Horticulture Innovation Australia

Final Report

Marker Assisted Breeding of Papaya to Develop new Commercial lines

Dr Roderick Drew
Griffith University

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Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

Contents

Summary

One of the challenges facing the papaya industry in Australia is to grow disease resistant varieties that produce fruit with superior eating quality all year round. This goal can only be achieved through the availability of disease resistant, high yielding and flavoursome varieties. Breeding to improve current commercial varieties of Australian papaya is a necessity. The research presented here had two major aims.

A challenge for the Australian papaya industry is to improve eating quality of commercial varieties of both yellow and red papayas, and to enhance uniformity and yield of red papayas. Twenty-seven lines and breeding selections of papaya were evaluated for 11 productivity traits and 13 characteristics related to fruit quality based on both grower and consumer preferences. Trees were evaluated at three different harvesting times, April 2012, October 2012 and May 2013, to determine the extent to which differences represented the genetics of the individuals rather than environmental effects. Selection of parents for the next phase of a breeding program to develop segregating populations was based on consistent expression of the attributes of interest over the three harvest seasons. Five groups of traits (flavour, skin quality, eating quality of red papaya, eating quality of yellow papaya and yield) were chosen for the production of segregating populations, which could be used for the development of DNA markers for those traits. Twenty-three representative trees were selected as parental lines and 17 crosses were made for the breeding programs to develop improved commercial lines and for a program of DNA marker-assisted selection.

To demonstrate the application of DNA markers to improve efficiency of the seedling selection process, flesh fruit colour was chosen, as it is one of the important traits for papaya production in Australia. DNA markers with diagnostic alleles associated with red flesh, CPFC2-R, and yellow flesh, CPFC2-Y were developed by using information from published DNA markers. These markers identified the attribute at 95.75% in 330 breeding lines, where CPFC2-R and CPFC2-Y showed 97% and 93% association with red- and yellow-fleshed plants, respectively.

Information from this research can be applied to plant improvement through conventional breeding, marker assisted breeding and genetic modification. In addition, segregation populations have been developed for future breeding work and for the development of molecular markers for a wide range of character traits. This will facilitate future research and enhance selection of new commercial varieties.

Work is progressing for the development of PRSV-P resistant genotypes that can be used to cross this virus resistance into elite papaya lines. Resistance genes from V. pubescens have been crossed into susceptible species V. parviflora. The resistant V. parviflora has been crossed top papaya and plants containing the R genes have been identified in vitro by a DNA marker after embryo rescue of hybrid embryos. These plants will be tested for fertility in the next phase of the project.

Keywords

Papaw, papaya, DNA markers, MAS, breeding, papaya ringspot virus, micropropagation, plant tissue culture

Introduction

Breeding programs in other major papaya producing countries have produced commercially named varieties (e.g. Solo, Tainung, Exotica), and promising breeding lines. However, Australia industry funded breeding programs undertaken by Queensland Government researchers for the last 20 years have not produced any new commercial or useful breeding lines. The commercial lines that are grown in Queensland were produced in a private breeding program. Currently, the commercial lines preferred by growers are 1B (yellow) and RB1, RB2 and RB4 (red). These lines are high yielding and produce fruit with clean skins, with good shape, and weight. This breeding program used a different approach to previous methods in Queensland. We aimed at maintaining the good characteristics of B1, RB1, RB2 and RB4 but incorporating some of the better traits of other available lines such as improved flavour, flesh texture, higher brix levels, longer shelf life, antracnose and phytophthora resistance. HAL projects FR99018 and PP04004 micropropagated and evaluated breeding lines that were produced in previous programs. These micropropagation systems were also employed in this project to maintain and multiply all parent lines, F1 plants and others that showed potential, and elite trees from grower properties. Although superior lines can be multiplied in tissue culture, the long term goal of this project is to produce hybrid lines that can be grown from seed OR micropropagated by tissue culture.

Rod Drew has had comprehensive breeding programs to develop PRSV-P resistance in papaya (papaw) genotypes for 20 years and recently achieved the first successful transfer of PRSV-P resistance from a *Vasconcellea* species (V. quercifolia) to papaya *(Carica papaya* L.). There are conflicting reports on PRSV-P resistance of *V. quercifolia*. It has been reported to be resistant to PRSV-P in Florida (Conover, 1964), Hawaii (Manshardt and Wenslaff, 1989b), and Australia (Drew et al., 2006a), but susceptible to PRSV-P in Venezuela (Horovitz and Jimenez, 1967). However, *V. pubescens* has been reported to be resistant against all strains of PRSV-P in all countries for more than 60 years. Therefore in this project, we continued a breeding program aimed at transferring PRSV-P resistance from *V. pubescens* to *papaya* using *V. parviflora* as a bridging species.

Previously at Griffith University, a genetic map of two *Vasconcellea* species was generated using Randomly Amplified DNA (RAF) polymorphisms (Dillon et al., 2005). DNA markers linked to a single dominant PRSV-P resistant gene locus (*prsv-1*) in *V. pubescens* were identified (Dillon et al., 2005). A SCAR (Sequence Characterised Amplified Region) marker and a codominant CAPS (Cleavage Amplified Polymorphic Sequence) marker were developed, are diagnostic for PRSV-P resistance in *V. pubescens* and its progeny (Dillon et al., 2006) and were used in subsequent breeding programs (O'Brien and Drew, 2009). In this project, a PhD student (Chutchamas Kanchana-udomkan**)** enrolled at Griffith University and worked on continued development of molecular markers for disease resistance and other useful agronomic traits that could be used in marker assisted selection.

Methodology

Breeding for New Commercial Lines

Parent lines were obtained from collections (at GU and from growers in Australia) and lines that were available in Queensland from Philippines, Malaysia, Taiwan, Thailand, Vietnam, Hawaii, Brazil and commercial lines in Queensland. Breeding lines produced in previous breeding trials were used as parents.

Lines of papaya were grown for evaluation leading to selection of parental lines for two main purposes; firstly to improve eating-quality in commercial varieties and secondly to establish segregating populations to use for identifying DNA markers for other traits.

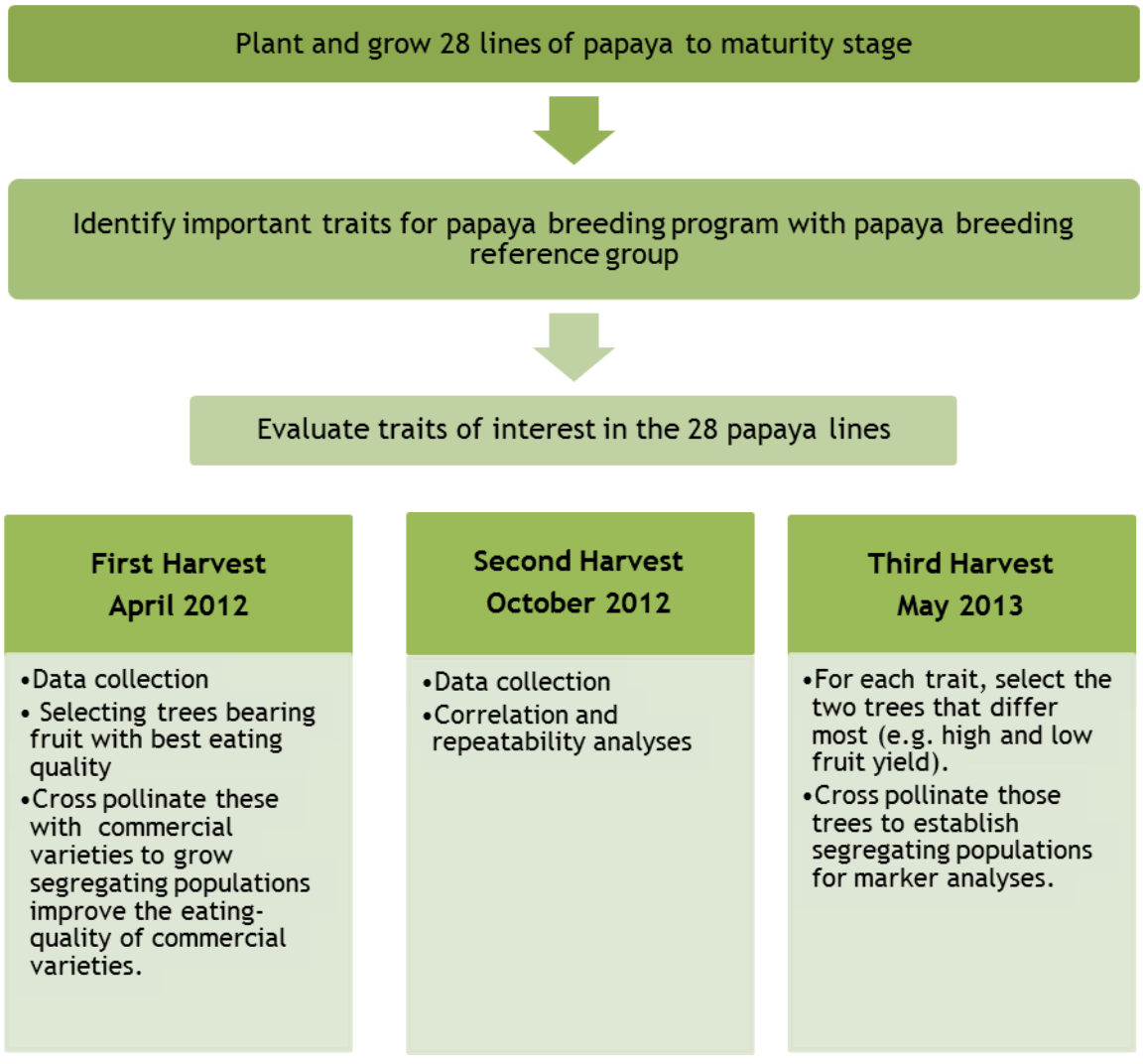

Figure 1: Steps of work for developing papaya breeding population and analysis of productivity and fruit quality traits and phenotypes

Table1: Identity, ancestry and source of 28 papaya lines planted at Lecker Farming, Mareeba, Queensland

Line number[1/]	Ancestry or variety name[2/]	Source of seed
R01	RB1 x 18-45	Cross of commercial red papaya RB1 and DAFFQ line #18-45
R02	RB1 x 24-29	Cross of commercial red papaya RB1 and DAFFQ line #24-29
R04	RB2 x 18-45	Cross of commercial red papaya RB2 and DAFFQ line #18-45
R06	RB2 x 25-5	Cross of commercial red papaya RB2 and DAFFQ line #25-5
R09	25-5 x RB1	Cross of DAFFQ line #25-5 and commercial red papaya RB1
Y11	24-29 x RB2	Cross of DAFFQ line #24-29 and commercial red papaya RB2
Y15	1B x 33-66	Cross of commercial yellow papaya 1B and DAFFQ line #33-66
Y16	7-82 x 1B	Cross of DAFFQ line #7-82 and commercial yellow papaya 1B
Y17	24-87 x 1B	Cross of DAFFQ line #24-87 and a commercial yellow papaya 1B
R19	24-29 Self	Self pollination of DAFFQ line #24-29
Y20	JC2	[3/]
R21	25-5 Self	Self pollination of a DAFFQ line #25-5
R22	TS2	[3/]
R23	Malaysian Red 1	[3/]
R24	Malaysian Red 2	[3/]
R25	Malaysian Red 3	[3/]
Y26	1B	A commercial yellow papaya
R27	RB2	A commercial red papaya
R28	RB4	A commercial red papaya
R29	Sunrise Solo	[3/]; it is a commercial red papaya in Hawaii
R30	Solo Linda	[3/]
R31	RD6 Self	[3/]
R33	Brazilian Solo	[3/]
Y34	2.54-14 self	[3/]
Y35	2.54-12 self	[3/]
R41	JC2 x Vietnam Red	[3/]
R42	TS2 Self	[3/]
R48	Red Lady, Taiwan	[3/]

[1/] The prefix R refers to red fleshed fruit; Y to yellow

[2/] Female parent is noted first in each cross.

[3/] From a collection of seed held by Professor Rod Drew, Griffith University, Brisbane.

Important commercial traits were identified during discussions with local commercial papaya producers. Traits were grouped into those relating to tree productivity and those relating to fruit quality. Ten fruit-bearing trees of each line were selected at random to evaluate traits. They were evaluated at three different harvesting times, April 2012, October 2012 and May 2013, to confirm that the data represented the genetics of the trees rather than environmental effects.

Nine productivity traits that related to performance of the trees were identified and recorded. They were:

i. Sex type: Height to the first flower
ii. Height to the first mature fruit
iii. Height to the first marketable fruit
iv. Peduncle length was recorded using a 1,3 and 5 rating scale; where
v. Yield of fruit of marketable fruit
vi. Yield gap
vii. Number of carpelliod fruits was counted for each tree.

Thirteen traits that related to fruit quality were identified and recorded:

i. Fruit shape
ii. Teat shape
iii. Stalk insertion
iv. Skin quality
v. Skin freckle
vi. Skin colour
vii. Cavity shape
viii. Consistency in flesh colour
ix. Flesh colour
x. Flesh firmness
xi. Useable flesh thickness.
xii. Flesh sweetness
xiii. Fruit flavour

Trees that good flavour flavour and other eating quality traits were selected as based on the result in the first harvest due to time limitation and the commitment to the project fund to produce crosses as soon as possible. Crosses were made from these selected trees to red and yellow fruited commercial papayas. The best three lines that were selected on the first evaluation (April 2012) were TS2, Malaysian Red 2 and Sunrise Solo. They exhibited excellent fruit eating quality (flesh flavour, firmness and thickness) and were selected to cross with Australian commercial lines 1B, RB1 and RB2. Of these 16 crosses from the second breeding population, seven trees, two and five of yellow and red papaya, respectively, were selected for their flavour and overall yield. These trees were used as parental lines to backcross to commercial varieties 1B, RB1 and RB2 and to sib cross to produce new hybrids.

A full description of materials and methods is presented in appendix 2.

Breeding for Papaya Ringspot Resistance (PRSV-P)

BC_4 (*V. parviflora* x *V. pubescens*) plants were produced by backcrossoing a BC_3 (*V. parviflora* x *V. pubescens*) plant to *V. pubescens*. They were evaluated for PRSV-P resistance and fertility. One line (clone 113) was selected as it produced a high yield of viable pollen and was PRSV-P resistant. Embryos were rescued from a cross of a BC_4 (*V. parviflora* x *V. pubescens* clone 113) X papaya to breed for PRSV-P resistance by introgression of the resistance from *V. pubescens*. They have been micropropagated and will be evaluated for fertility and resistance to PRSV-P. These plants could be included in the next phase of the breeding program. Molecular markers for PRSV-P resistance have been developed and refined and are available for use in evaluating these lines and future progeny.

Development of Molecular Markers

A segregating population was evaluated both for commercial traits and MAS. Selected parental trees for each trait including flavour, flesh quality, skin quality and yield, from the first breeding trial were cross-pollinated to produce F_1 plants, Seventeen crosses have been made. Seeds have been collected and cleaned.

DNAs of approximately 350 trees of the first breeding trial were extracted; and, a DNA marker linked to flesh fruit colour was selected to validate in these plant material. Co-dominant markers were developed and they showed 93% and 99% association to yellow and red flesh colour, respectively. The linkage between the markers and trait will be analysed in a F2 segregating population. This marker will be useful to aid selection for flesh colour when crossing unknown genotypes of red flesh and yellow flesh. It can also be used to help plan for on farm plantings and management.

Micropropagation / Plant Tissue Culture

Apically dominant plants were dissected into nodal sections and sub-cultured in a multiplication medium for 4 weeks (Appendix 1). Shoots were removed from the nodes and transferred to a root induction medium for 3 days (Appendix 1). They were then transferred to a plant growth regulator-free medium (Appendix 1), also known as "single shoot medium" for 3-4 weeks. Then, the cycle of micropropagation was repeated by multiplication, root induction and shoot induction (Figure 2.1) to multiply the number of plants that were required. All plant sections were incubated under conditions of 16 hours light from fluorescent lamps and 8 hours dark at 25ºC ± 1ºC.

Rooted plants from shoot induction medium were acclimatized following the procedure of Drew (1988). Roots were washed under tap water to remove residual agar. Each plant was placed in a 25mm square by 150mm tall black tube containing steam-pasteurized potting mix (peat: perlite: polystyrene balls in the ratio of 1: 1: 1). Plants were gown initially at 90% humidity with a gradual decrease by 5% every day for 7 days or until the humidity in the cabinet reached ambient relative humidity.A liquid fertilizer (Aquasol® 23N: 4P: 18K) was applied to the plants initially at a quarter-strength and increased at weekly intervals to full strength after 3 weeks.

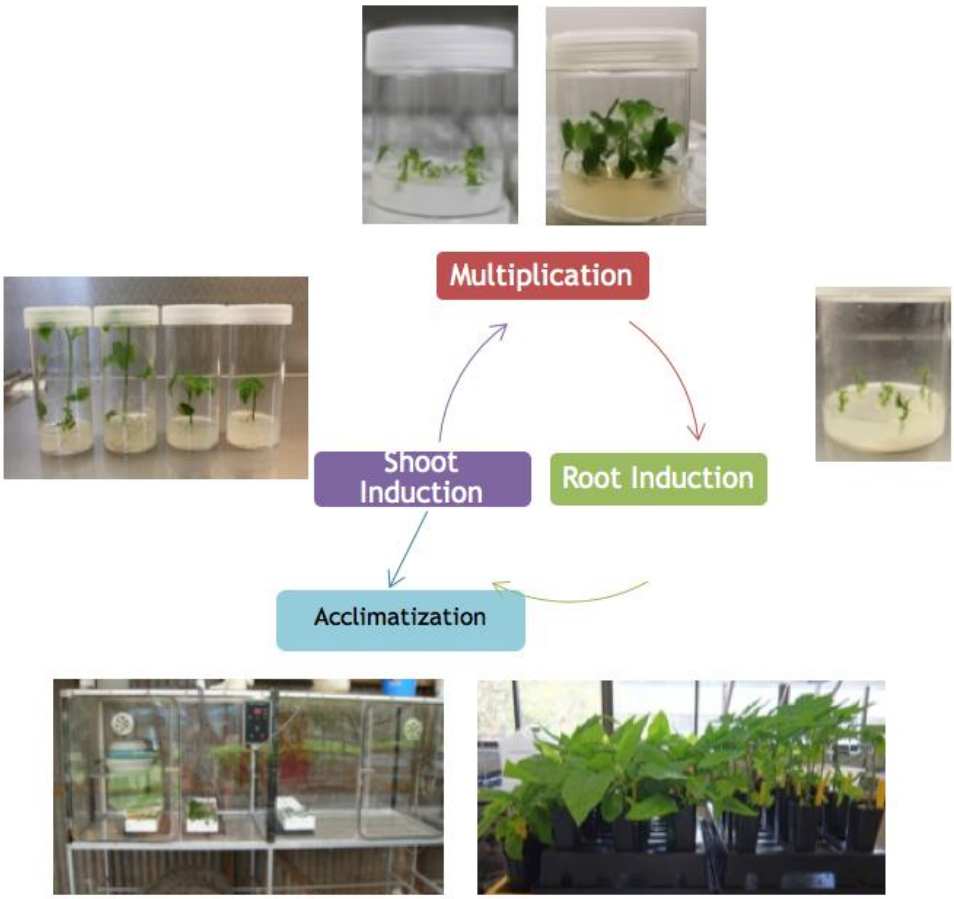

Figure 2: Procedure of *In vitro* propagation of *C. papaya* and *Vasconcellea spp.*

Outputs

1) Twenty-seven lines and breeding selections of papaya were evaluated for 11 productivity traits and 13 characteristics related to fruit quality based on both grower and consumer preferences. Results were used to determine selection of best parents to be used in the next stages of the breeding programs.
2) Establishment of potential parental lines, best F1 plants and selected plants from elite commercial lines in tissue culture.
3) Production and plantation of F_2 population of improved lines in two regions to be evaluated in the next phase of the project.
4) Evaluation of papaya germplasm and data was generated and stored in database system.
5) Development and validation of DNA markers linked to flesh colour and virus resistance.
6) Establishment of segregating populations of a wide range of traits.
7) Production of potential new line of papayas that contained PRSV-P resistance.
8) A comprehensive literature review has been produced – full details in appendix 1.

