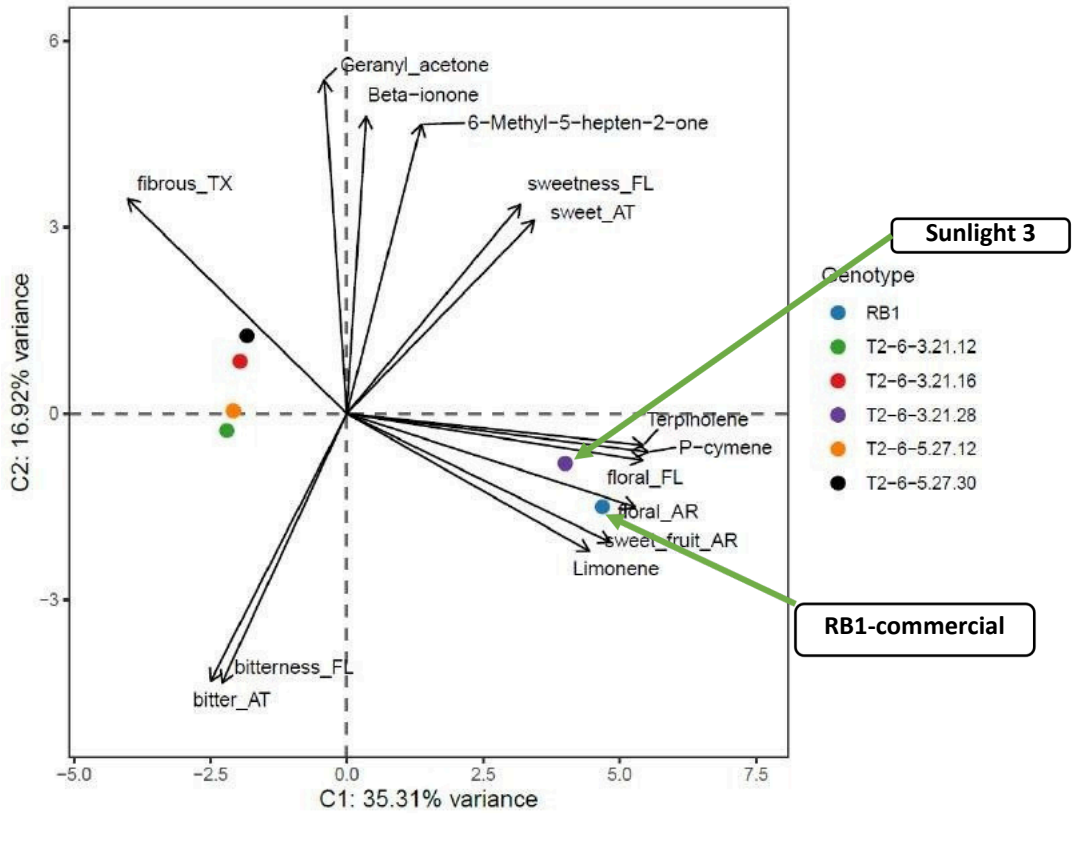


Figure 2: **Sensory characteristics** of papaya samples from the **February 2023** sensory panel.

Volatile compounds characteristics of papaya samples
PCA (Attributed variation observation)



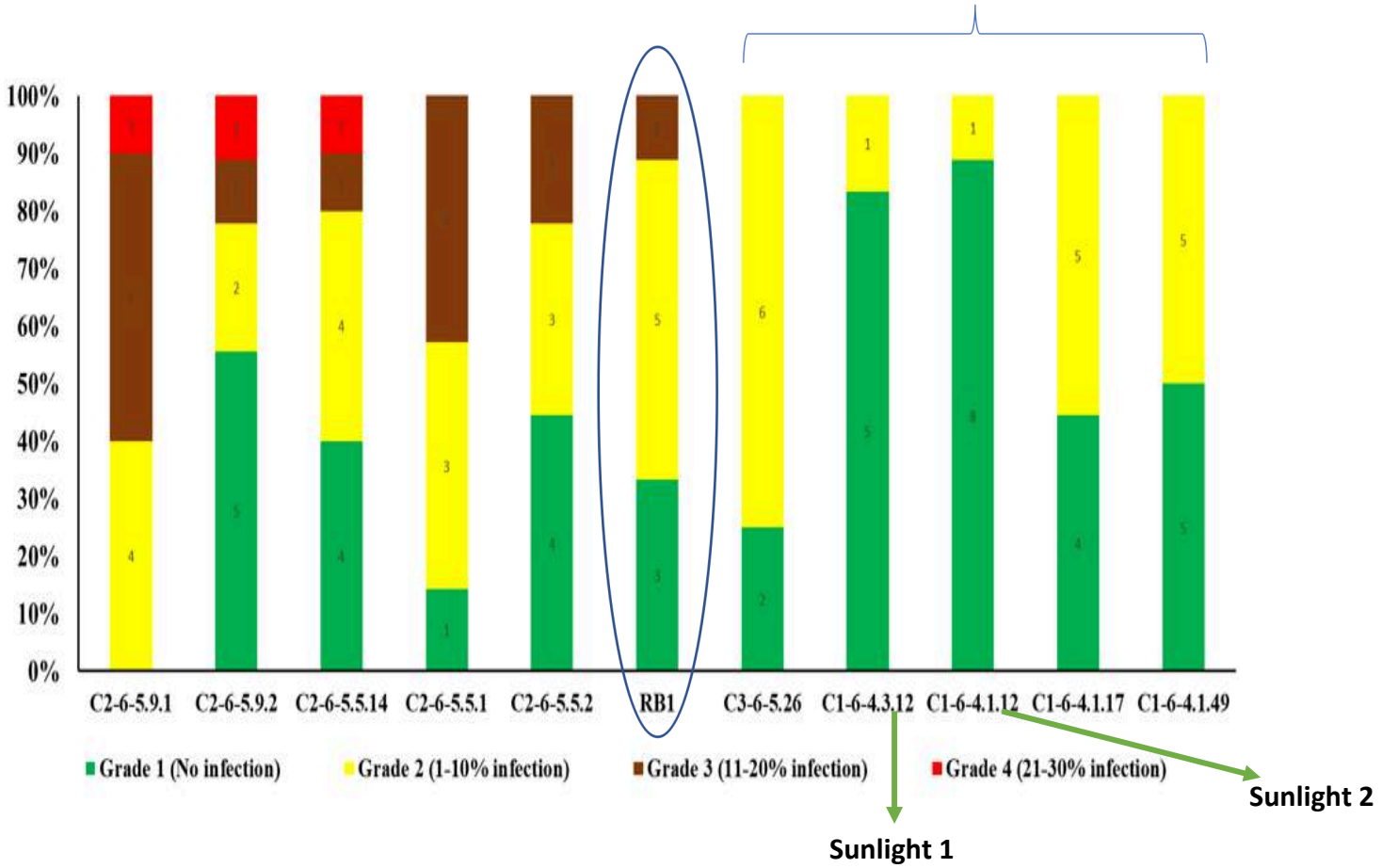
Volatile organic compounds of papaya samples from the **August 2022** sensory panel.