International Symposium on papaya

A papaya farm visit was organized in North Queensland after the International Papaya symposium so that researchers could meet growers. The event was held during last week of August aiming to exchange knowledge and build on papaya cultivation techniques.

A group of researchers from Hawaii and Australia were supported from a HNRN/HAL project to visit the main papaya-growing region in North QLD, Australia. The participants were Dr Maureen Fitch, Dr Judy Zhu and Ron Fitch from Hawaii; Dr Rebecca Ford, Chat Kanchana-udomkan and Mai Nantawan from Australia; and a grower from Mexico, Diego Urena.

The tour was organised to visit growing regions in Innisfail and Mareeba after the 4th Internatioanl Symposium on papaya which was held during IHC2014. There were three farm visits in Innisfail (growers: Mark Darveniza, Hayden Darveniza and Michael Oldano) on 24 August 2014 and a BBQ dinner at South Johnstone research station, which was supported by the Innisfail Papaw/Papaya Growers Association at an INC papaya meeting. Researchers from Hawaii presented their works in the topic of the current Hawaii papaya and GMO situation by Dr Maureen Fitch and papaya diseases in Hawaii by Dr Judy Zhu. Also, Diego, a grower from Maxico, showed papaya plantation and growing system in Mexico for export market to the USA. A discussion panel was held after the presentations between researcher and growers. It focused on many aspects of papaya production such as future of GMO papaya, disease and pest control, farm management and papaya marketing.

On 25 August 2014, two farms in Mareeba (Lecker Farming and Skybury Farmgate) were visited. Both farms showed their plantation and packing systems. They also presented their own breeding programs to improve flavour. At Lecker Farming, this group of researchers met with another group of researchers who took a North QLD tour which was organized by Yan Diczbalis, DAFFQ. Both groups visited plantation at Lecker Farming and a papaya tissue culture laboratory. Chat Kanchana-udomkan presented the Australian papaya breeding programs for PRSV-P resistance and improvement of fruit quality, and tissue culture of papaya for commercial scale.

Both growing regions showed their production systems from seed germination, planting, harvest to packing systems. Problems of papaya production in Innisfail are Phytophthora infection, black spots, fruit spotting bugs and use of suitable fallow crops. The same disease and insect problems are also found in Mareeba plantations. Other existing issues are an increase cost of production from chemical sprays to control pest and disease, labour costs, and seed price.

Figure 3: Group photo at Hayden Darveniza's farm, Innisfail, QLD.

Outcomes

Fruit flavour has been improved in both red and yellow fleshed papaya from the breeding program to improve flavour in commercial varieties. Trees with better flavour and high yield were crossed back to commercial varieties to keep improving the Australian genetics. A potential new variety of yellow papaya was established in tissue culture and can be field tested in different regions in the next phase of breeding program. Germplasm of 26 lines were evaluated and identified for potential parental lines for each trait of interest. Crosses to represent each trait were made and will be used for genetics study and development of DNA markers linked to each trait in the future. This represents a strong base for future papaya breeding programs.

DNA markers linked to fruit flesh colour, sex and virus resistance were developed and improved. They can be used to assist the selection at seedling stage in the next phase of breeding program and to improve farm management practices by planting red and yellow papaya of one sex in separate plantings.. The markers can also save time and cost in labour, evaluation and selection.

Crosses of the PRSV-P resistant line (clone 113) to cultivated papaya contained promising genetics of the resistant line as confirmed by DNA markers and morphology. These lines are being micropropagated in tissue culture and will be inoculated to screen for PRSV-P susceptibility/resistance and fertility.

Knowledge of techniques related to papaya breeding such as pollination, *in vitro* propagation, molecular techniques, gene expression analysis and bioinformatics, was transferred to new researchers. Dr Chat Kanchana-udomkan completed her Ph.D. under the support of this project. Local people on the Tableland learnt tissue culture techniques. This has stimulated new papaya research in Australia.

Evaluation and Discussion

The main purpose of this research was to evaluate germplasm in order to identify parents to develop populations for papaya breeding programs. The aim was to improve Australian commercial papaya varieties in terms of fruit quality and productivity. Phenotypic data for fruit quality and productivity traits were collected at three harvest times (April 2012, October 2012 and May 2013) to minimise environmental effects on the traits of interest. Some traits, such as number of carpelliod fruits, were believed to be affected by season (OCDE 2005), however this study showed no significant differences for this trait at the three harvest times. This suggests there should be a major gene involved in the expression of the trait, which opens up the possibility for improvement through breeding and selection. However, environmental factors remain important in trait expression as suggested by low repeatabilities in all traits and they need to be closely monitored. Fruit quality traits are complex traits and most of them are controlled by additive genes and quantitative trait loci. Parental lines were selected for traits of interest and crosses were made for crop improvement and establishment of segregating populations, to be used for the development of DNA markers to assist breeding selection.

A demonstration of the application of DNA markers to assist selection was applied and two DNA markers, CPFC-R and CPFC-Y, were developed to distinguish fruit flesh colour between red and yellow-fleshed varieties. The high percentage of association at 95.75% between trait and markers was presented in the germplasm representing a wide range of genetic backgrounds. In the future, these markers should be validated in segregating populations to calculate linkage distance between markers and the trait.

Full results and discussions are presented in appendix 2 and 3.

Recommendations

1. Genetic inheritance should be studied to enable design of suitable breeding strategies for each trait.
2. Bioassays for fruit quality and productivity traits should be standardised for future breeding research.
3. Commercial varieties from other countries should be included in germplasm evaluation to broaden the genetic base of papaya in Australia.
4. Chemical composition of flesh flavour of different flavours should be identified and evaluated for consumer perception and preference.
5. New varieties should be able to reproduced by seed production and micropropagation.
6. A second phase of the project should be started in 2016.

Scientific Refereed Publications

O'Brien, C.M. and Drew, R.A. 2009. Potential for using *Vasconcellea parviflora* as a bridging species in intergeneric hybridisation between *V. pubescens* and *Carica papaya.* Australian Journal of Botany 57:592-601.

O'Brien, C.M. and Drew, R.A. 2010. Marker-assisted hybridisation and backcrossing between *Vasconcellea* species and *Carica papaya* for PRSV-P resistance. Acta Horticulturae 859:361-368.

Scheldeman, X., Kyndt, T., Coppens d'Eeckenbrugge, G., Ming, R., Drew, R., van Droogenbroeck, B., van Damme, P. and Moore, P. 2011. Vasconcellea. In: Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits. (Cole, C. ed) p213-249. Springer Verlag, Berlin Heidelberg.

Siar, S.V., Beligan, G.A., Sajise, A.G.C., Villegas, V.N. and Drew, R.A. 2011. Papaya ringspot virus resistance in *Carica papaya* via introgression from *Vasconcellea quercifolia*. Euphytica 181:159-168.

Sair, S.V., Drew, R.A., Razali, R.M. and Villegas, V.N. 2012. Gene for PRSV-P Resistance in Vasconcellea species and Development of PRSV-P Resistant Papaya via Intergeneric Hybridisation. Acta Horticulturae: 929:335-342.

Razali, R.M. and Drew. R.A. 2013. A refined protocol for embryogenesis to transfer PRSV-P resistance genes from *Vasconcellea pubescens* to *Carica papaya*. Acta Horticulturae: 1022:47-53.

Kaity, A.; Drew, R.A.; Ashmore, S.E. 2013. Genetic and Epigenetic Integrity Assessment of Acclimatised Papaya Plants Regenerated Directly From Shoot-tips Following Short- and Long-term Cryopreservation. Plant Cell Tiss Organ Cult 112:75-86.

Alamery, S. and Drew. R.A. 2014. Studies on the genetics of PRSV-P resistance genes in intergeneric hybrids between Carica papaya and Vasconcellea quercifolia. Acta Horticulturae: 1022:55-62.

#Drew, R.A., 2014. The use of non-transgenic technologies for the development of Papaya Ringspot resistance in Carica papaya (L). Acta Horticulturae: 1022:23-30.

Razali, R.M. and Drew, R.A. 2014. Isolation and Characterisation of PRSV-P Resistance Genes in *Carica* and *Vasconcellea.* International Journal of Genomics: Volume 2014 (2014), Article ID 145403, 1-8.

Coppens d'Eeckenbrugge, G., Scheldeman, X., Drew, R.A. and Kyndt, T. 2014. *Vasconcellea* for papaya improvement. In: Genetics and Genomics of Papaya. (R. Ming and P. Moore. Eds) :47-80.

Razali, R.H.M. and Drew, R.A. 2014. A Review of PRSV-P Resistance Genes in Vasconcellea Species and Their Application to PRSV-P Resistance in Carica papaya. Acta Horticulturae: 1048:65-74.

Kanchana-udomkan, C., Ford, R. and Drew, R.A. 2014. Molecular Markers in Papaya. In: Genetics and Genomics of Papaya. (R. Ming and P. Moore. Eds) :355-376.

Razali, R.M. and Drew, R.A. 2015. Identification and Characterisation of PRSV-P Resistance Genes in Carica and Vasconcellea. Acta Horticulturae: in press

Kanchana-udomkan C., Razean M.R., Peace C. and Drew R. 2015. Developing DNA Markers for a Wild Source of Resistance to Papaya Ringspot Virus. Acta Horticulturae:in press.

Kanchana-udomkan C., Razean M.R., Peace C. and Drew R. 2015. Developing DNA Markers for a Wild Source of Resistance to Papaya Ringspot Virus. Acta Horticulturae: in press

Nantawan, U., Kanchana-udomkan C., and Drew R.2015. Progress in Marker Assisted Breeding of Papaya in Australia. Acta Horticulturae: in press.

Intellectual Property/Commercialisation

No commercial IP generated

References

References used in this report and the literature review in Appendix 1.

Afzal, I, Basra, SMA, Ashraf, M, Hameed, A & Farooq, M 2006, 'Physiological enhancements for alleviationof salt tolerance in spring wheat', *Pak. J. Bot*, vol. 38, pp. 1649–59.

Akamine, EK 1966, 'Respiration of fruits of papaya (*Carica papaya L.* var. Solo) with reference to effect of quarantine disinfestation treatments', *Proc. Amer. Soc. Hort. Sci*, vol. 89, pp. 231-6.

Albrecht, M & Takken, FLW 2006, 'Update on the domain architectures of NLRs and R proteins', *Biochemical and Biophysical Research Communications*, vol. 339, no. 2, pp. 459-62.

Allan, P 1963, 'Pollen studies in *Carica papaya*. II: Germination and storage of pollen', *South African Journal of Agricultural Science*, vol. 6, pp. 613-24.

Allan, P 2002, 'Carica papaya responses under cool subtropical growth conditions', Acta Horticulturae, no. 575, pp. 757-63.

Almeida, FT, Marinho, CS & Souza EF, GS 2003, 'Expressão sexual do mamoeiro sob diferentes lâminas de irrigação na Região Norte Fluminense', *Rev Brasil Fruticult*, vol. 25, pp. 383–5.

Almora, K, Pino, JA, Hernandez, M, Duarte, C, Gonzalez, J & Roncal, E 2004, 'Evaluation of volatiles from ripening papaya (*Carica papaya L.*, var. Maradolroja)', *Food Chemistry*, vol. 86, pp. 127-30.

Anderson, JP, Badruzsaufari, E, Schenk, PM, Manners, JM, Desmond, OJ, Ehlert, C, Maclean, DJ, Ebert, PR & Kazan, K 2004, 'Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis', *Plant Cell*, vol. 16, no. 12, pp. 3460-79.

Anh, NT, Trang, PN, Hong, NTB & Hoan, NV 2011, 'Evaluating agronomic characteristics of twelve local papaya (*Carica papaya L.*) varieties in Viet Nam', *Bull. Inst. Trop. Agr*, vol. 34, pp. 15-22.

Aradhya, MK, Manshardt, RM, Zee, F & Morden, CW 1999, 'A phylogenetic analysis of the genus *Carica L.* (Caricaceae) based on restriction fragment length variation in a cpDNA intergenic spacer region', *Genet. Resour. Crop Evol.*, vol. 46, no. 6, pp. 579-86.

Arkle, TD & Nakasone, HY 1984, 'Floral differentiation in the hermaphroditic papaya', *HORTSCIENCE*, vol. 19, pp. 832-4.

Artico, S, Nardeli, SM, Brilhante, O, Grossi-de-Sa, MF & Alves-Ferreira, M 2010, 'Identification and evaluation of new reference genes in Gossypium hirsutum for accurate normalisation of real-time quantitative RT-PCR data', *BMC Plant Biol*, vol. 10, no. 49, p. 49.

Arumuganathan, K & Earle, E 1991, 'Nuclear DNA content of some important plant species', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 9, no. 3, pp. 208-18.

Badillo, VM 1993, 'Caricaceae, segundo esquema.', *Revista de la Facultad de Agronomía de la Universidad de Venezuela*, vol. 43, pp. 1-111.

—— 2000, '*Carica* L. vs. *Vasconcellea* St.-Hil. (Caricaceae) con la rehabilitacion de este ultimo', *Ernstia*, vol. 10, no. 2, pp. 74-9.

Badillo, VM 2001, 'Nota correctiva *Vasconcellea* St. Hill. y no Vasconcella (Caricaceae). ', *Ernstia*, vol. 11, pp. 75-6.

Bateson, MF, Henderson, J, Chaleeprom, W, Gibbs, AJ & Dale, JL 1994, 'Papaya ringspot potyvirus-isolate variability and the origin of PRSV type P (Australia)', *Journal of General Virology*, vol. 75, pp. 3547-53.

Bau, H-J, Cheng, Y-H, Yu, T-A, Yang, J-S & Yeh, S-D 2003, 'Broad-Spectrum Resistance to Different Geographic Strains of Papaya ringspot virus in Coat Protein Gene Transgenic Papaya', Phytopathology, vol. 93, no. 1, pp. 112-20.

Bau, H-J, Cheng, Y-H, Yu, T-A, Yang, J-S, Liou, P-C, Hsiao, C-H, Lin, C-Y & Yeh, S-D 2004, 'Field Evaluation of Transgenic Papaya Lines Carrying the Coat Protein Gene of Papaya ringspot virus in Taiwan', Plant Disease, vol. 88, no. 6, pp. 594-9.

Benson, CW & Poffley, M 1998, *Growing pawpaws*, Northern Territory Government Department of Primary Industry Fisheries and Mines.

Blas, AL, Ming, R, Liu, Z, Veatch, OJ, Paull, RE, Moore, PH & Yu, Q 2010, 'Cloning of the papaya chromoplast-specific lycopene β-cyclase, CpCYC-b, controlling fruit flesh color reveals conserved microsynteny and a recombination hot spot', *Plant Physiology*, vol. 152, no. 4, pp. 2013-22.

Blas, AL, Yu, Q, Chen, C, Veatch, O, Moore, PH, Paull, RE & Ming, R 2009, 'Enrichment of a papaya high-density genetic map with AFLP markers', *Genome*, vol. 52, no. 8, pp. 716-25.

Blas, AL, Yu, Q, Veatch, QJ, Paull, RE, Moore, PH & Ming, R 2012, 'Genetic mapping of quantitative trait loci controlling fruit size and shape in papaya', *Mol Breed*, vol. 29, no. 801, pp. 457–66.

Broughton, WJ, Hashim, AM, Shen, TC & Tan, IKP 1977, 'Maturation of Malaysian fruits. I. Storage conditions and ripeningof papaya (*Carica papaya* L. CV. Sunrise Solo)', *Malaysian Agr. Res. & Dev. Inst. Res. Bul*, vol. 5, pp. 59-72.

Brown, PO & Botstein, D 1999, 'Exploring the new world of the genome with DNA microarrays', *Nat Genet*, vol. 21, no. 1 Suppl, pp. 33-7.

Bruening, G 2006, 'Resistance to infection', in G Loebenstein & JP Carr (eds), *Natural Resistance Mechanisms of Plants to Viruses*, Springer, Dordrecht, The Netherlands, pp. 211-40.

Bustin, SA, Benes, V, Garson, JA, Hellemans, J, Huggett, J, Kubista, M, Mueller, R, Nolan, T, Pfaffl, MW, Shipley, GL, Vandesompele, J & Wittwer, CT 2009, 'The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments', *Clin Chem.*, vol. 55, no. 4, pp. 611-22.

Cai, WQ, Gonsalves, C, Tennant, P, Fermin, G, Souza, M, Sarindu, N, Jan, FJ, Zhu, HY & Gonsalves, D 1999, 'A protocol for efficient transformation and regeneration of *Carica papaya* L.', *In Vitro Cellular & Developmental Biology-Plant*, vol. 35, no. 1, pp. 61-9.

Cao, J, Cao, Z & Wu, T 2007, 'Generation of antibodies against DMRT1 and DMRT4 of Oreochromis aurea and analysis of their expression profile in Oreochromis aurea tissues', *J Genet Genomics*, vol. 34, pp. 497 - 509.

Carrington, JC, Kasschau, KD, Mahajan, SK & Schaad, MC 1996, 'Cell-to-Cell and Long-Distance Transport of Viruses in Plants', *Plant Cell*, vol. 8, no. 10, pp. 1669-81.
Carvalho, FA & Renner, SS 2012, 'A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history', *Molecular Phylogenetics and Evolution*, vol. 65, no. 1, pp. 46-53.

Chan, HT, Kenneth, LH, Goo, T & Akamine, EK 1979, 'Sugar composition of papaya during fruit development', *HORTSCIENCE*, vol. 14, pp. 140–1.

Chaves-Bedoya, G & Nuñez, V 2007, 'A SCAR marker for the sex types determination in Colombian genotypes of *Carica papaya*', *Euphytica*, vol. 153, no. 1, pp. 215-20.

Chay-Prove, P, Ross, P, O'Hare, P, Macleod, N, Kernot, I, Evans, D, Grice, K, Vawdrey, L, Richards, N, Blair, A & Astridge, D 2000, *Your Growing Guide to Better Farming, Papaw Information Kit*, Queensland Horticulture Institute and Department of Primary Industries, Nambour, Qld.

Chen, C, Yu, Q, Hou, S, Li, Y, Eustice, M, Skelton, RL, Veatch, O, Herdes, RE, Diebold, L, Saw, J, Feng, Y, Qian, W, Bynum, L, Wang, L, Moore, PH, Paull, RE, Alam, M & Ming, R 2007, 'Construction of a sequence-tagged high-density genetic map of papaya for comparative structural and evolutionary genomics in brassicales', *Genetics*, vol. 177, no. 4, pp. 2481-91.

Clontech Laboratories Inc. 2012. Smarter™ RACE cDNA amplification kit user manual. Cat no:634923& 634924.

Conover, RA 1964, 'Mild mosaic and faint mottle ringspot, two papaya virus diseases of minor importance in Florida', *Proc. of the Fla. State Hort. Soc*, vol. 77, pp. 444-8.

Crute, IR & Pink, D 1996, 'Genetics and Utilization of Pathogen Resistance in Plants', *Plant Cell*, vol. 8, no. 10, pp. 1747-55.

Cunningham, FX & Gantt, E 1998, 'Genes and enzymes of carotenoid biosyntesis in plants', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 49, no. 1, pp. 557-83.

Czechowski, T, Stitt, M, Altmann, T, Udvardi, MK & Scheible, WR 2005, 'Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis', *Plant Physiol*, vol. 139, no. 1, pp. 5-17.

d'Eeckenbrugge, GC, Drew, RA, Kyndt, T & Scheldeman, X 2014, '*Vasconcellea* for Papaya Improvement', in R Ming & PH Moore (eds), *Genetics and Genomics of Papaya*, Springer New York, vol. 10, pp. 47-79.

Dangl, JL & Jones, JDG 2001, 'Plant pathogens and integrated defence responses to infection', *Nature*, vol. 411, no. 6839, pp. 826-33.

Dardick, C 2007, 'Comparative expression profiling of Nicotiana benthamiana leaves systemically infected with three fruit tree viruses', *Mol Plant Microbe Interact*, vol. 20, no. 8, pp. 1004-17.

de Oliveira, EJ, Amorim, VBO, Matos, ELS, Costa, JL, Castellen, MD, Padua, JG & Dantas, JLL 2010a, 'Polymorphism of Microsatellite Markers in Papaya (*Carica papaya* L.)', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 28, no. 3, pp. 519-30.

de Oliveira, EJ, Dantas, JLL, Castellen, MDS, de Lima, DS, Barbosa, HD & Motta, TBN 2007, 'Molecular markers for sex identification in papaya', *PESQUISA AGROPECUARIA BRASILEIRA*, vol. 42, no. 12, pp. 1747-54.

De Oliveira, EJ, Dantas, JLL, Castellen, MDS & Machado, MD 2008, 'Identification of microsatellites for papaya by DNA data bank exploration', *REVISTA BRASILEIRA DE FRUTICULTURA*, vol. 30, no. 3, pp. 841-5.

de Oliveira, EJ, dos Santos Silva, A, de Carvalho, FM, dos Santos, LF, Costa, JL, de Oliveira Amorim, VB & Dantas, JLL 2010b, 'Polymorphic microsatellite marker set for L. and its use in molecular-assisted selection', *Euphytica*, vol. 173, no. 2, pp. 279-87.

del Pozo, O, Pedley, KF & Martin, GB 2004, 'MAPKKKalpha is a positive regulator of cell death associated with both plant immunity and disease', *EMBO J*, vol. 23, no. 15, pp. 3072-82.

Dellaporta, SL, Wood, J & Hicks, JB 1983, 'A plant DNA minipreparation: Version II', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 1, no. 4, pp. 19-21.

Department of Agriculture, Forestry and Fisheries, South Africa, 2009 http://www.nda.agric.za/docs/Infopaks/papayas.pdf

Deputy, JC, Ming, R, Ma, H, Liu, Z, Fitch, MMM, Wang, M, Manshardt, R & Stiles, JI 2002, 'Molecular markers for sex determination in papaya (*Carica papaya* L.)', *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, vol. 106, no. 1, pp. 107-11.

DeYoung, BJ & Innes, RW 2006, 'Plant NBS-LRR proteins in pathogen sensing and host defense', *Nat Immunol*, vol. 7, no. 12, pp. 1243-9.

Dickinson, M 2003, *Molecular Plant Pathology*, Taylor & Francis.

Diczbalis, Y, Williams, B & Hickey, M 2012, *Papaya*, The Australian Papaya Industry, National Horticultural Research Network.

Dillon, S 2006a, 'Characterisation, genetic mapping and development of marker selection strategies for resistance to the Papaya ringspot virus type P (PRSV-P) in "highland papaya"', Ph.D. thesis, Griffith University.

Dillon, S, Ramage, C, Ashmore, S & Drew, RA 2006b, 'Development of a codominant CAPS marker linked to PRSV-P resistance in highland papaya', *Theor Appl Genet*, vol. 113, no. 6, pp. 1159-69.

Dillon, S, Ramage, C, Drew, R & Ashmore, S 2005, 'Genetic mapping of a PRSV-P resistance gene in "highland papaya" based on inheritance of RAF markers', *Euphytica*, vol. 145, no. 1-2, pp. 11-23.

Dillon, S, Ramage, C, O'Brien, CM & Drew, RA 2006c, 'Application of SCAR marker linked to a putative PRSV-P resistance locus for asissted breeding of resistant *C. papaya* cultivars', *Acta Hort. (ISHS)*, vol. 725, pp. 627-34.

Dixon, MS, Jones, DA, Keddie, JS, Thomas, CM, Harrison, K & Jones, JD 1996, 'The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins', *Cell*, vol. 84, no. 3, pp. 451-9.

Drew, RA 1988, 'Rapid clonal propagation of papaya *in vitro* from mature field-grown trees', *HORTSCIENCE*, vol. 23, no. 3, pp. 609-11.

—— 1992, 'Improved techniques for *in vitro* propagation and germplasm storage of papaya', *HORTSCIENCE*, vol. 27, pp. 1122-4.

—— 2005, *Development of new papaya varieties for Southeast and Central Queensland. Report No. FR02024*, Horticulture Australia Limited, Sydney, Australia.

Drew, RA, Godwin, ID, Magdalita, PM & Adkins, SW 1998a, 'Efficient interspecific hybridisation protocol for *Carica papaya* L. x *C. Cauliflora* Jacq', *Australian Journal of Experimental Agriculture (CSIRO)*, vol. 38, pp. 523-30.

Drew, RA, O'Brien, CM & Magdalita, PM 1998b, *Development of Carica interspecific hybrids*, International Society for Horticultural Science, Leuven, Belgique.

Drew, RA, Persley, DM, O'Brien, C & Bateson, MF 2005, 'Papaya ringspot virus in Australia and the development of virus resistant plant', *Acta Hort. (ISHS)*, vol. 692, pp. 101-6.

Drew, RA, Siar, SV, Dillon, S, Ramage, C, O'Brien, C & Sajise, AGC 2007, 'Intergeneric

hybridisation between *Carica papaya* and Wild *Vasconcellea* Species and Identification of a PRSV-P resistance Gene', *Proceedings of the International Symposium on Biotechnology of Temperate Fruit Crops and Tropical Species*, no. 738, pp. 165-9.

Drew, RA, Siar, SV, O'Brien, CM, Magdalita, PM & Sajise, AGC 2006a, 'Breeding for Papaya ringspot virus resistance in *Carica papaya* via hybridisation with *Vasconcellea quercifolia*', *Australian Journal of Experimental Agriculture*, vol.46, no. 3, pp.413-8.

Drew, RA, Siar, SV, O'Brien, CM & Sajise, AGC 2006b, 'Progress in backcrossing between *Carica papaya* × *Vasconcellea quercifolia* intergeneric hybrids and *C. papaya*', *Australian Journal of Experimental Agriculture*, vol. 46, no. 3, pp. 419-24.

Elder, RJ, Macleod, WNB, Bell, KL, Tyas, JA & Gillespie, RL 2000a, 'Growth, yield and phenology of 2 hybrid papayas (*Carica papaya* L.) as influenced by method of water application', *Australian Journal of Experimental Agriculture*, vol. 40, pp. 739-46.

Elder, RJ, Macleod, WNB, Reid, DJ & Gillespie, RL 2000b, 'Growth and yield of 3 hybrid papayas (*Carica papaya* L.) under mulched and bare ground conditions.', *Australian Journal of Experimental Agriculture*, vol. 40, pp. 747-54.

Elder, RJ, Reid, DJ, Macleod, WNB & Gillespie, RL 2002, 'Post-ratoon growth and yield of three hybrid papays (Carica papaya L.) under mulched and bare-ground conditions', Australian Journal of Experimental Agriculture, vol. 42, pp. 71-81.

Ellis, J & Jones, D 2003, 'Plant Disease Resistance Genes', in RA Ezekowitz & J Hoffmann (eds), *Innate Immunity*, Humana Press, pp. 27-45.

Eloisa, M, Reyes, Q & Paull, RE 1994, 'Skin freckles on solo papaya fruit', *SCIENTIA HORTICULTURAE*, vol. 58, no. 1, pp. 31-9.

Eustice, M, Yu, Q, Lai, CW, Hou, S, Thimmapuram, J, Liu, L, Alam, M, Moore, PH, Presting, GG & Ming, R 2008, 'Development and application of microsatellite markers for genomic analysis of papaya', *Tree Genetics & Genomes*, vol. 4, no. 2, pp. 333-41.

Fabi, JP, Seymour, G, Graham, N, Broadley, M, May, S, Lajolo, FM, Cordenunsi, BR & Oliveira do Nascimento, JR 2012, 'Analysis of ripening-related gene expression in papaya using an Arabidopsis-based microarray', *BMC Plant Biology*, vol. 12, no. 1, p. 242.

Falconer, DS 1960, *Introduction to Quantitative Genetics*, Oliver and Boyd, London.

Fan, M-J, Chen, S, Kung, Y-J, Cheng, Y-H, Bau, H-J, Su, T-T & Yeh, S-D 2009, 'Transgene-specific and event-specific molecular markers for characterization of transgenic papaya lines resistant to Papaya ringspot virus', *Transgenic research*, vol. 18, no. 6, pp. 971-86.

Fermin, G, Inglessis, V, Garboza, C, Rangle, S, Dagert, M & Gonsalves, D 2004, 'Engineered resistance against Papaya ringspot virus in Venezuelan transgenic papaya', *Plant Dis*, vol. 88, pp. 516-22.

Fernandez-Trujillo, JP, Picó, B, Garcia-Mas, J, M., ÁJ & Monforte, AJ 2011, 'Breeding for fruit quality in melon.', in MA Jenks & PJ Bebeli (eds), *Breeding for fruit quality*, John Wiley & Sons, pp. 261–78.

Fitch, MM, Manshardt, RM, Gonsalves, D, Slightom, JL & Sanford, JC 1990, 'Stable transformation of papaya via microprojectile bombardment', *Plant Cell Reports*, vol. 9, no. 4, pp. 189-94.

Fitch, MMM 2005, *'Carica papaya* Papaya', in RE Litz (ed.), *Biotechnology of Fruit and Nut Crops*, CABI Publishing, pp. 174-207.

Flath, RA & Forrey, RR 1977, 'Volatile components of papaya (*Carica papaya L.*, Solo variety)', *Journal of Agricultural and Food Chemistry*, vol. 25, no. 1, pp. 103-9.

Flor, HH 1971, 'Current status of the gene-for-gene concept', *Annu Rev Phytopathol*, vol. 9, pp. 275–96.

Francisco, FS, Messias, GP, Helaine, CCR, Pedro, CDJ, Telma, NSP & Carlos, DI 2007, 'Genotypic correlations of morpho-agronomic traits in papaya and implications for genetic breeding', *CROP BREEDING AND APPLIED BIOTECHNOLOGY*, vol. 7, pp. 345-52.

Fraser, RSS 1986, 'Genes for resistance to plant viruses', *CRC Crit. Rev. Plant Sci*, vol. 3, no. 6-15.

Fraser, RSS 1990, 'The genetics of resistance to plant viruses', *Annual Review of Phytopathology*, vol. 28, pp. 179-200.

Galván-Ampudia, CS & Offringa, R 2007, 'Plant evolution: AGC kinases tell the auxin tale', *Trends in Plant Science*, vol. 12, no. 12, pp. 541-7.

Gangopadhyay, G, Roy, SK, Ghose, K, Poddar, R, Bandyopadhyay, T, Basu, D & Mukherjee, KK 2007, 'Sex detection of *Carica papaya* and Cycas circinalis in pre-flowering stage by ISSR and RAPD', *CURRENT SCIENCE*, vol. 92, no. 4, pp. 524-6.

Garcia, A, Al-Yousif, M & Hirt, H 2012, 'Role of AGC kinases in plant growth and stress responses', *Cellular and Molecular Life Sciences*, vol. 69, no. 19, pp. 3259-67.

Garrett, A 1995, 'The pollination biology of papaw (*Carica papaya* L.) in central Queensland', PhD Thesis thesis, Central Queensland University.

Gilliland, FD, Islam, T, Berhane, K, Gauderman, WJ, McConnell, R, Avol, E & Peters, JM 2006, 'Regular smoking and asthma incidence in adolescents', *Am J Respir Crit Care Med*, vol. 174, no. 10, pp. 1094-100.

Goldbach, R, Bucher, E & Prins, M 2003, 'Resistance mechanisms to plant viruses: an overview', *Virus Research*, vol. 92, no. 2, pp. 207-12.

Gomez-Gomez, L & Boller, T 2000, 'FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis', *Mol Cell*, vol. 5, no. 6, pp. 1003-11.

Gonsalves, D 1998, 'Control of papaya ringspot virus in papaya: A case study', *Annual Review of Phytopathology*, vol. 36, no. 1, pp. 415-37.

Gonsalves, D & Ishii, M 1980, 'Purification and serology of papaya ringspot virus', *Phytopathology*, vol. 70, pp. 1028–32.

Gonzalez-Jara, P, Fraile, A, Canto, T & Garcia-Arenal, F 2009, 'The multiplicity of infection of a plant virus varies during colonization of its eukaryotic host', *J Virol*, vol. 83, no. 15, pp. 7487-94.

Grandillo, S, Ku, HM & Tanksley, SD 1999, 'Identifying the loci responsible for natural variation in fruit size and shape in tomato', *Theoretical and Applied Genetics*, vol. 99, no. 6, pp. 978-87.

Gu, C, Chen, S, Liu, Z, Shan, H, Luo, H, Guan, Z & Chen, F 2011, 'Reference gene selection for quantitative real-time PCR in Chrysanthemum subjected to biotic and abiotic stress', *Mol Biotechnol*, vol. 49, no. 2, pp. 192-7.

Gutha, L, Casassa, L, Harbertson, J & Naidu, R 2010, 'Modulation of flavonoid biosynthetic pathway genes and anthocyanins due to virus infection in grapevine (Vitis vinifera L.) leaves',

BMC Plant Biology, vol. 10, no. 1, p. 187.

Gutierrez, L, Mauriat, M, Guénin, S, Pelloux, J, Lefebvre, J-F, Louvet, R, Rusterucci, C, Moritz, T, Guerineau, F, Bellini, C & Van Wuytswinkel, O 2008, 'The lack of a systematic validation of reference genes: a serious pitfall undervalued in reverse transcription-polymerase chain reaction (RT-PCR) analysis in plants', *Plant Biotechnology Journal*, vol. 6, no. 6, pp. 609-18.

Hall, CB 1964, 'The effect of short periods of high temperature on the ripening of detached tomato fruits', *Proc. Amer. Soc. Hort. Sci*, vol. 84, pp. 501-6.

Hammond, RW & Zhao, Y 2000, 'Characterization of a tomato protein kinase gene induced by infection by Potato spindle tuber viroid', *Molecular Plant-Microbe Interactions*, vol. 13, pp. 903-10.

Hammond-Kosack, KE & Jones, JD 1996, 'Resistance gene-dependent plant defense responses', *The Plant Cell*, vol. 8, no. 10, pp. 1773-91.

Hammond-Kosack, KE & Kanyuka, K 2001, 'Resistance Genes (R Genes) in Plants', in JW Sons (ed.), *Encyclopedia of Life Sciences*, John Wiley & Sons, Ltd.

Hanks, SK & Hunter, T 1995, 'Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification', *FASEB J.*, vol. 9, no. 8, pp. 576-96.

Hansen, V 2005, *Papaya breeding and variety development, Report No. FR99018*, FR99018, Horticulture Australia Limited, Sydney, Australia.

Hellemans, J, Mortier, G, De Paepe, A, Speleman, F & Vandesompele, J 2007, 'qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data', Genome biology, vol. 8, no. 2, p. R19.

Hofmeyr, JDJ 1938, 'Genetical studies of *Carica papaya L.*', *S. Afr. J. Sci*, vol. 35, pp. 300-4.

—— 1939, 'Sex-linked inheritance in *Carica papaya* L.', *S Afr J Sci*, vol. 36, pp. 283–5.

Hong, SY, Seo, PJ, Yang, MS, Xiang, F & Park, CM 2008, 'Exploring valid reference genes for gene expression studies in Brachypodium distachyon by real-time PCR', *BMC Plant Biol*, vol. 8, p. 112.

Horovitz, S & Jimenez, H 1967, 'Cruzamientos interespecíficos e intergenéricos en Caricaceas y sus implicaciones fitotécnicas', *Agronomía Tropical*, vol. 17, no. 323-344.

Hu, L, Benson, ML, Smith, RD, Lerner, MG & Carlson, HA 2005, 'Binding MOAD (mother of all databases)', *Proteins: Structure, Function, and Bioinformatics*, vol. 60, no. 3, pp. 333-40.

Idstein, H, Keller, T & Schreier, P 1985, 'Volatile constituents of mountain papaya (*Carica candamarcensis*, syn. *C. pubescens* Lenne et Koch) fruit', *Journal of Agricultural and Food Chemistry*, vol. 33, no. 4, pp. 663-6.

Inohara, Chamaillard, McDonald, C & Nunez, G 2005, 'NOD-LRR proteins: role in host-microbial interactions and inflammatory disease', *Annu Rev Biochem*, vol. 74, pp. 355-83.

International Board for Plant Genetic Resources (IBPGR) 1988, *Descriptors for Papaya*, IBPGR, Rome.

Ishitani, M, Majumder, AI, Bornhouser, A, Michalowski, CB, Jensen, RG & Bohnert, HJ 1996, 'Coordinate transcriptional induction of myo-inositol metabolism during environmental stress', *Plant J*, vol. 9, no. 4, pp. 537-48.

Iwaki, M & Arakawa, Y 2006, 'Transformation of Acinetobacter sp. BD413 with DNA from commercially available genetically modified potato and papaya', *Letters in applied microbiology*, vol. 43, no. 2, pp. 215-21.

Jain, M, Tyagi, A & Khurana, J 2006, 'Molecular characterization and differential expression of cytokinin-responsive type-A response regulators in rice (Oryza sativa)', *BMC Plant Biology*, vol. 6, no. 1, p. 1.

Jarosova, J & Kundu, JK 2010, 'Validation of reference genes as internal control for studying viral infections in cereals by quantitative real-time RT-PCR', *BMC Plant Biol*, vol. 10, p. 146.
Jensen, DD 1949, 'Papaya virus diseases with special reference to papaya ringspot', *Phyt pathology*, vol. 39, pp. 191-211.

Jobin-Decor, MP, Graham, GC, Henry, RJ & Drew, RA 1997, 'RAPD and isozyme analysis of genetic relationships between *Carica papaya* and wild relatives', *Genet. Resour Crop Evol*, vol. 44, no. 5, pp. 471-7.

Jones, DA & Jones, JDG 1996, 'The role of leucine-rich repeat proteins in plant defences', *Adv. Bot. Res. incorporating Adv. Plant Pathol*, vol. 24, pp. 89–167.

Jones, JDG & Dangl, JL 2006, 'The plant immune system', *Nature*, vol.444, no.7117, pp.323-9.

Jost, W, Baur, A, Nick, P, Reski, R & Gorr, G 2004, 'A large plant beta-tubulin family with minimal C-terminal variation but differences in expression', *Gene*, vol. 340, no. 1, pp. 151-60.

Kajava, A 1998, 'Structural diversity of leucine-rich repeat proteins', *Journal of Molecular Biology*, vol. 277, no. 3, pp. 519-27.

Kanchana-udomkan, C, Ford, R & Drew, R 2014, 'Molecular Markers in Papayas', in R Ming & PH Moore (eds), *Genetics and Genomics of Papaya*, Springer New York, vol. 10, pp. 355-75.

Kang, BC, Yeam, I, Jahn, MM & Jahn, MM 2005, 'Genetics of plant virus resistance', *Annu Rev Phytopathol*, vol. 43, pp. 581-621.

Keen, NT 1990, 'Gene-for-gene complementarity in plant-pathogen interactions', *Annu Rev Genet*, vol. 24, pp. 447-63.

Kim, MS, Moore, PH, Zee, F, Fitch, MM, Steiger, DL, Manshardt, RM, Paull, RE, Drew, RA, Sekioka, T & Ming, R 2002, 'Genetic diversity of *Carica papaya* as revealed by AFLP markers', *Genome 503-12.*, vol. 45, no. 3, pp. 503-12.

Kinkade, MP & Foolad, MR 2013, 'Validation and fine mapping of lyc12.1, a QTL for increased tomato fruit lycopene content', *Theor Appl Genet*, vol. 126, no. 8, pp. 2163-75.

Klinge, S, Voigts-Hoffmann, F, Leibundgut, M, Arpagaus, S & Ban, N 2011, 'Crystal structure of the eukaryotic 60S ribosomal subunit in complex with initiation factor 6', *Science*, vol. 334, no. 6058, pp. 941-8.