Volatile organic compounds of papaya samples from the **Feb 2023** sensory panel.

Phytophthora disease incidence

Single assessment at a high inoculum site



In summary

We have advanced Red and Yellow papaya breeding lines for every house AND farm-hold with:

-Far better agronomic performance

-Far better consumer-desirable

-Taste really sweet and good

These traits are:

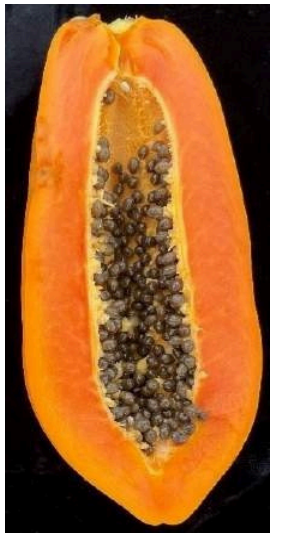
-Generally, stably inherited within a location

-Some vary across locations/environments

-Plant Breeder Rights Stage 1 has been applied **(Sunlight 1, Sunlight 2 and Sunlight 3)**

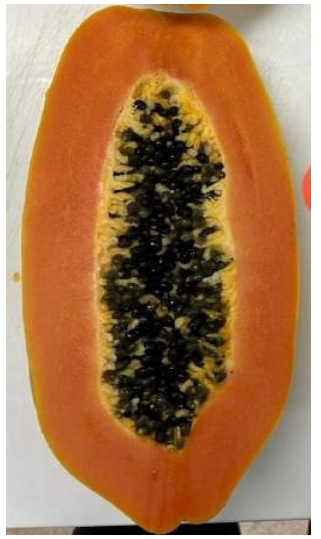
Moonlight 1

Moonlight 2



Sunlight 1

Sunlight 2



Sunlight 3

1B commercial

RB1 commercial

Red papaya F1 hybrid RH9

Red papaya F1 hybrid RH10

Commercial red papaya RB1

Papaya Team-Griffith University

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Griffith University

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Griffith University

Dr Ido Bar
Griffith University

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Kasetsart University, Thailand

Acknowledgement

Papaya Grower and industry leader – Mr Gerard Kath

(Left-Phil Slocombie; Director PSA) and (Right- Papaya Grower – Mr Chris Maisel (Rocky Top Farms)

Papaya Grower – Mr Joe Zappala (Zappala Farms)

Possible future directions for follow on R&D activities

Proposed title: *Seed multiplication and commercial assessment of elite papaya genotypes.*

- Assessing the **stability of traits in F1 hybrid** in fully **commercial trials**.
- **Optimising farming practices**, e.g. Nitrogen use, pests and diseases, etc.
- **Deeper investigation** for more accurate future selection of the **genetic components** of the top preferred traits. Implementing the **bio-economic quantitative genetic model (developed by Dr Fawad Ali**, Papaya Breeder and submitted in M110 to HortInnovation) will rank traits in elite papaya genotypes.
- Development of **novel mapping populations**
- Development of **novel F1 hybrids** (red-and-yellow papaya types)
- Out-crossing with targeted (**that favourite types**)
- **Linking Frontiers funds** guided by **industry/breeder needs**

- A **further collaboration** to introduce **new** international germplasm.
- Germplasm from South America (focusing on **Brazil and Mexico**) and Indo-Pacific regions (**Malaysia, Philippines and Thailand**) based on the **relationship** developed **through PP18000**.

Recent developments for the future of the papaya industry

- F7 red for Tablelands breeding lines
- Five (5) new F1 hybrids were developed for the Tablelands
- Seed bulking crosses were done for RH9 and RH10 for commercial trials
- Three new mapping populations were developed using RH1, RH9 and RH10 (flavourprofile, tree agronomy and productivity)
- F7 yellow breeding lines (Moonlight 1 and Moonlight 2)
- Three new F1 yellow papaya hybrids
- Sex-determination assay: technology transfer to papaya industry