Kohler, A, Rinaldi, C, Duplessis, S, Baucher, M, Geelen, D, Duchaussoy, F, Meyers, BC, Boerjan, W & Martin, F 2008, 'Genome-wide identification of NBS resistance genes in Populus trichocarpa', Plant molecular biology, vol. 66, no. 6, pp. 619-36.

Kørner, CF, Klauser, D, Niehl, A, Dominguez-Ferreras, A, Chinchilla, D, Boller, T, Heinlein, M & Hann, DR 2013, 'The immunity regulator BAK1 contributes to resistance against diverse RNA viruses', *Mol Plant Microbe Interact*, vol. 26, no. 11, pp. 1271-80.

Kozera, B & Rapacz, M 2013, 'Reference genes in real-time PCR', *J Appl Genet*, vol. 54, no. 4, pp. 391-406.

Kung, Y-J, Bau, H-J, Wu, Y-L, Huang, C-H, Chen, T-M & Yeh, S-D 2009, 'Generation of Transgenic Papaya with Double Resistance to Papaya ringspot virus and Papaya leaf-distortion mosaic virus', Phytopathology, vol. 99, no. 11, pp. 1312-20.

Kyndt, T, Romeijn-Peeters, E, Van Droogenbroeck, B, Romero-Motochi, JP, Gheysen, G & Goetghebeur, P 2005, 'Species relationships in the genus *Vasconcellea* (*Caricaceae*) based on molecular and morphological evidence.', *Am. J. Bot.*, vol. 92, no. 6, pp. 1033-44.

Lai, CWJ, Yu, Q, Hou, S, Skelton, RL, Jones, MR, Lewis, KLT, Murray, J, Eustice, M, Guan, P, Agbayani, R, Moore, PH, Ming, R & Presting, GG 2006, 'Analysis of papaya BAC end sequences reveals first insights into the organization of a fruit tree genome', *Molecular Genetics and Genomics*, vol. 276, no. 1, pp. 1-12.

Lawton, MA, Yamamoto, RT, Hanks, SK & Lamb, CJ 1989, 'Molecular cloning of plant transcripts encoding protein kinase homologs', *Proc Natl Acad Sci USA*, vol. 86, pp. 3140-4.

Leipe, DD, Koonin, EV & Aravind, L 2004, 'STAND, a Class of P-Loop NTPases Including Animal and Plant Regulators of Programmed Cell Death: Multiple, Complex Domain Architectures, Unusual Phyletic Patterns, and Evolution by Horizontal Gene Transfer', *Journal of Molecular Biology*, vol. 343, no. 1, pp. 1-28.

Lemos, EGM, Silva, CLSP & Zaidan, HA 2002, 'Identification of sex in *Carica papaya* L. using RAPD markers', *Euphytica*, vol. 127, no. 2, pp. 179-84.
Lilly, ST, Drummond, RS, Pearson, MN & MacDiarmid, RM 2011, 'Identification and validation of reference genes for normalization of transcripts from virus-infected Arabidopsis thaliana', *Mol Plant Microbe Interact*, vol. 24, no. 3, pp. 294-304.

Liu, D, Shi, L, Han, C, Yu, J, Li, D & Zhang, Y 2012a, 'Validation of reference genes for gene expression studies in virus-infected Nicotiana benthamiana using quantitative real-time PCR', *PLoS ONE*, vol. 7, no. 9, p. e46451.

Livak, KJ & Schmittgen, TD 2001, 'Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method', *Methods*, vol. 25, no. 4, pp. 402-8.

Loebenstein, G & Akad, F 2006, 'The local lesion response', in G Loebenstein & JP Carr (eds), *Natural Resistance Mechanisms of Plants to Viruses*, Springer, Dordrecht, The Netherlands, pp. 99-124.

Ma, H, Moore, PH, Liu, Z, Kim, MS, Yu, Q, Fitch, MMM, Sekioka, T, Paterson, AH & Ming, R 2004, 'High-density linkage mapping revealed suppression of recombination at the sex determination locus in papaya', *Genetics*, vol. 166, no. 1, pp. 419-36.

Mafra, V, Kubo, KS, Alves-Ferreira, M, Ribeiro-Alves, M, Stuart, RM, Boava, LP, Rodrigues, CM & Machado, MA 2012, 'Reference genes for accurate transcript normalization in citrus genotypes under different experimental conditions', *PLoS ONE*, vol. 7, no. 2, p. e31263.

Magdalita, PM, Drew, RA, Adkins, SW & Godwin, ID 1997, 'Morphological, molecular and cytological analyses of *Carica papaya* × *C. cauliflora* interspecific hybrids', *Theoretical and Applied Genetics*, vol. 95, no. 1-2, pp. 224-9.

Magdalita, PM, Drew, RA, Godwin, ID & Adkins, SW 1998, 'An efficient interspecific hybridisation protocol for *Carica papaya* L. x C. cauliflora Jacq', *Australian Journal of Experimental Agriculture*, vol. 38, pp. 523-30.

Mangrauthia, S, Singh Shakya, V, Jain, RK & Praveen, S 2009, 'Ambient temperature perception in papaya for papaya ringspot virus interaction', *Virus Genes*, vol. 38, no. 3, pp. 429-34.

Manshardt, RM 1992, 'Papaya', in FA Hammerschlag & RE Litz (eds), *Biotechnology in Agriculture No. 8 Biotechnology of Perennial Fruit Crops*, Alden Press, Oxford.

Manshardt, RM & Drew, RA 1998, 'Biotechnology of Papaya', *International Symposium on Biotechnology of Tropical and Subtropical Species - Part Ii*, no. 461, pp. 65-73.

Manshardt, RM & Wenslaff, TF 1989, 'Interspecific hybridization of papaya with other *Carica* species', *J. Amer. Soc. Hort. Sci.*, vol. 114, no. 4, pp. 689-94.

Marathe, R & Dinesh-Kumar, SP 2003, 'Plant defense: one post, multiple guards?', *Mol Cell*, vol. 11, no. 2, pp. 284-6.

Marcotte, EM 2000 'Computational genetics: finding protein function by nonhomology methods', *Curr Opin Struct Biol*, vol. 10, no. 3, pp. 359-65.

Maróstica, MR & Pastore, GM 2007, 'Tropical fruit flavor', in RG Berger (ed.), *Flavours and fragrances: Chemistry, bioprocessing and sustainability* Springer, Berlin, p. 189–201.

Martin, G, Brommonschenkel, S, Chunwongse, J, Frary, A, Ganal, M, Spivey, R, Wu, T, Earle, E & Tanksley, S 1993, 'Map-based cloning of a protein kinase gene conferring disease resistance in tomato', *Science*, vol. 262, no. 5138, pp. 1432-6.

Martin, GB 1999 'Functional analysis of plant disease resistance genes and their downstream effectors', *Curr Opin Plant Biol.*, vol. 2, no. 4, pp. 273-9.

Martin, GB, Bogdanove, AJ & Sessa, G 2003, 'Understanding the functions of plant disease resistance proteins', *Annu Rev Plant Biol*, vol. 54, pp. 23-61.

Mascia, T, Santovito, E, Gallitelli, D & Cillo, F 2010, 'Evaluation of reference genes for quantitative reverse-transcription polymerase chain reaction normalization in infected tomato plants', *Molecular plant pathology*, vol. 11, no. 6, pp. 805-16.

Matsui, H, Miyao, A, Takahashi, A & Hirochika, H 2010b, 'Pdk1 kinase regulates basal disease resistance through the OsOxi1-OsPti1a phosphorylation cascade in rice', *Plant Cell Physiol*, vol. 51, no. 12, pp. 2082-91.

Matsui, H, Yamazaki M Fau - Kishi-Kaboshi, M, Kishi-Kaboshi M Fau - Takahashi, A, Takahashi A Fau - Hirochika, H & Hirochika, H 2010a, 'AGC kinase OsOxi1 positively regulates basal resistance through suppression of OsPti1a-mediated negative regulation', *Plant Cell Physiol*, vol. 51, no. 10, pp. 1731-44.

Mauch-Mani, B & Mauch, F 2005, 'The role of abscisic acid in plant-pathogen interactions', *Curr Opin Plant Biol*, vol. 8, no. 4, pp. 409-14.

McDowell, JM & Woffenden, BJ 2003 'Plant disease resistance genes: recent insights and potential applications', *Trends Biotechnol*, vol. 21, no. 4, pp. 178-83.

Meyers, BC, Kozik, A, Griego, A, Kuang, H & Michelmore, RW 2003, 'Genome-Wide Analysis of NBS-LRR–Encoding Genes in Arabidopsis', *The Plant Cell*, vol. 15, no. 4, pp. 809-34.

Mezard, C 2006, 'Meiotic recombination hotspots in plants', *Biochem Soc Trans*, vol. 34, no. Pt 4, pp. 531-4.

Michelmore, RW & Meyers, BC 1998, 'Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process', *Genome Res*, vol. 8, no. 11, pp. 1113-30.

Ming, R, Hou, S, Feng, Y, Yu, Q, Dionne-Laporte, A, Saw, JH, Senin, P, Wang, W, Ly, BV, Lewis,

KLT, Salzberg, SL, Feng, L, Jones, MR, Skelton, RL, Murray, JE, Chen, C, Qian, W, Shen, J, Du, P, Eustice, M, Tong, E, Tang, H, Lyons, E, Paull, RE, Michael, TP, Wall, K, Rice, DW, Albert, H, Wang, M-L, Zhu, YJ, Schatz, M, Nagarajan, N, Acob, RA, Guan, P, Blas, A, Wai, CM, Ackerman, CM, Ren, Y, Liu, C, Wang, J, Wang, J, Na, J-K, Shakirov, EV, Haas, B, Thimmapuram, J, Nelson, D, Wang, X, Bowers, JE, Gschwend, AR, Delcher, AL, Singh, R, Suzuki, JY, Tripathi, S, Neupane, K, Wei, H, Irikura, B, Paidi, M, Jiang, N, Zhang, W, Presting, G, Windsor, A, Navajas-P√©rez, R, Torres, MJ, Feltus, FA, Porter, B, Li, Y, Burroughs, AM, Luo, M-C, Liu, L, Christopher, DA, Mount, SM, Moore, PH, Sugimura, T, Jiang, J, Schuler, MA, Friedman, V, Mitchell-Olds, T, Shippen, DE, dePamphilis, CW, Palmer, JD, Freeling, M, Paterson, AH, Gonsalves, D, Wang, L & Alam, M 2008, 'The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus)', *Nature*, vol. 452, no. 7190, pp. 991-6.

Ming, R, Moore, PH, Zee, F, Abbey, CA, Ma, H & Paterson, AH 2001, 'Construction and characterization of a papaya BAC library as a foundation for molecular dissection of a tree-fruit genome', *Theoretical and Applied Genetics*, vol. 102, no. 6, pp. 892-9.

Ming, R, Yu, Q & Moore, PH 2007, 'Sex determination in papaya', *Seminars in cell & developmental biology*, vol. 18, no. 3, pp. 401-8.

Moore, GA & Litz, RE 1984, *Biochemical markers for Carica papaya, C. cauliflora, and plants from somatic embryos of their hybrid*, vol. 109, American Society for Horticultural Science, Alexandria, VA.

Mora, A, Komander, D, van Aalten, DMF & Alessi, DR 2004, 'PDK1, the master regulator of AGC kinase signal transduction', *Semin Cell Dev Biol*, vol. 15, no. 2, pp. 161-70.

Morel, J-B & Dangl, JL 1997, 'The hypersensitive response and the induction of cell death in plants', *Cell death and differentiation*, vol. 4, no. 8, pp. 671-83.

Morillo, SA & Tax, FE 2006 'Functional analysis of receptor-like kinases in monocots and dicots', *Curr Opin Plant Biol*, vol. 9, no. 5, pp. 460-9.
Morton, JF 1987, 'Papaya *Carica papaya* L', in NC Winterville (ed.), *Fruits of Warm Climates*, Creative Resources Inc, pp. 336-46.

Nagarajan, N, Navajas-Pérez, R, Pop, M, Alam, M, Ming, R, Paterson, A & Salzberg, S 2008, 'Genome-Wide Analysis of Repetitive Elements in Papaya', *Tropical Plant Biology*, vol. 1, no. 3, pp. 191-201.

Nakajima, Y, Iwakabe, H, Akazawa, C, Nawa, H, Shigemoto, R, Mizuno, N & Nakanishi, S 1993, 'Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate', *J Biol Chem*, vol. 268, no. 16, pp. 11868-73.

Nakasone, HY & Paull, RE 1998, *Tropical fruits*, CAB International, Oxon, UK.

Nazeeb, M & Broughton, WJ 1978., 'Storage conditions and ripening of papaya 'Bentong' and 'Taiping.' ', *Sci. Hort*, vol. 9, pp. 265-77.
Nelson, RS, McCormic, SM, Delannay, X, Dube, P, Layton, J, Anderson, EJ, Kaniewska, M, Proksch, RK, Horsch, RB, Rogers, SG, Fraley, RT & Beachy, RN 1988, 'Virus tolerance, plant growth and field performance of transgenic tomato plants expressing coat protein from tobacco mosaicvirus', *Biotechnology*, vol. 6, pp. 403-9.

Newton, AC 2003, 'Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm', *Biochemical Journal*, vol. 370, no. Pt 2, pp. 361-71.

Nicot, N, Hausman, J, Hoffmann, L & Evers, D 2005, 'Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress', *J Exp Bot.*, vol. 56, no. 421, pp. 2907-14

Niroshini, E, Everard, J, Karunanayake, EH & Tirimanne, TLS 2008, 'Detection of sequence characterized amplified region (SCAR) markers linked to sex expression in *Carica papaya* L', *JOURNAL OF THE NATIONAL SCIENCE FOUNDATION OF SRI LANKA*, vol. 36, no. 2, pp. 145-50.

Noorda-Nguyen, K, Jia, R, Aoki, A, Yu, Q, Nishijima, W & Zhu, YJ 2010, 'Identification of disease tolerance loci to *Phytophthora palmivora* in *Carica papaya* using molecular marker approach', *Acta Hort*, no. 851, pp. 189-96.

O'Brien, CM & Drew, RA 2010, 'Marker-assisted hybridisation and backcrossing between *Vasconcellea* species and *Carica papaya* for PRSV-P resistance', *Acta Hort. (ISHS)*, vol. 859, pp. 361-8.

O'Brien, CM & Drew, RA 2009, 'Potential for using *Vasconcellea parviflora* as a bridging species in intergeneric hybridisation between *V. pubescens* and *Carica papaya*', *Australian Journal of Botany*, vol. 57, no. 7, pp. 592-601.

Ocampo Pérez, J, Coppens d'Eeckenbrugge, G, Risterucci, AM, Dambier, D & Ollitrault, P 2007, 'Papaya genetic diversity assessed with microsatellite markers in germplasm from the Caribbean region', *Acta Hort*, vol. 740, pp. 93-101.

OECD 2005, *Consensus document on the Biology of papaya (Carica papaya)* Organisation for Economic Co-operation and Development, Paris, France.

O'Hare, P 1993, *Growing Papayas in South Queensland*, Queensland Government Department of Primary Industries, Brisbane, Queensland.

Oliveira, EJ, Amorim, VO, Matos, EL, Costa, JL, Silva Castellen, M, Pádua, J & Dantas, J 2010, 'Polymorphism of microsatellite markers in papaya (*Carica papaya L.*)', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 28, no. 3, pp. 519-30.

Palukaitis, P & Carr, JP 2008, 'Plant resistance responses to viruses', *J Plant Pathol*, vol. 90, pp. 153–71.

Papaya-Seed-Australia 2007, *Information*, viewed 10 March 2014, http://www.papayaseed.com.au/information.htm.

Parasnis, AS, Gupta, VS, Tamhankar, SA & Ranjekar, PK 2000, 'A highly reliable sex diagnostic PCR assay for mass screening of papaya seedlings', *Molecular Breeding*, vol. 6, no. 3, pp. 337-44.

Parasnis, AS, Ramakrishna, W, Chowdari, KV, Gupta, VS & Ranjekar, PK 1999, 'Microsatellite (GATA)n reveals sex-specific differences in Papaya', *Theoretical and Applied Genetics*, vol. 99, no. 6, pp. 1047-52.

PâRez, JO, Dambier, D, Ollitrault, P, DÄo Eeckenbrugge, GC, Brottier, P, Froelicher, Y & Risterucci, AÄ 2006, 'Microsatellite markers in *Carica papaya* L.: isolation, characterization and transferability to *Vasconcellea* species', *Molecular Ecology Notes*, vol. 6, no. 1, pp. 212-7.

Parker, PJ & Parkinson, SJ 2001, 'AGC protein kinase phosphorylation and protein kinase C', *Biochem Soc Trans*, vol. 29, no. 6, pp. 860-3.

Pearce, LR, Komander, D & Alessi, D, R. 2010, 'The nuts and bolts of AGC protein kinases', *Nat Rev Mol Cell Biol*, vol. 11, no. 1, pp. 9-22.
Pegg, AE 2009, 'S-Adenosylmethionine decarboxylase', *Essays Biochem*, vol. 46, pp. 25–46

Peterson, TR, Laplante, M, Thoreen, CC, Sancak, Y, Kang, SA, Kuehl, WM, Gray, NS & Sabatini, DM 2009, 'DEPTOR Is an mTOR Inhibitor Frequently Overexpressed in Multiple Myeloma Cells

and Required for Their Survival', *Cell*, vol. 137, no. 5, pp. 873-86.

Pfaffl, M, Tichopad, A, Prgomet, C & Neuvians, T 2004, 'Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations', *Biotechnology Letters*, vol. 26, no. 6, pp. 509-15.

Pfaffl, MW 2001, 'A new mathematical model for relative quantification in real-time RT-PCR', *Nucleic Acids Res.*, vol. 29, no. 9, p. e45.

Pino, E, Campos, AM & Lissi, EA 2003, '8-Hydroxy- 1,3,6-pyrene trisulfonic acid (pyranine) bleaching by 2,2'-azobis(2-amidinopropane) derived peroxyl radicals', *Int J Chem Kinetics*, vol. 35, pp. 525-31.

Pino, JA, Almora, K & Marbot, R 2003, 'Volatile components of papaya (*Carica papaya L.*, Maradol variety) fruit.', *Flavour Fragrance J*, vol. 18, no. 6, pp. 492–6.

Pitzschke, A & Hirt, H 2009, 'Disentangling the Complexity of Mitogen-Activated Protein Kinases and Reactive Oxygen Species Signaling', *Plant Physiology*, vol. 149, no. 2, pp. 606-15.

Pollination Aware Report 2008, https://rirdc.infoservices.com.au/downloads/10-129

Porter, BW, Paidi, M, Ming, R, Alam, M, Nishijima, WT & Zhu, YJ 2009a, 'Genome-wide analysis of *Carica papaya* reveals a small NBS resistance gene family', *Molecular Genetics and Genomics*, vol. 281, no. 6, pp. 609-26.

Porter, BW, Zhu, YJ, Webb, DT & Christopher, DA 2009b, 'Novel thigmomorphogenetic responses in *Carica papaya*: touch decreases anthocyanin levels and stimulates petiole cork outgrowths', *Annals of botany*, vol. 103, no. 6, pp. 847-58.

Purcifull, DE, Edwardson, JR, Hiebert, E & Gonsalves, D 1984, *Papaya ringspot virus CMI/AAB despriptions of plant viruses No. 292*, CAB International, Wallingford, UK.

Quemada, HD, Gonsalves, D & Slightom, JL 1991, 'Expression of coat protein gene from cucumber mosaic virus strain C in tobacco: Protection against infections by CMV strains transmitted mechanically or by aphids', *Phytopathology*, vol. 81, pp. 794-802.

Rairdan, GJ & Moffett, P 2006, 'Distinct domains in the ARC region of the potato resistance protein Rx mediate LRR binding and inhibition of activation', *Plant Cell*, vol. 18, no. 8, pp. 2082-93.

Ratchadaporn, J, Sureeporn, K & Khumcha, U 2007, 'An analysis on DNA fingerprints of thirty papaya cultivars (*Carica papaya* L.), grown in Thailand with the use of amplified fragment length polymorphisms technique', *Pakistan journal of biological sciences: PJBS*, vol. 10, no. 18, p. 3072.

Razean Haireen, MR 2013, 'Identification, characterisation and expression of PRSV-P resistance genes in *Carica* and *Vasconcellea*', Ph.D. thesis, Griffith University, Brisbane.

Razean Haireen, MR & Drew, RA 2014, 'Isolation and characterisation of PRSV-P resistance genes in *Carica* and *Vasconcellea*', *International Journal of Genomics*, vol. 2014, p. 8.

Reis, FO, Campostrini, E, Sousa, EF & Silva, MG 2006, 'Sap flow in papaya plants: Laboratory calibrations and relationships with gas exchanges under field conditions', *Sci Hortic*, vol. 110, no. 3, pp. 254–9.

Remans, T, Smeets, K, Opdenakker, K, Mathijsen, D, Vangronsveld, J & Cuypers, A 2008, 'Normalisation of real-time RT-PCR gene expression measurements in Arabidopsis thaliana exposed to increased metal concentrations', *Planta*, vol. 227, no. 6, pp. 1343-9.

Rentel, MC, Lecourieux, D, Ouaked, F, Usher, SL, Petersen, L, Okamoto, H, Knight, H, Peck, SC, Grierson, CS, Hirt, H & Knight, MR 2004, 'OXI1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis', *Nature*, vol. 427, no. 6977, pp. 858-61.

Romeis, T, Ludwig, AA, Martin, R & Jones, JDG 2001, 'Calcium-dependent protein kinases play an essential role in a plant defence response', *The EMBO Journal*, vol. 20, no. 20, pp. 5556-67.

Rozen, S & Skaletsky, H 2000, 'Primer3 on the WWW for general users and for biologist programmers', *Methods Mol Biol.*, vol. 132, pp. 365-86.

Ruiz, D & Egea, J 2008, 'Phenotypic diversity and relationships of fruit quality traits in apricot (*Prunus armeniaca L.*) germplasm', *Euphytica*, vol. 163, pp. 143–58.

Sanders, PR, Sammons, B, Kaniewski, W, Haley, L, Layton, J, Lavallee, BJ, Delannay, X & Tumer, NE 1992, 'Field resistance of transgenic tomatoes expressing the tobacco mosaic virus or tomato mosaic virus coat protein genes', *Phytopathology*, vol. 82, pp. 683-90.

Santos, SC, Ruggiero, C, Silva, CLSP & Lemos, EGM 2003, 'A microsatellite library for *Carica papaya* L. cv. Sunrise solo', *REVISTA BRASILEIRA DE FRUTICULTURA*, vol. 25, no. 2, pp. 263-7.

Scheel, D 1998, 'Resistance response physiology and signal transduction', *Curr Opin Plant Biol*, vol. 1, no. 4, pp. 305-10.

Schenk, PM, Kazan, K, Manners, JM, Anderson, JP, Simpson, RS, Wilson, IW, Somerville, SC & Maclean, DJ 2003, 'Systemic Gene Expression in Arabidopsis during an Incompatible Interaction with Alternaria brassicicola', *Plant Physiology*, vol. 132, no. 2, pp. 999-1010.

Sessa, G & Martin, GB 2000, 'Protein kinases in the plant defense response', in JA Callow, M Kreis & JC Walker (eds), *Advances in Botanical Research*, Academic Press, London, UK, vol. 32, pp. 379–404.

Sharon, D, Hillel, J, Vainstein, A & Lavi, U 1992, 'Application of DNA fingerprints for identification and genetic analysis of *Carica papaya* and other *Carica* species', *Euphytica*, vol. 62, no. 2, pp. 119-26.

Shi, C, Ingvardsen, C, Thümmler, F, Melchinger, A, Wenzel, G & Lübberstedt, T 2005, 'Identification by suppression subtractive hybridization of genes that are differentially expressed between near-isogenic maize lines in association with sugarcane mosaic virus resistance', *Molecular Genetics and Genomics*, vol. 273, no. 6, pp. 450-61.

Shiu, SH & Bleecker, AB 2003, 'Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis', *Plant Physiol*, vol.132, no.2, pp. 530-43.

Siar, SV, Beligan, GA, Sajise, AJC, Villegas, VN & Drew, RA 2011, 'Papaya ringspot virus resistance in *Carica papaya* via introgression from *Vasconcellea quercifolia'*, *Euphytica*, vol. 181, no. 2, pp. 159-68.

Simmonds, NW 1979, 'Principles of crop improvement', in *Simmonds, NW: Principles of crop improvement.*

Singh, I 1990, *Papaya*, Oxford and IBH Publishing, New Delhi.

Singh, R & Green, MR 1993, 'Sequence-specific binding of transfer RNA by glyceraldehyde-3-phosphate dehydrogenase', *Science*, vol. 259, pp. 365–8.

Skelton, RL, Yu, Q, Srinivasan, R, Manshardt, R, Moore, PH & Ming, R 2006, 'Tissue differential expression of lycopene [beta]-cyclase gene in papaya', *Cell Res*, vol. 16, no. 8, pp. 731-9.

Somsri, S & Bussabakornkul, S 2008, ' Identification of certain papaya cultivars and sex identification in papaya by DNA Amplification Fingerprinting (DAF). Acta Hort 787:197-206', *Acta Hort. (ISHS)*, vol. 787, pp. 197-206.

Sondur, SN, Manshardt, RM & Stiles, JI 1996, 'A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers', *Theoretical and Applied Genetics*, vol. 93, no. 4, pp. 547-53.

Song, W-Y, Wang, G-L, Chen, L-L, Kim, H-S, Pi, L-Y, Holsten, T, Gardner, J, Wang, B, Zhai, W-X, Zhu, L-H, Fauquet, C & Ronald, P 1995, 'A Receptor Kinase-Like Protein Encoded by the Rice Disease Resistance Gene, Xa21', *Science*, vol. 270, no. 5243, pp. 1804-6.

Sooriyapathirana, SS, Khan, A, Sebolt, AM, Wang, D, Bushakra, JM, Lin-Wang, K, Allan, AC, Gardiner, SE, Chagné, D & Iezzoni, AF 2010, 'QTL analysis and candidate gene mapping for skin and flesh color in sweet cherry fruit (*Prunus avium* L.)', *Tree Genetics & Genomes*, vol. 6, no. 6, pp. 821-32.

Souza, BSd, Durigan, JF, Donadon, JR & Teixeira, GHdA 2005, 'Conservação de mamão 'Formosa' minimamente processado armazenado sob refrigeração', *REVISTA BRASILEIRA DE FRUTICULTURA*, vol. 27, pp. 273-6.

Stiles, JI, Lemme, C, Sondur, SN, Morshidi, MB & Manshardt, R 1993, 'Using randomly amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars', *Theoretical and Applied Genetics*, vol. 85, no. 6, pp. 697-701.

Storey, WB 1938, 'Segregation of sex types in solo papaya and their application to the selection of seed', *Proc. Amer. Soc. Hort. Sci*, vol. 35, pp. 83-5.

—— 1969, 'Papaya', in FP Ferwerda & F Wit (eds), *Outlines of Perennial Crop Breeding in the Tropics*, H. Vienman and Zonen N. V. Wageningen, pp. 389-407.

—— 1976, 'Papaya', in NW Simmonds (ed.), *Evolution of crop plants* Longman, England, UK., pp. 21-4.

Takken, FLW, Albrecht, M & Tameling, WIL 2006, 'Resistance proteins: molecular switches of plant defence', *Current Opinion in Plant Biology*, vol. 9, no. 4, pp. 383-90.

Tan, SC & Weinheimer, EA 1976, 'The isoenzyme patterns of developing fruit and mature leaf of papaya (*Carica papaya* L.)', *Sains Malays J Nat Sci*, vol. Jan 5, no. 1, pp. 7-14.

Tennant, P, Fermin, G, Fitch, MM, Manshardt, RM, Slightom, JL & Gonsalves, D 2001, 'Papaya ringspot virus resistance of transgenic Rainbow and SunUp is affected by gene dosage, plant development, and coat protein homology', *European Journal of Plant Pathology*, vol. 107, no. 6, pp. 645-53.

Tennant, PF, Gonsalves, C, Ling, KS, Fitch, MM, Manshardt, RM, Slightom, LJ & Gonsalves, D 1994, 'Differential protection against papaya ringspot virus isolates in coat protein gene transgenic papaya and classically cross-protected papaya', *Phytopathology*, vol. 84, no. 11, pp. 1359-66.

Thellin, O, Zorzi, W, Lakaye, B, De Borman, B, Coumans, B, Hennen, G, Grisar, T, Igout, A & Heinen, E 1999, ' Housekeeping genes as internal standards: use and limits', *J Biotechnol*, vol. 75, pp. 291-5.

Ton, J, Flors, V & Mauch-Mani, B 2009, 'The multifaceted role of ABA in disease resistance', Trends Plant Sci, vol. 14, no. 6, pp. 310-7.

Torii, KU 2004, 'Leucine-rich repeat receptor kinases in plants: structure, function, and signal

transduction pathways', *Int Rev Cytol*, vol. 234, pp. 1- 46.

Tripathi, S, Ferreira, SA & Gonsalves, D 2008, 'Papaya ringspot virus-P: characteristics, pathogenicity, sequence variability and control', *Molecular plant phatology*, vol. 9, no. 3, pp. 269-80.

Ulrich, D & Olbricht, K 2011, 'Fruit Organoleptic Properties and Potential for Their Genetic Improvement', in MA Jenks & P Bebeli (eds), *Breeding for Fruit Quality*, Wiley-Blackwell, pp. 41-56.

Urasaki, N, Tarora, K, Uehara, T, Chinen, I, Terauchi, R & Tokumoto, M 2002a, 'Rapid and highly reliable sex diagnostic PCR assay for papaya (*Carica papaya* L.)', *BREEDING SCIENCE*, vol. 52, no. 4, pp. 333-5.

Urasaki, N, Tokumoto, M, Tarora, K, Ban, Y, Kayano, T, Tanaka, H, Oku, H, Chinen, I & Terauchi, R 2002b, 'A male and hermaphrodite specific RAPD marker for papaya (*Carica papaya* L.)', *TAG Theoretical and Applied Genetics*, vol. 104, no. 2, pp. 281-5.

Van der Biezen, EA & Jones, JD 1998, 'Plant disease-resistance proteins and the gene-for-gene concept', *Trends Biochem Sci*, vol. 23, no. 12, pp. 454-6.

Van der Knaap, E & Tanksley, SD 2001, 'Identification and characterization of a novel locus controlling early fruit development in tomato', *Theoretical and Applied Genetics*, vol. 103, no. 2-3, pp. 353-8.

Van Droogenbroeck, B, Breyne, P, Goetghebeur, P, Romeijn-Peeters, E, Kyndt, T & Gheysen, G 2002, 'AFLP analysis of genetic relationships among papaya and its wild relatives (Caricaceae) from Ecuador', *Theoretical and Applied Genetics*, vol. 105, no. 2, pp. 289-97.

Van Ooijen, G, Mayr, G, Kasiem, MMA, Albrecht, M, Cornelissen, BJC & Takken, FLW 2008, 'Structure–function analysis of the NB-ARC domain of plant disease resistance proteins', *Journal of Experimental Botany*, vol. 59, no. 6, pp. 1383-97.

Vandesompele, J, De Preter, K, Pattyn, F, Poppe, B, Van Roy, N, De Paepe, A & Speleman, F 2002, 'Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes', *Genome biology*, vol. 3, no. 7, p. research0034.

Vandesompele, J, Kubista, M & Pfaffl, MW 2009, 'Reference gene validation software for improved normalization', *Real-time PCR: current technology and applications*, pp. 47-64.

Wai, CM, Ming, R, Moore, PH, Paull, RE & Yu, Q 2010, 'Development of Chromosome-specific Cytogenetic Markers and Merging of Linkage Fragments in Papaya', *Tropical Plant Biology*, vol. 3, no. 3, pp. 171-81.

Waigmann, E, Ueki, S, Trutnyeva, K & Citovsky, V 2004 'The ins and outs of non-destructive cell-to-cell and systemic movement of plant viruses', *Crit Rev Plant Sci*, vol. 23, pp. 195–250.

Walton, D & Yi, L 1995, 'Abscisic Acid Biosynthesis and Metabolism', in P Davies (ed.), *Plant Hormones*, Springer Netherlands, pp. 140-57.

Wan, H, Zhao, Z, Qian, C, Sui, Y, Malik, AA & Chen, J 2009, 'Selection of appropriate reference genes for gene expression studies by quantitative real-time polymerase chain reaction in cucumber', *Anal Biochem*, vol. 399, no. 2, pp. 257-61.

Wan, H, Zhao, Z, Qian, C, Sui, Y, Malik, AA & Chen, J 2010, 'Selection of appropriate reference genes for gene expression studies by quantitative real-time polymerase chain reaction in cucumber', *Analytical Biochemistry*, vol. 399, no. 2, pp. 257-61.

Wang, J, Chen, C, Na, J-K, Yu, Q, Hou, S, Paull, RE, Moore, PH, Alam, M & Ming, R 2008, 'Genome-Wide Comparative Analyses of Microsatellites in Papaya', *Tropical Plant Biology*, vol. 1, no. 3, pp. 278-92.

Warmke, H, Cabanillas, E & Cruzado, H 1954, 'A new interspecific hybrid in the genus Carica', in *Proceedings of the American Society for Horticultural Science*, vol. 64, pp. 284-8.

Watson, B 1997, *Agronomy/agroclimatology notes for the production of papaya*, MAFFA, Australia.

World Health Organization 2007, *Micronutrient Deficiencies-Vitamin A Deficiency*, World Health Organization (WHO), viewed 21 December 2014, <http://www.who.int/nutrition/topics/vad/en/>.

Yamamoto, HY 1964, 'Comparison of the carotenoid in yellow-and red-fleshed *Carica papaya*', *Nature*, vol. 201, pp. 1049–50.

Yang, Y, Shah, J & Klessig, DF 1997, 'Signal perception and transduction in plant defense responses', *Genes Dev.*, vol. 11, no. 13, pp. 1621-39.

Ying, KC 2008, 'Breeding Papaya (*Carica papaya L.*)', in *Breeding Plantation Tree Crops: Tropical species*, Springer, pp. 128-31.

Yu, Q, Tong, E, Skelton, RL, Bowers, JE, Jones, MR, Murray, JE, Hou, S, Guan, P, Acob, RA, Luo, M-C, Moore, PH, Alam, M, Paterson, AH & Ming, R 2009, 'A physical map of the papaya genome with integrated genetic map and genome sequence', *BMC genomics*, vol. 10, no. 1, pp. 371-.

Zaitlin, M & Hull, R 1987, 'Plant Virus-Host Interactions', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 38, pp. 291–315.

Zeevaart, JAD & Creelman, RA 1988, 'Metabolism and physiology of abscisic acid', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 39, no. 1, pp. 439-73.

Zeng, S, Liu, Y, Wu, M, Liu, X, Shen, X, Liu, C & Wang, Y 2014, 'Identification and Validation of Reference Genes for Quantitative Real-Time PCR Normalization and Its Applications in Lycium', *PLoS ONE*, vol. 9, no. 5, p. e97039.

Zhang, Y, He, J & McCormick, S 2009, 'Two Arabidopsis AGC kinases are critical for the polarized growth of pollen tubes', *Plant J*, vol. 58, no. 3, pp. 474-84.

Zhu, X, Li, X, Chen, W, Chen, J, Lu, W, Chen, L & Fu, D 2012, 'Evaluation of new reference genes in papaya for accurate transcript normalization under different experimental conditions', *PLoS ONE*, vol. 7, no. 8, p. e44405.

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Appendices

Appendix 1

Chapter 1 of PhD thesis of Dr. Chat Kanchana-udomkan

This chapter provides a complete literature review of publications relevant to the research in this report and additional research undertaken in the PhD program.

Chapter 1:

Literature Reviews

1.1 Published reviews by Kanchana-udomkan et al. (2014)

1.1.1 Background

Molecular markers are effective tools and have been used to facilitate genetic improvement in many crop species including *Carica papaya* L. (Eustice et al. 2008). The main purpose of using molecular markers within a breeding program is to either determine the relatedness among genotypes for germplasm resource management and parental choice, for true-to-type and hybrid identification; or, to identify and select for particular sequences that are associated with traits of interest. DNA markers are generally stable, unaffected by environment, present at all stages of plant growth and in all tissue types. They have been adopted within papaya breeding programs as accurate selection tools for traits of interest (Eustice et al. 2008; Ma et al. 2004; Porter et al. 2009a; de Oliveira et al. 2010b; Deputy et al. 2002; Dillon et al. 2006b).

Recent research has led to some important developments in this field. In the search for DNA markers linked to the genes that condition the traits of interest, a genetic and physical map of the papaya genome was developed (Yu et al. 2009). The papaya genome was sequenced and has been used to identify a library of SSR (Simple Sequences Repeat; microsatellite) loci (Eustice et al. 2008; Santos et al. 2003; Wang et al. 2008). In addition, several gene sequences with associated functions have become available through the papaya genome project database (Ming et al. 2008).

This chapter will focus on the development and application of molecular markers that have been used to assess genetic diversity and to improve papaya breeding objectives.

Table 1.1 DNA markers and their primer sequences linked to sex type identification in *Carica papaya*

Marker name	Primer sequence (5' ---> 3')	Product size	Type of marker	Sex detection	Note	Reference
OPT12	OPT12:GGGTGTGTAG	Na	RAPD		*Sex1*: 7cM	Sondur et al. (1996)
OPT1C	OPT1: GGGCCACTCA	na	RAPD		*Sex1*: 7cM	Sondur et al. (1996)
T1	T1F: TGCTCTTGATATGCTCTCTG T1R: TACCTTCGCTCACCTCTGCA	1.3 kb	SCAR	All sex	*Sex1*: n/a	Deputy et al. (2002)
T12	T12F: GGGTGTGTAGGCACTCTCCTT T12R: GGGTGTGTAGCATGCATGATA	800 bp	SCAR	Hermaphrodite and Male	*Sex1*: 0.3 cM	Deputy et al. (2002)
W11	W11F: CTGATGCGTGTGTGGCTCTA W11R: CTGATGCGTGATCATCTACT	800 bp	SCAR	Hermaphrodite and Male	*Sex1*: 0.3 cM	(Deputy et al. 2002)
PSDM	IBRC-RP07: TTGGCACGGG	450 bp	RAPD	Hermaphrodite and Male	*Sex1*: n/a	(Urasaki et al. 2002b)
SCARps	SDP-1: GCACGATTTAGATTAGATGT SDP-2: GGATAGCTTGCCCAGGTCAC	225 bp	SCAR	Hermaphrodite and Male	*Sex1-H* and *Sex1-M*: n/a	(Urasaki et al. 2002b)
Papain	P5': GGGCATTCTCAGCTGTTGTA P3': CTCCCTTGAGCGACAATAAC	221 bp	EST		Papain	(Urasaki et al. 2002a)
SCARpm	SDP-2: GGATAGCTTGCCCAGGTCAC SDP-3: GGTAAGAGTTTTTCCCAAGC	347 bp	SCAR	Hermaphrodite and Male		(Urasaki et al. 2002a)
$BC210_{438}$	BC210: GCACCGAGAG	438 bp	RAPD	Hermaphrodite		(Lemos et al. 2002)
$OP\text{-}Y7_{900}$	OP-Y7: AGAGCCGTCA	900 bp	RAPD	Male		(Chaves-Bedoya and Nuñez 2007)
SCAR SDSP	CFw: AAACTACCGTGCCATTATCA CRv: AGAGATGGGTTGTGT CACTG	369 bp	SCAR	Hermaphrodite and Male		(Chaves-Bedoya and Nuñez 2007)

$(GATA)_4$	Genomic DNA digested with *Hin*fI	5 kb	RFLP	Hermaphrodite and Male		(Parasnis et al. 1999)
$(GATA)_4$	Genomic DNA digested with *Hae*III	4 kb	RFLP	Hermaphrodite and Male		(Parasnis et al. 1999)
OPC09-1.7	OPC09: CTCACCGTCC	1.7 kb	RAPD			(Niroshini et al. 2008)
OPE03-0.4	OPE03: CCAGATGCAC	0.4 kb	RAPD			(Niroshini et al. 2008)
C09/20	C09/20FP: CTCACCGTCCATTTTAATTA	1.7 kb	SCAR	Hermaphrodite and Male		(Niroshini et al. 2008)
	C09/20RP: CTCACCGTCCGCGGCATCAATGTA	978 bp				
$(GACA)_4$	(GACA)4: GACAGACAGACAGACA		ISSR			(Gangopadhyay et al. 2007)
OPF2-0.8	OPF2: GAGGATCCCT	831 bp	RAPD	Male	Acc#AF148540	(Parasnis et al. 2000)
	F-Napf-76: GAGGATCCCTATTAGTGTAAG	831 bp	SCAR	Male		(Parasnis et al. 2000)
	R-Napf-77: GAGGATCCCTTTTGCACTCTG					
	F-Napf-70: GGATCCCT ATTAG	0.83 kb	SCAR	Male	Mass screening	(Parasnis et al. 2000)
	R-Napf-71: GAGGATCCCTTTTGC					
GC	F-GN-C: CGAAATCGGTAGACGATACG	0.6 kb		Sex neutral		(Parasnis et al. 2000)
	R-GN- D: GGGGATAGAGGGACTTGAAC					
OPA06	OPA 06: 5' GGT CCC TGAC 3'	365 bp	DAF	Hermaphrodite and Male		(Somsri and Bussabakornkul 2008)
		360 bp				

1.1.2 Development of molecular marker in papaya

The first markers were morphological, which Hofmeyr (1938) mapped onto the initial papaya genetic map in 1938. Subsequently, from the 1970s to the 1990s, biochemical markers (isozymes) were used to study the development of papaya fruits and mature leaves (Tan and Weinheimer 1976); hybridity of *C. papaya* and *C. cauliflora* (Jacq.) A.DC. (Moore and Litz 1984); and genetic relationships between *C. papaya* and wild *Vasconcellea* relatives (Jobin-Decor et al. 1997).

The first report of the use of DNA markers in a papaya genomic study was in 1992. Southern blot detection of restriction fragment length polymorphisms (RFLPs), produced by digesting total genomic DNA with restriction enzymes and detection by micro- and mini-satellite probes, were used to detect polymorphisms between *C. papaya* genotypes and between *C. papaya* and its related species (Sharon et al. 1992). The report suggested that *C. papaya* genotypes and their related species could be identified and distinguished by their unique DNA fingerprints.

The invention of the polymerase chain reaction (PCR) in the early 1990s then led to rapid advances in the development and application of molecular markers. The first PCR-based markers for papaya were random amplified polymorphic DNA (RAPD) markers, which are dominant markers that are amplified by arbitrary short primer sequences. These were used to evaluate genetic relationships among papaya cultivars (Stiles et al. 1993) and between papaya and wild related *Vasconcellea* species. RAPD sourced markers were also applied to determine sex of papaya prior to flowering. However, RAPD markers can be difficult to reproduce on different equipment and by different researchers. In addition, although assumptions have been made in the past, RAPD markers cannot be used to determine allele differences at a particular locus without fragment sequencing. A variation of this technique, that employs increased annealing temperature and polyacrylamide gel detection, randomly amplified DNA fingerprinting (RAF), identified markers linked to Papaya Ringspot Virus type P (PRSV-P) resistance in the related *Vasconcellea cundinamarcensis* Badillo, also known as *V. pubescens* (Dillon et al. 2005).

Subsequently, amplified fragment length polymorphism (AFLP) markers, integrating RFLP with PCR, were applied to assess genetic relationships within the Caricaceae (Kim et al. 2002; Van Droogenbroeck et al. 2002; Ocampo Pérez et al. 2007; Ratchadaporn et al. 2007). AFLP markers have also been placed on papaya genome maps (Ma et al. 2004; Blas et al. 2009). Approximately 1500 AFLP markers were mapped onto 12 linkage groups (LGs) (Ma et al. 2004). However, using the same population, only 20% of these markers were mapped along with other types of markers into 9 major and 5 minor linkage groups (Blas et al. 2009). These markers, RAPD, RAF and AFLP, which are non-specific and dominant markers, could be useful for papaya crop improvements as they do not require much genetic information i.e. DNA sequence analysis. However, the amplification patterns of these markers are complicated and they can not identify single loci and alleles. Hence, more reliable, stable and useful markers were needed.

Simple sequence repeats (SSR), otherwise known as microsatellite markers have many advantages over anonymous dominant markers. They are targeted to flanking sequences to amplify the tandem short repeat units dispersed throughout the genome. Therefore, they are generally locus specific and may be co-dominant. Also, due to the phenomenon of conserved slippage they may be highly polymorphic among individuals within a species although they are generally not well transferred between species. The *C. papaya* SSR

markers have been used for sex identification (Parasnis et al. 1999), in genetic diversity studies (Pérez et al. 2006; Ocampo Pérez et al. 2007; de Oliveira et al. 2008; Eustice et al. 2008; de Oliveira et al. 2010a; de Oliveira et al. 2010b), for the construction of genetic maps (Blas et al. 2009; Chen et al. 2007) and integration to a physical map, and for comparing cytogenetic markers to merge linkage fragments (Yu et al. 2009; Wai et al. 2010).

1.1.3 Application

1.1.3.1 Molecular markers and papaya genomic studies

Papaya belongs to the order Brassicales which comprises 17 families including Caricaceae that contains papaya, and Arabidopsis, the model plant in Brassicaceae. The family Caricaceae contains six genera and 35 species including *Carica papaya*. Papaya is the only member in the genus *Carica*, and has a relatively small genome size of 372 Mb (Arumuganathan and Earle 1991); diploid 2n = 18. Papaya has been identified as a model for biotechnology applications in tropical fruit species because it is an economically important fruit crop in tropical and subtropical regions, it has a small genome size; and it has a short generation time (9-15 months). The genome was successfully sequenced in 2008 for 'SunUp', which is a commercial virus-resistant transgenic genotype of papaya (Ming et al. 2008). The papaya genome was the fifth flowering plant to be sequenced. Compared to the other four plant genomes that were sequenced, papaya contains 24746 genes, which is 20%, 34%, 46% and 19% less than *Arabidopsis*, rice, poplar and grape, respectively.

The first genetic map of papaya comprised three morphological markers; sex type, flower color and stem color, covering 41 cM (Hofmeyr 1939) of the genome. The second papaya genetic map (F_2: Hawaiian cultivar 'Sunrise' x UH breeding line 356) was established 60 years later comprising 61 RAPD markers and one morphological locus (*SEX1*) within 11 linkage groups and comprising a total map distance of 999.3 cM (Sondur et al. 1996). In 2004, another map was produced using 54 F_2 plants derived from cultivars Kapoho x Sunup and containing 1498 AFLP markers, a PRSV-P coat protein marker and two morphological markers; that determined sex type and fruit flesh colour (Ma et al. 2004). These markers were mapped into 12 linkage groups with a total length of 3294.2 cM and an average distance of 2.2 cM. This map was then integrated into a recent genetic map (Blas et al. 2009).

SSR markers have been used widely in papaya research and SSR libraries for papaya have been developed (Eustice et al. 2008; Santos et al. 2003; Wang et al. 2008). The patterns of SSR distribution are similar within genomic or genic regions and the most abundant motif repeats are dinucleotides. The AT/TA motif repeats are predominant across several studies (Eustice et al. 2008; Santos et al. 2003; Wang et al. 2008; Nagarajan et al. 2008). The most abundantly detected trinucleotides motif differs among studies (AAG/TTC (44.1%; Eustice et al. 2008); AAT/TTA (55.8%; Wang et al. 2008); and TAC/ATG, AGA/TCT and ATT/TAA were reported to be very common in the papaya genome sequence (Nagarajan et al. 2008). This is likely due to the different methods used to isolate the SSR sequences. SSRs were identified from the papaya genome sequence using Microsatellite Analysis Server (Eustice et al. 2008), Perl program, Simple Sequence Repeat Identification Tool (SSRIT; Wang et al. 2008) and the Tandem Repeats Finder software (Nagarajan et al. 2008).

SSR markers were included within a subsequent map constructed with 54 F_2 plants that were derived from AU9 x SunUp. This comprised 706 SSR markers and one morphological marker for fruit flesh colour within 12 linkage groups over 1068.9 cM and an average distance of 1.51 cM (Chen et al. 2007). The nine major linkage groups represent nine chromosomes in the papaya genome, covered 993.5 cM with 683 map loci with an average marker density of 1.45 cM. The three short linkage groups covered 75.4 cM with 24 mapped loci and an average distance of 3.1 cM between adjacent markers.

Codominant markers have greatly increased the resolution and accuracy of papaya genetic maps. The map containing SSR markers was far more compact (1068.9 cM) than that produced by AFLP markers (3294.2 cM). The threefold reduction in genetic distance was due to the ability of codominant markers to separate the three classes of genotypes within the F_2 population; homozygous dominant, homozygous recessive and heterozygous.

A recent genetic map of papaya was saturated with 712 SSR, 277 AFLP and one morphological markers spanning 945.2 cM (Blas et al. 2009). This was constructed with the same 54 F_2 AU9 x Sunup population and comprised 14 linkage groups; nine major and five minor. The nine major linkage groups incorporated 939 marker loci over a total 849.4 cM at 1.6 cM or less intervals. The five minor linkage groups comprised 51 loci and spanned 95.8 cM with 3.0 cM or less intervals. Comparing the previous map (Chen et al. 2007) to this recent map, the addition of AFLP markers allowed six unmapped SSR markers from previous map to be linked, but did not join the gap between three previous minor linkage groups and the nine major linkage groups. However, the number of gaps that were greater than 5 cM between adjacent loci was reduced from 48 to 27 and the total map length was reduced by approximately 11.5%. The addition of AFLP markers resulted in an order of locus rearrangement.

Prior to the availability of the whole genome sequence, bacterial artificial chromosome clones containing papaya genomic DNA were produced and assembled. The first papaya BAC library consisted of 39168 clones from two separate ligation reactions (Ming et al. 2001). The average insert size of 18700 clones from the first ligation was 86 kb, while 20468 clones from the second ligation contained inserts twice as large, averaging 174 kb. The entire BAC library was estimated to provide a 13.7 x papaya genome coverage. In 2006, a total of 50661 BAC end sequence (BES) chromatograms were generated from 26017 BAC clones (Lai et al. 2006) from the BAC clone library by Ming et al (2001). After eliminating all unused sequences, 35472 high-quality sequences from 20842 BAC clones were generated. The total number of high-quality bases was 17483563 or 4.7% of the papaya genome. Two years later, a 3x draft genome of cultivar 'SunUp' was reported within which 1.6 million high-quality reads were assembled into contigs containing 271 Mb and scaffolds spanning 370 Mb (Ming et al. 2008). Subsequently, 652 BAC and whole genome sequence derived SSRs were used to anchor 167 Mb of contigs and 235 Mb of scaffolds to the 12 linkage groups of papaya on the current genetic map (Ming et al. 2008).

A physical map of the papaya genome, that integrated with the genetic map and genome sequence, was published in 2009 (Yu et al. 2009). The BAC-based physical map of papaya covered 95.8% of the genome, while 72.4% was aligned to a sequence-tagged SSR genetic map (Chen et al. 2007). This BAC library initially included 39168 BAC clones;

however, after evaluation and reviews, 26466 BAC clones were assembled into 963 contigs having an average number of fragments for each clone of 69.4. The average physical distance per centimorgan was approximately 348 kb. The integrated genetic and physical map when aligned with the genome sequence revealed recombination hot spots as well as regions suppressed for recombination across the genome, particularly on the sex chromosome, namely LG1 (Yu et al. 2009). A total of 1181 overgos representing conserved sequences of *Arabidopsis* and genetically mapped *Brassica* loci were anchored on the integrated genetic and physical map and the draft genome sequence of papaya. These overgos are direct links among papaya, *Arabidopsis* and *Brassica* genomes for comparative genomic research among species within the order Brassicales. The combined information of physical and genetic maps will enhance the capacity for map-based cloning and identification of underlying genes controlling traits of interest in papaya. It will also expedite the mapping and cloning of target genes and promote marker-assisted selection for papaya breeding.

Recently, chromosome-specific cytogenetic markers were developed and merged with linkage groups of papaya using the integrated technique of fluorescence in situ hybridisation (FISH) and BAC clones harboring mapped SSR markers as probes (Wai et al. 2010). Minor linkage groups 10, 11 and 12 from the previous map were assigned to major LGs 8, 9 and 7, respectively. Thus, the nine linkage groups in the genetic map corresponded to the haploid number of papaya chromosomes. This integrated map will facilitate genome assembly, quantitative trait locus mapping, and the study of cytological, physical and genetic distance relationships between papaya chromosomes. It is an even more powerful and accurate tool for trait selection.

1.1.3.2 Molecular markers and genetic diversity in Caricaceae

Variations in *Carica papaya* in terms of phenotypic, morphological and horticultural characteristics such as fruit size, fruit shape, flesh colour, texture, flavour and sweetness, stamen carpellody and carpel abortion, sex type, length of juvenile period, plant stature, plant canopy size, can be detected at high levels between different genotypes of papaya in the field. However, most of this morphological diversity in papayas has not been correlated to genetic diversity, specific genes or molecular markers despite many studies on genetic diversity. Different techniques have been used to study genetic diversity in papaya and relationships between plants within *Caricaceae* including AFLPs, isozymes, RAPDs, microsatellites and SSRs.

Kim et al. (2002) studied genetic relationships between papayas and related *Vasconcellea* species using samples which had a wide range of morphology and climate adaptation variation, from tropical, subtropical and temperate regions. But only 12% of genetic variation was detected among this diverse group of material using AFLP markers, and it was not representative of the wide range of morphological characteristics that were observed in the field. The accessions that Kim et al. (2002) used in their study consisted of breeding lines, unimproved germplasm and related species. Five cultivars of papayas were initially screened for polymorphisms by 64 sets of *Eco*RI-*Mse*I primers with three nucleotide extensions. The number of polymorphic markers ranged from 0 to 9 with an average of 3.2. Nine primers were selected to assess all samples and generated 186 polymorphic markers (42%) from 445 readable fragments. The estimation of genetic similarity using pairwise comparison among 63 papaya accessions ranged between 0.74

and 0.98 (mean 0.88). Cluster analysis of 71 papaya accessions and related species showed the genetic relationship among individual genotypes which developed in different geographic regions. The first cluster included all 15 Solo-type cultivars and breeding lines. The second cluster included dioecious Australian cultivars and Indian cultivars that grow in subtropical or temperate regions. The third cluster was a group of cultivars originating from different countries. AFLP markers were used to study genetic relationships between papaya and wild relatives by Van Droogenbroeck et al. (2002) who analysed 95 accessions of papayas and wild relatives from Ecuador. Five primer combinations were used and revealed 951 bands ranging in length from 50 to 500 base pairs. Only 512 bands were scored of which 16 were monomorphic. All papaya genotypes were clustered separate to two other clusters comprising individuals from *Jacaratia* or *Vasconcellea*. Genetic similarity ranged from 0.39 to 0.81 among the *Vasconcellea* species that were assessed.

Genetic relationships between *C. papaya* and wild relatives were studied by Jobin-Décor et al. (1997) by comparing isozyme and RAPD techniques. A total of 47 bands were generated by nine enzymes and 188 bands were generated from 14 RAPD primers. Both techniques gave similar measures of genetic distance of 70% dissimilarity between *C. papaya* and the other *Carica* species (later renamed *Vasconcellea* species) and approximately 50% dissimilarities among *Vasconcellea* species. RFLP markers were used to study phylogenetic relationships using the chloroplast DNA (cpDNA) of 12 wild and cultivated species of *Cairca* (Aradhya et al. 1999). Twenty-three accessions, representing 14 taxa, were analysed in the cpDNA intergenic spacer region by amplification of the region via PCR technique and then the PCR product was digested by 14 restriction enzymes. A total of 138 fragments accounting for 137 restriction sites were examined and the results confirmed the close association among South American wild *Carica* (*Vasconcellea*) species.

From a microsatellite-enriched library developed using $(GA)_n$ and $(GT)_n$ probes (Pérez et al. 2006), 45 primer pairs giving the best resolution and allelic differentiation were used for evaluation of 29 accessions of *C. papaya* and 11 accession of *Vasconcellea*. Of these, 24 revealed polymorphisms between these two genera. A total of 99 alleles were observed in papaya with an average of 3.8 alleles per locus. In the *Vasconcellea* samples, 22 alleles were identified from four loci. These two genera had a clear allelic divergence for the loci that they shared. This strong differentiation gave further support to the hypothesis of the early divergence of *Vasconcellea* from *Carica* (Pérez et al. 2006). Many more SSR markers were identified from the genome sequence project (Eustice et al. 2008). These were tested for polymorphism in seven genotypes of papaya. Of the 938 SSR markers that were defined, 812 were from genomic sequences and 126 from genic sequences (Eustice et al. 2008). Overall, 52.9% were polymorphic. SSR primers developed in 2008 (Oliveira et al. 2008) were screened on 30 papaya accessions and 18 landraces (de Oliveira et al. 2010b). Of the 100 SSR primers, 81 successfully amplified PCR products of high quality and were selected for further studies. Of these, 59 produced easily scorable markers and detected a total of 237 alleles with 2 to 11 per locus. In a separate study using 27 of the same SSR loci developed in 2008 (Oliveira et al. 2008), the relationships among 83 papaya lines were assessed (de Oliveira et al. 2010b). Of the 27 primers, 20 were polymorphic and identified a total of 86 alleles, with an average of 3.18 alleles per primer. Since cultivated *C. papaya* is proposed to have a low or narrow genetic base (Ratchadaporn et al. 2007; Stiles et al. 1993), many important genes conditioning traits of interest (such as disease resistances and abiotic stress tolerances) may have been lost or excluded in the pursuit of other traits (such as fruit colour and

sweetness). Indeed, many disease resistances are found in wild relative *Vasconcellea* species and future breeding may require interspecific recombination to reintrogress these back into the elite cultivated genomes (d'Eeckenbrugge et al. 2014).

A number of taxonomy studies have confirmed the diversity between *C. papaya* and *Vasconcellea* species and have supported the separation into two genera by Badillo (2000). Most studies supported the early divergence of *C. papaya* from the wild relatives and this has been verified by different molecular marker techniques; isozyme and RAPD (Jobin-Decor et al. 1997), RFLP (Aradhya et al. 1999), AFLP (Kim et al. 2002; Van Droogenbroeck et al. 2002). The most closely related species were reported as *V. stipulata* (Badillo) and *V. pubescens* (Jobin-Decor et al. 1997; Sharon et al. 1992); and *V. goudotiana* Tr. et Pl. and *V. pubescens* (Kim et al. 2002). The species most distant from *C. papaya* were reported to be *V. cauliflora* (Jobin-Decor et al. 1997) and *V. goudotiana* (Sharon et al. 1992; Kim et al. 2002). In particular, Kim et al. (2002) reported that the average genetic similarity between papaya and other *Vasconcellea* species was 0.43 and the average similarity among six different species of *Vasconcellea* was 0.73, much closer to each other than to *C. papaya*. *C. goudotiana* was the most distantly related species to papaya (0.36 similarity), while it was closely related to *C. pubescens* (*V. pubescens*) with a similarity of 0.87.

Previously, Jobin-Décor et al. (1997) had reported *C. papaya* to be distinct from the other *Carica* species, *C. cauliflora*, *C. parviflora*, *C. pubescens*, *C. goudotiana*, *C. stipulata* and *C. quercifolia* with a mean dissimilarity of 0.73 and 0.69 using isozyme and RAPD analysis, respectively. As this work was done before 2000 all these species were considered to be in *Carica* genus at that time. The other *Carica* (*Vasconcellea*) species were more closely related to each other with a mean dissimilarity of 0.46. The closest two species were *V. stipulata* and *V. pubescens* with the dissimilarity of 0.13 and 0.18 0.87 and 0.82 using isozyme and RAPD analysis respectively. However, from RAPD analysis, the *Vasconcellea* species most distant from *C. papaya* was *cauliflora.* It was reported that *C. papaya* and other species had band sharing between 25% and 48% (Sharon et al. 1992). *C. goudotiana* is more distant from *C. papaya* with band sharing of 25%. *C. stipulata* and *C. pubescens* were very closely related species with band sharing of 71%.

In summarising their genetic diversity, it should be noted that the *Vasconcellea* species have recently been divided into three clades (D'Eeckenbrugge et al. 2014). They are (1) *V. weberbaueri* (Harms)*, V. stipulata, V. x heilbornii* (Badillo) Badillo and *V. parviflora* A.DC.*;* (2) *V. chilensis* (Planch. ex A.DC.) A.DC.*, V. candicans* (Gray) A.DC.*, V. quercifolia* St.-Hil. and *V. glandulosa* A.DC. and (3) a clade holding all other species of the genus (D'Eeckenbrugge et al. 2014).

1.1.3.3 Molecular marker and sex determination in *Carica papaya*

Carica papaya is a polygamous species with three basic sex types; female, male and hermaphrodite. Although the male plants occasionally produce hermaphrodite flowers on the abaxial end of inflorescences, they do not produce commercial fruit. However, in dioecious plantings, which are common in subtropical regions, male plants are still needed for pollen. Usually the ratio of male: female plants is 1:10, thus multiple seedlings are planted at each site and then the plants are thin to achieve the required ratio of female and male plants. In tropical regions hermaphrodite trees are preferred

because every tree is capable of producing marketable fruits. In plantations comprising hermaphrodite plants, female plants are unwanted and removed after flowering. Commercial papaya growers have to plant 3-5 plants per site, then evaluate sex type after flowering and cull the undesired plants. The process is time consuming, laborious, and cost ineffective. In addition, competition between multiple plants at each planting site causes poor root systems, elongated plants and increased height to the first flower and fruit. Therefore, the use of DNA markers to discriminate sex of papaya plants at the earliest plant growth stage has the potential to greatly increase efficiency within the papaya production system. Thus much research has been applied to this subject in recent years.

Genetic control of sex of papaya has been studied since 1938. Hofmeyr (1938) and Storey (1938) independently proposed the hypothesis that sex determination in papaya is controlled by a single dominant gene with three alleles, named M^1, M^2 and m by Hofmeyr, and M, M^h and m by Storey. They proposed the genotype of male, hermaphrodite and female plants are Mm (M^1m), M^hm (M^2m) and mm respectively, and explained that homozygous dominant alleles are lethal. Therefore segregation of sex type from selfed hermaphrodite trees is 2 hermaphrodites: 1 female. Whereas seeds from female trees segregate at the ratio of 1:1 female: hermaphrodite if the plant is crossed with a hermaphrodite tree, but that of 1:1 female: male when it is crossed with a male tree. Subsequently, other researchers have proposed other hypotheses for genetic control of sex in papaya and this was reviewed by Ming et al. (2007).

Most research on DNA marker-assisted sex selection in papaya has been done on Hawaiian papaya genotypes. The first report of a sex linked marker in papaya was reported by Sondur et al. in 1996. They created a genetic linkage map using RAPD markers and investigated the genetics of sex determination in papaya using an F_2 population of Hawaiian cultivar Sunrise x IH breeding line 356. Of 596 10-base primers screened, two; OPT1 and OPT12, produced two marker bands; OPT1C and OPT12, flanking the *SEX1* locus at 7 cM for both markers. RAPD and DNA amplification fingerprinting (DAF) were compared and showed that DAF reactions produced at least five times more fragments than equivalent RAPD reactions in terms of ability to detect variation. They also revealed that DAF reactions were more reliable (Somsri et al. 1998). Bulk segregant analysis was used to define a large number of DAF markers present in only male or hermaphrodite pooled DNAs. Preliminary analyses for linkage associations indicated these markers were closely linked to the sex- determining alleles. Ten years later, Somsri and Bussabakornkul (2008) employed DAF to study the relationships between fourteen cultivars of papaya in Thailand. Using 11 primers, a total of 129 distinct fragments were amplified. Primer OPA06 could be used to identify the sex type of papaya plants. This primer produced two polymorphic bands: at 365 bp from the hermaphrodite bulk DNA and 360 bp from the male bulk DNA. Neither band was detected for females. Evaluation of the accuracy of OPA 06 analysis was verified using 254 plants of different generations and their original parents, and the analysis correctly identified sex type for 88.18% of the plants. In the final experiment, 47 hermaphrodite plants of the Khaeg Dum cultivar, that were grown in tissue culture, were examined using OPA 06, and the sex type was identified correctly for 100% of the plants.

From 2000 onwards, the sequence characterized amplified region (SCAR) technique has been used to increase specificity of priming sites from RAPD primers to the target DNA for sex determination of papaya. Eighty RAPD primers from Operon kits were screened on 12 different papaya varieties and the marker OPF2-0.8 kb was identified as male-

specific (Parasnis et al. 2000). The marker was converted into a SCAR marker, by designing a 20-bp primer pair as a sex specific primer. They also developed an internal control for the PCR reaction using primer GC, which is a neutral sex, meaning the marker presents in all sex types of papaya. For mass screening, they developed a single step DNA extraction and used a 15 bp-SCAR primer at a lower annealing temperature for sex detection.

The information from Sondur et al. (1996) was applied by cloning three RAPD products and SCAR primers were designed on these sequences. Two RAPD markers, OPT1C and OPT12, flanked the *SEX1* gene (Sondur et al. 1996), however Deputy et al. (2002) identified another marker, W11, that did not show recombination in the population that they used. So, W11 was included in this study along with T1 and T12. A SCAR T1 primer was designed on the interval sequences of the T1 marker, however SCAR T1 can amplify all sex types; female, male and hermaphrodite at 1300 bp. This could be because one or more point mutations exist in the original 10-base primer site that can distinguish females from hermaphrodites. Therefore, T1 was used as positive (or internal) control for PCR amplification. T12 and W11 were designed on the original 10-base plus a further 10-11 bases to make the primer more specific to papaya sequences. Both primers successfully differentiated sex type male/hermaphrodite from female plants generating the marker at 800 bp for both primers. The linkage analysis of SCAR markers W11 and T12 in 182 F_2 plants from the cross of Sunup x Kapaho indicated that these markers were within 0.3 cM of *SEX1*.

RAPD techniques were used to determine the sex of 11 Hawaiian cultivars of *C. papaya* for three sex types, male female and hermaphrodite (Urasaki et al. 2002b). Twenty-five arbitrary 10-mer primers were tested with papaya DNA. The IBRC-RP07 primer produced a fragment named PSDM at 450 bp in all male and hermaphrodite but not female plants. The fragment was analysed and a SCAR marker named SCARps was designed and produced a PCR product of 225 bp in the male and hermaphrodite plants only. A multiplex-PCR assay was developed for a sex-specific SCARpm marker and a marker for a papain gene as an internal control to minimize false negatives (Urasaki et al. 2002a). The marker was tested for amplification on a hermaphrodite plant of *C. papaya* 'Sunrise Solo'. This marker differentiated hermaphrodite and male plants from female plants.

Lemos et al. (2002) screened 152 RAPD primers on female and hermaphrodite plants of *C. papaya* cv. Baixinho de Santa Amália, cv. Sunrise Solo and cv. Improved Sunrise Solo 72/12. Primer BC210 produced a marker band at 438 bp ($BC210_{438}$) that was present in hermaphrodite but not in female plants. The marker was tested with 195 different samples from three cultivars and was present only in hermaphrodite plants. Published sex linked markers were validated on a selection of Brazilian commercial genotypes; two varieties of a Solo group and 2 hybrids of the Formosa group (de Oliveira et al. 2007). Four SCAR markers (Deputy et al. 2002; Parasnis et al. 2000; Urasaki et al. 2002b; Chaves-Bedoya and Nuñez 2007) revealed the presence of both false positives and negatives in some varieties, while the RAPD marker $BC210_{438}$ (Lemos et al. 2002) could predict papaya sex type correctly.

In another study, the sex linked markers that were described previously were tested on three Columbian papayas, but could not distinguish between male/hermaphrodite and female plants (Chaves-Bedoya and Nuñez 2007). Therefore, 32 arbitrary 10-mer Operon primers were screened and the OP-Y7 primer that generated a PCR product that had 900 bp and was present only in male plants and absent in female and hermaphrodite plants.

The marker was analysed and converted into a SCAR marker that could differentiate female plants from male and hermaphrodite plants. The SCAR SDSP marker at 369 bp, was present in male and hermaphrodite but not female plants.

In Sri Lanka, Niroshini et al. (2008) screened 100 arbitary decamer primers in ten plants of each papaya sex type. The plants were selected from home gardens near Kadawatha, Sri Lanka. Of the 100 primers that were tested, two primers; OPC09 and OPE03 produced two DNA maker bands specific to male/hermaphrodite plants at 1.7 and 0.4 kb, respectively. The markers were then sequenced and SCAR primers were designed by use of extension oligonucleotides at both ends of the sequence. SCAR primer C09/20 amplified two fragments of length 1.7 kb and 978 bp in both male and hermaphrodite plants. However, SCAR primer E03/20FP and E03/20RP that were designed from OPE03-0.4 did not detect polymorphisms among plants of different sex types.

Other markers based on microsatellites have been used for sex detection in papaya by Santos et al. (2003). It was possible to design primers from the library from sequences enriched with the probe $(TCA)_{10}$. Thirty-two pairs of SSR primers were designed, however, none of them could identify sex type in this study. Commercial cultivars of papaya and a wild species *V. cauliflora* were screened by digesting genomic DNA with various restriction enzymes and microsatellite sequences were used as probes by Parasnis et al. (1999). The microsatellite repeats $(GATA)_4$ and $(GAA)_6$ detected sex-specific differences in *Hin*fI or *Hae*III digested samples. However, only the repeat $(GATA)_4$ showed male/hermaphrodite specific bands at 5 and 4 kb after digesting the genomic DNA with *Hin*fI and *Hae*II, respectively, while the repeat $(GAA)_6$ could detect polymorphisms for sex in some cultivars only.

Inter simple sequence repeat (ISSR) and RAPD techniques were used to determine sex of 200 seedlings of a local variety of papaya (Gangopadhyay et al. 2007) in Kolkata, India. Ten RAPD primers (OPA01-OPA05 and OPB01-OPB05) failed to show polymorphisms among the three sex types. Of three ISSR primers used in this study, one ISSR primer, $(GACA)_4$, could distinguish female or hermaphrodite from male plants.

In conclusion, much marker research has been applied to the identification of DNA markers for the differentiation and selection of male, female and hermaphrodite plants. Although many of these markers can be used for marker assisted selection in breeding programs, there is a need to adapt some of these markers into low-cost techniques that can identify sex of seedlings and thus facilitate commercial production of papayas. Over planting, then thinning to achieve the desired sex ratios after plants reach flowering stage is still a major and potentially unnecessary cost for papaya producers in both tropical and subtropical regions.

1.1.3.4 Molecular marker assisted selection for papaya breeding

1.1.3.4.1 Disease resistance

Lack of disease resistance genotypes in *Carica* is the major problem for crop improvement, while resistance to all diseases that attack papaya can be found in wild relative *Vasconcellea* species (D'Eeckenbrugge et al. 2014). Therefore the relationship between these two genera has been studied with the aim of transferring resistant genes from *Vasconcellea* spp. to papaya.

Papaya ringspot disease, caused by *Papaya ringspot virus* type P; PRSV-P, is widely reported as the most devastating disease of papaya production worldwide. The sustainable method to control this disease in the papaya industry is to produce improved papaya varieties that are resistant to the pathogen. A transgenic papaya variety that was resistant to PRSV-P was successfully developed in 1990 (Fitch et al. 1990), however GMOs plants are not accepted in many countries, and transgenic resistant varieties can be virus-strain specific in their resistance (Tennant et al. 1994). Therefore, conventional breeding for PRSV-P resistance in papaya is a viable option for long-term control of the disease, and until GMO food crops are more universally accepted worldwide.

Resistance to PRSV-P has been reported in *V. cauliflora, V. stipulata, V. pubescens and V. quercifolia* (Manshardt and Drew 1998). The gene for PRSV-P resistance was successfully backcrossed from *V. pubescens* into *V. parviflora* from F_3 interspecific hybrids containing the homozygous dominant allele of the gene (O' Brien and Drew 2009). This suggested that a single dominant gene controlled PRSV-P resistance in *V. pubescens* and was consistent with earlier reports on the PRSV-P resistance in a generic hybrid between *V. pubescens* and *C. papaya* (Drew et al. 1998b). This is consistent with reports of single gene dominance regulating the PRSV-P resistance in F_1 intergeneric hybrids of *C. papaya* and *V. cauliflora* (Magdalita et al. 1997). Similar results were reported in interspecific hybrids between *V. pubescens* x *V. parviflora* (Dillon et al. 2006a; b). Genetic mapping studies of these hybrids further supported the concept that PRSV-P resistance in *V. pubescens* is controlled by a single dominant gene (Dillon et al. 2006c). This was confirmed with molecular markers which were linked to a single locus resistance gene (*prsv-1*) that was identified in *V. pubescens* (Dillon et al. 2005; Dillon et al. 2006b).

A genetic map of PRSV-P resistance gene(s) based on RAF markers was constructed from100 F_2 plants of *V. cundinamarcensis* (*V. pubescens*) and *V. parviflora* (Dillon et al. 2005). Mapping of dominant markers in repulsion phase in F_2 populations can cause an incorrect estimation of genetic distance, therefore, RAF markers were mapped to separate parental maps. For *V. pubescens*; markers were mapped to ten linkage groups that covered 745.4 cM with an average distance of 9.68 cM between adjacent markers. The PRSV-P resistance locus (*prsv-1*) was mapped within 4 and 2.8 cM of adjacent markers Pbw15_40 and OPA15_8 on LG7. For *V. parviflora*, markers were mapped to ten linkage groups that covered 630.2 cM separated by an average distance of 7.95 cM between adjacent markers. The markers Pbw15_40 and OPA15_8, flanking *prsv-1* locus, were near but not on the resistance gene-coding region, as they did not co-locate with the resistant phenotype.

Markers linked to *prsv-1* have been used in the marker-assisted breeding programs described above because of their dominant inheritance, and because resistance to the Australian strain of PRSV-P imparted by *prsv-1* has been shown to be robust (Drew et al. 1998a; Magdalita et al. 1997). Five DNA markers, which were developed by use of RAF on bulked segregants of virus resistant and susceptible populations, were identified in the cross of *V. parviflora* x *V. pubescens* (Dillon et al. 2006b). The markers were mapped to the same linkage group, LG7, flanking prsv-1 at the distance of 2.1, 5.4, 9.7 and 12.0 cM for the marker Opa_16r, Opk4_1r, Opk4_2r and Opb8_1r, respectively, while the another marker, Opa11_5R co-located with prsv-1. The two

candidate markers, Opa11_5R and Opk4_1r, were sequenced and converted to SCAR markers. A SCAR marker, Opk4_1r, was converted into a CAPS marker, *Psilk4*, by digesting the amplicon with *Psi*1 and was shown to be diagnostic for the 3 alleles of *prsv-1*. The SCAR marker Opk4_1r detected similar band sizes for *V. pubescens, V. cauliflora* and *V. goudotiana* with the size of 360 bp, 360 bp and 361 bp, respectively. However, the amplicons of *V. parviflora*, *V. quercifolia* and *V. stipulata* were slightly larger with the size of 372 bp. The application of this SCAR and CAPS marker for marker assisted breeding was confirmed in research on interspecific populations of *V. pubescens* x *V. parviflora*; F_2 and F_3 populations produced from the *V. pubescens* and *V. parviflora* F_1; and, BC_1 and BC_2 generations when *V. pubescens* x *V. parviflora* F_3 RR plants were backcrossed to *V. parviflora* (O'Brien and Drew 2010). The Opk4_1r SCAR marker amplified in other *Vasconcellea* spp*. quercifolia, goudotiana,* and *cauliflora* however, the CAPS marker was not consistent in determining the allele of *prsv-1* in crosses involving *Vasconcellea* spp. other than *pubescens* and *parviflora* (O'Brien and Drew 2010).

Transgenic papayas, resistant to PRSV-P have been developed in many countries; and, DNA markers have been used to detect the transgenic plants. A detection protocol for characterisation of PRSV coat protein transgenic papaya lines was demonstrated by use of PCR (Fan et al. 2009). PCR patterns using primers designed from the left or right flanking DNA sequence of the transgene insert in transgenic papaya lines were specific and reproducible.

In addition to PRSV-P, papayas are susceptible to many other pathogens. However, there are few reports on the use of DNA markers to identify other disease genes. However, tolerance to *Phytophthora palmivora* in papaya was identified and molecular markers linked to this resistance were developed by use of AFLPs (Noorda-Nguyen et al. 2010). Several polymorphic bands linked with the tolerance trait in a F_2 population, derived from an F_1 of the most tolerant Hawaiian cultivar Kamiya, crossed with a highly susceptible cultivar SunUp, have been identified. These markers were further characterized to form sequence characterized amplified region markers. Nineteen genes were selected for gene expression analysis for resistance to *P. palmivora* (Porter et al. 2009b). Of these genes, a predicted peroxidase, β-1,3-glucanase, ferulate 5-hydroxylase and hypersensitive-induced response protein were pathogen upregulated, while a second peroxidase (Cp9) and aquaporin (Cp15) were downregulated.

1.1.3.4.2 Hybrid identification

To be able to identify hybrids at an early stage of plant growth is preferable in crop improvement studies, especially confirmation of F_1 hybrids in populations to facilitate further crossing. Seventeen RAPD primers were selected and screened to confirm hybridity of 120 putative interspecific cross of *C. papaya* x *C. cauliflora* (whereas, nowadays, they are intrageneric cross of *C. papaya* x *V. cauliflora*; Magdalita et al. 1997). A range of 1-5 primers consistently confirmed that all 120 plants were genetic hybrids. A single primer can not guarantee accurate results thus more than one marker is necessary to analyse for hybridity. This is because chromosome elimination can occur during meiosis thus absence of a single marker may represent elimination of part of a chromosome.

1.1.3.4.3 Fruit quality traits

Even though papaya genomic research is exceptionally advanced in many aspects, there are many other characteristics of papaya that need to be studied. Very little information on other characteristics of papaya has been studied in the past few years. One of the characteristics that need to be identified for papaya marketing is fruit flesh colour. The Australian papaya industry is clearly split between growers who grow either the yellow-fleshed (commonly described as Paw Paw) or the red-fleshed varieties (known as Papaya) or both types (Australian Papaya Industry Strategic Plan 2008-2012). A single major gene for yellow flesh that is dominant over red flesh color, has been found in a simple Mendelian segregation in flesh fruit colour of papaya by recognized papaya breeders (Blas et al. 2010). The carotenoid composition profiles of red- and yellow-fleshed Hawaiian Solo papayas showed a strong accumulation of lycopene in red-fleshed fruit, while none was detected in yellow flesh (Yamamoto 1964). The flesh colour locus was mapped near the end of LG7 and the two flanking markers were located at 3.4 and 3.7 cM, respectively (Ma et al. 2004). Recently, a high-density genetic map of papaya using SSR markers was established, fruit flesh colour was mapped at the end of LG5 and the closet marker was located at 13 cM (Chen et al. 2007). Blas et al. (2010) reported the cloning and characterisation of the papaya chromoplast-specific lycopene β-cyclase, *cpCYC-b*, and geneomic analysis of the surrounding region included a recombination hot spot in papaya. They found tomato chromoplast-specific lycopene β-cyclase (*CYC-b*) and SunUp *CpCYC-b*, shared 75% sequence identity over a 682-bp genomic sequence length. Quantitative RT-PCR analysis and subsequent functional analysis in bacteria confirmed the role of *CpCYC-b* in controlling fruit flesh color in papaya. The elevated expression of *CpCYC-b* and papaya β-carotene hydroxylase (*CpCHY-b*) between yellow-fleshed Kapoho and red-fleshed SunUp validated activation of the carotenoid biosynthesis pathway in yellow-fleshed papaya. The disruption of this pathway in red flesh varieties is caused by a frame-shift mutation induced by a 2-bp insertion (Blas et al. 2010). A PCR-based marker was developed the CPFC1 marker (Table 1.2), which is 530 bp away from *CpCYC-b*. The marker showed approximately 98% recombination frequency to flesh colour in 219 F_2 (KD3 x 2H94). It should be noted that this tightly linked marker, only 580 bp away from the target gene, is still not 100% accurate due to the extremely high recombination rate in this region of the genome.

Table 1.2: DNA markers for PRSV-P and other genetic traits in *Carica papaya*

Characteristics	Markers name	Primer sequence	Reference
PRSV-P coat protein region detection	Cp	F: GAGAAGTGGTATGAGGGAGTG R: CCATACCTGCCGTCACAATCA	Dillon et al. (2005)
PRSV-P resistance; *prsv-1* locus	Opa5_11R	PBA115R F: CAATCGCCGTAGGAAAATTC PBA115R R: CAATCGCCGTAGAGGAGGAGG	Dillon et al. (2006a)
PRSV-P resistance; *prsv-1* locus	Opk4_1r	PBK41R F: CCGCCCAAACTGCGGAACAC PBK41R R: CCGCCCAAACCCCCAACTAG	Dillon et al. (2006a)
Flesh colour; CpCYC-b	CPFC1	F: GACGTGTTAGTGTCCGACAA R: GACCAGGAAGCAAATTTTGTAA	Blas et al. (2012)

1.1.4 Quantitative trait loci in papaya

Many agronomically important traits, i.e. fruit size, fruit shape, flesh flavour and skin quality are quantitative traits that are influenced by multiple genes. Various quantitative trait loci (QTLs) have been identified in many crops; however, surprisingly not many QTLs have been identified in papaya breeding research. Recently, in 2012, a QTL analysis for papaya fruit size and shape has been reported (Blas et al. 2012). Fifty-four SSR markers, the morphological flesh colour locus and CPF1 and CPF2 SCAR markers were mapped to 11 LGs using a population of 219 F_2 plants (KD X 2H94). Fourteen QTLs having phenotypic effects ranging from 5 to 23% were identified across six linkage groups. These loci contain homologs to the tomato fruit QTL *ovate*, *sun* and *fw2.2* regulating fruit size and shape.

1.1.5 Potential and future

Papaya genomic research is exceptionally advanced in many aspects. There is a recent genetic map which correlates with the number of chromosomes. A physical map was analysed, a draft genome of the plant was sequenced and anchored on the genetic map, the genome was thoroughly analysed for simple sequence repeats and *NBS* gene families, and SSR libraries have been developed. Thus much basic information has been revealed in the past few years. However, there are many other characteristics of papaya that need to be studied and the molecular markers that have been identified, particularly the SSR markers, need to be linked to the many important traits of interest for MAS in papaya breeding.

Future papaya genomics approaches to develop more precise tools for trait selection are likely to involve the identification and mapping of candidate genes from the full genome sequence. These must then be tested for functional validation to the trait of interest, potentially through expression analysis. Meanwhile, with affordability becoming a reality, it is likely that expression of representative RNA sequences of the geneome will be analysed and functional genes isolated directly from the transcriptome in response to a particular target trait, such as disease resistance. We predict that in the very near future, that suits of expressed papaya genes and their predicted pathways will be commonly available for e-mapping and trait-associated marker-assisted selection.

1.2 Additional information from the previous reviews

1.2.1 *Vasconcellea quercifoia*

V. quercifolia was reported to be resistant to PRSV-P virus in Florida (Conover 1964), Hawaii (Manshardt and Wenslaff 1989) and Australia (Drew et al. 2006a; b). However, another report classified *V. quercifolia* as one of the susceptible species in Venezuela (Horovitz and Jimenez 1967). This reflects variation in resistance within the species and/or varying response to different PRSV-P strains. *V. quercifolia* was classified in clade 2 of *Vasconcellea* along with *V. chilensis*, *V. candicans* and *V. glandulosa* (d'Eeckenbrugge et al. 2014). This clade is the basal clade of genus *Vasconcellea* which means that they diverged early in evolution from other species of the genus. *V. quercifolia* was successfully crossed to papaya (Drew et al. 1998a; 2005; 2006a; 2006b; Manshardt and Wenslaff 1989; Siar 2011). It is proposed that this species is genetically less distant from *C. papaya* and hence the best option for intergeneric hybridisation with *C. papaya* (Siar et al. 2011).

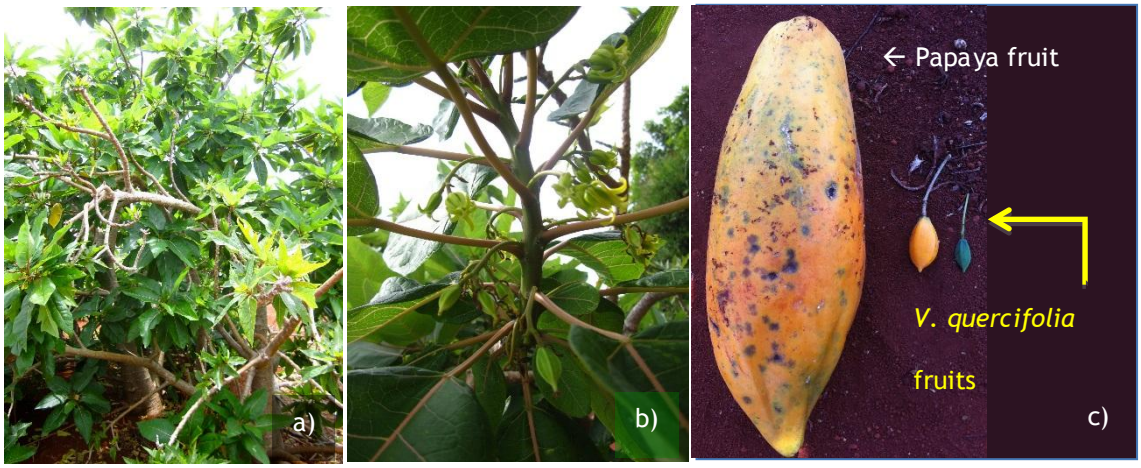

Figure 1.1: *V. quercifolia* a) tree, b) fruits and flowers and c) a comparison to a papaya fruit in Thornlands, Queensland

1.2.2 Papaya ringspot virus

Papaya ringspot virus (PRSV), a positive sense RNA, is a member of *Potyvirus*, the aphid-transmitted genus in the family *Potyviridae*. This virus can also be transmitted mechanically, and is typically not seed-transmitted. It is transmitted by many species of aphids (mainly *Myzus persicae* and *Aphis gossypii*) by sap sucking in a non-persistent manner.

The virus has and continues to be a destructive disease and a major biotic problem for papaya and cucurbit production worldwide (Purcifull et al. 1984). There are two types of PRSV based on host range infection; papaya-infecting type-P (PRSV-P) and non-papaya – infecting type-W (PRSV-W). PRSV-P isolates can infect plants in the family's Caricaceae, Cucurbitaceae and Chenopodiaceae, while the isolate of PRSV-W can infect plants in the families Cucurbitaceae and Chenopodiaceae. There was no significant difference between sequence of the coat protein gene for the Australian isolates, PRSV type P or W which suggested that PRSV-P could have arose from PRSV-W in Australia (Bateson et al. 1994).

The name of the disease, papaya ringspot, was taken from the ringed spots that develop on fruits and infected trees (Jensen 1949). In addition to the typical ringspots, PRSV infection produces a wide range of symptoms including leaf mosaic and chlorosis, water soaked oily streaks on the petiole and upper part of the trunk, distortion of young leaves, stunting of infected plants and flower abortion. Consequently, fruit production can be severely decreased and fruit quality can be reduced by decreased sugar concentration (Gonsalves 1998).

PRSV-P is considered an important threat to the Australian papaya industry although it has not yet occurred in the major growing region of North Queensland (Drew et al. 2006b). In 1991, the disease was detected in Australia for the first time in Southeast Queensland (Thomas and Dodman 1993), and could spread to North Queensland.

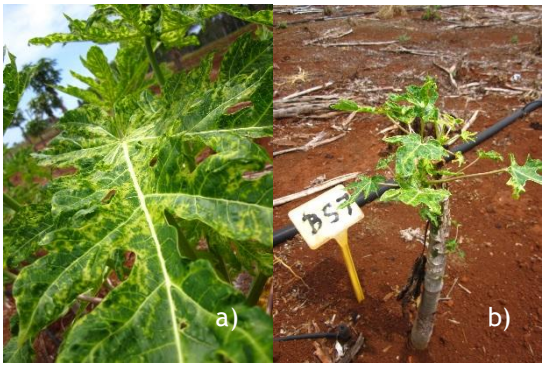

Figure 1.2: Papaya a) leaves and b) tree infected by PRSV-P in Thornlands, Queensland

1.2.3 Crop improvement for PRSV-P resistance in papaya

The sustainable method to control this disease in the papaya industry is to produce improved papaya varieties that are resistant to the pathogen. A transgenic papaya variety that was resistant to PRSV-P was successfully developed in 1990 (Fitch et al. 1990). The strategy is termed coat protein-mediated resistance and the first transgenic line was called 55-1. Transgenic papayas in other countries have been reported in Brazil for 'Sunrise' and 'Sunset' cultivars (Souza et al. 2005), Venezuela (Fermin et al. 2004), Jamaica (Cai et al. 1999), Taiwan (Bau et al. 2003; 2004; Kung et al. 2009) and Thailand (reviewed by Tripathi et al. 2008). These lines showed excellent PRSV-P resistance and horticultural characteristics. They had increased yields when compared to the non-transgenic controls that were infected with the virus.

Although GM is generally considered to be the best strategy for long-term virus control (Gonsalves 1998), they are not accepted in many countries (Tennant et al. 1994; 2001). Furthermore, transgenic resistant varieties can be virus-strain specific in their resistance (Nelson et al. 1988; Quemada et al. 1991; Sanders et al. 1992; Nakajima et al. 1993) as the virus and transgene must have more than 98% sequence homology for the technique to be effective (Gonsalves 1998). Therefore, conventional breeding for PRSV-P resistance in papaya is a viable option for long-term control of the disease. Reviews of breeding for PRSV-P resistance was covered in 1.1.

1.2.4 Molecular study of PRSV-P resistance genes in *V. pubescens*

Recent work was undertaken by Razean Haireen (2013) to further characterise PRSV-P resistance genes in *V. pubescens.* The marker Opa11_5r, which was shown to be linked to PRSV-P resistance in *V. pubescens* Dillon *et al.* (2006a), was sequenced, aligned to the NCBI database and shown to have similarity to a Kinase gene. Novel genes of VP_STK1, VP_STK2 and VP_LRR1 were characterized from *V. pubescens* (Razean Haireen, 2013). The full sequence of STK genes revealed one bp insertion/deletion in the coding region of *V. pubescens* and was predicted to be derived from an alternative splicing process. Gene expression supported the function of VP_STK2 in relation to resistance to PRSV-P in *V. pubescens* as it was up-regulated at 15 days after inoculation.

1.2.5 Resistance to plant virus

Resistance to disease of plants can be caused by the ability of the pathogen to infect a host (host and non-host resistance) or by physical/chemical response to pathogen (passive and active resistance). In plant viruses, host resistance has been investigated in detail because it is genetically accessible (Kang et al. 2005). The term host or specific resistance means a virus may or may not able to multiply to some extent, but the spread of viral particles is restricted relative to susceptible hosts. Active defense mechanisms are more related to resistance to a virus in a plant because typically the virus initiates infection by penetrating the plant cell wall through wounds either by mechanical abrasion or vectors such as insects (Goldbach et al. 2003; Kang et al. 2005)

Plant viruses have to undergo multiple steps to complete their life cycles. The processes include entry into plant cells, uncoating of nucleic acid, translation of viral proteins, replication of viral nucleic acid, assembly of progenies virions, cell-to-cell movement, systemic movement (long distance movement) and plant-to-plant movement (Carrington et al. 1996). Resistance can operate at four levels: inhibition of replication, cell-to-cell movement, long distance movement and defense responses restricting infection to a limited number of cells (Zaitlin and Hull 1987).

1.2.5.1 Type of resistance

Complete resistance to virus infection in plants is referred to as ***immunity*** (Bruening 2006). The immunity is usually a result of prevention of virus replication. If immunity occurs against all biotypes of a pathogen and in all cultivars or accessions of plant species, it is referred to as non-host resistance. The terms of ***extreme resistance*** (ER) or cellular resistance convey immunity (Fraser 1986; 1990). The most common mechanism associated with active defence is ***hypersensitive response*** (HR), which is a rapid development of cell death at and immediately surrounding infection sites (Morel and Dangl 1997). As a result, HR disrupts cell-to-cell movement of plant viruses and results in prevention of further spread of a virus (Kang et al. 2005). The HR and ER are referred to as a type of ***innate resistance*** where both are associated with a dominant resistance gene (reviewed by Gilliland et al. 2006; Loebenstein and Akad 2006). ***Tolerance*** is another level of resistance where plants may show mild or no symptoms. It is usually associated with a reduction of viral titre in the infected plants (Palukaitis and Carr 2008).

Because viruses are intercellular obligate parasites, they need the host cell to replicate themselves. Cell death or HR appears to be the best system to block multiplication of the viral particles. Fraser (1990) reported more than 65% of viral resistance genes were not associated with HR, but rather with the reduction of viral multiplication (Loebenstein and Akad 2006). Thus, HR is not the major resistance mechanism of a plant against viral infection (Morel and Dangl 1997). Local lesion infection was observed to be one of the most remarkable resistance responses of plant to virus and is used by breeders to obtain resistant cultivars. The resistance to PRSV-P in *V. pubescens* showed a sign of HR (Figure 1.3) in F_3 (RR), BC_2 (Rr) and BC_4 (Rr) plants in interspecific cross of *V. parviflora* (susceptible to PRSV-P) and *V. pubescens* (Razean Haireen 2013). However, self-limiting necrotic, chlorotic and ring-like patterns at local lesions has been described as localised acquired resistance rather HR (Loebenstein and Akad 2006).

Figure 1.3: A hypersensitive response showed rapid cell death and yellow spots in a BC_2F_3 plant of interspecific cross of *V. parviflora* and *V. pubescens* after inoculation with PRSV-P after 45 days (Picture from Razean Haireen, 2013)

1.2.5.2 Gene for gene interaction

The classic concept of "gene-for-gene" interaction has been used to explain resistance mechanisms in plants for the correspondence between plant a resistance gene (*R*) and a pathogen's avirulence gene (*Avr*) (Flor 1971). The model proposes the resistance reaction occurs when the complementary compatible of dominant *R* gene and *Avr* gene are present. Conversely, a loss or alteration to either the *R* gene or *Avr* gene leads to susceptibility to a disease (compatibility). This simple model has been used to explain many biotroph pathogens, including fungi, viruses, bacteria and nematodes (Crute and Pink 1996; Keen 1990).

1.2.5.3 Class of R genes

Despite the wide range of pathogens and their pathogenicity, *R* genes encode only five classes of protein (Dangl and Jones 2001). Figure 1.4 represents the five classes of proteins related to *R* genes. The largest class of R genes encodes a nucleotide-binding site plus leucine-rich repeat (NB-LRR) type of protein and all R genes conferring viral resistance have been identified in this class (Goldbach et al. 2003). In papaya, 54 NBS class resistance genes were identified (Porter et al. 2009a) and 61% of these had NBS domains homolog to *Vitis vinifera*. Of the 54 genes identified, 18 genes have only NBS domains, 23 genes have NBS-LRRs, seven genes show N-terminal TIR domains and six genes are predicted to encode CC motifs (Porter et al. 2009a).

LRR domains are also presented in the most class of R proteins. It shows function of protein-protein binding, peptide-ligand binding or protein-carbohydrate interaction (Jones and Jones 1996; Kajava 1998). *Cf* gene in tomato reported to be an extracellular receptor-like protein that involved in pathogen detection (reviewed by Dickinson 2003). Dixon et al. (1996) suggested LRR might involve in facilitating the interaction of *R* gene products with other proteins involved in defence signal transduction. The ARC domain in between the NBS and LRR domains has been identified to play a role in the recruitment of the LRR domain to the N-terminal region (Rairdan and Moffett 2006). LRR location is known to influence the timing of detection of the invading pathogen and affects the resistance response. This resulted in a variation in degrees of hypersensitive reactions and pathogen colonisation in different *R* gene/*Avr* gene-dependent interactions (Dickinson 2003).

Protein kinases play a central role for signalling transduction during pathogen recognition and the subsequent activation of plant defence mechanisms (Romeis et al. 2001). A

kinase gene may work together with an NBS-LRR gene by forming a molecular complex to detect more than one pathogenic organism. This resulted in initiation of defence response to multiple pathogens, which is known as a guarding mechanism (Jones and Dangl 2006). In papaya, fourteen of the flanking genes have significant similarity to the gene encoding kinases, including receptor kinases and kinases associated with LRRs (Porter et al., 2009a). Furthemore, Dillon et al. (2006c) described a marker, Opa11_5r collocated with the *prsv-1* resistance locus and is homologous to a serine/threonine protein kinase gene. Recently, two STK genes, VP_STK1 and VP_STK2 which were characterized from SC28.106 and SC28.105, showed relation to PRSV-P resistance in *V. pubescens* (Razean Haireen, 2013).

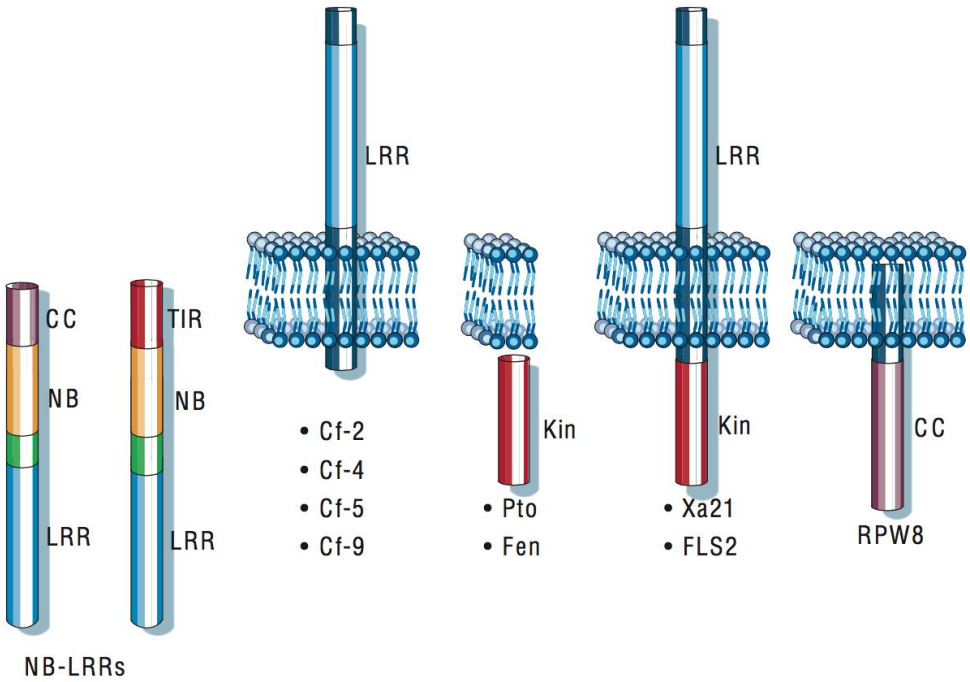

Figure 1.4: Representative functional domains of five main classes of disease resistance proteins (Dangl and Jones 2001)

1) NB-LRRs: are cytoplasmic and carry distinct N-terminal domain,
2) Cf-X proteins: extracellular LRR,
3) *Pto* gene: intracellular or cytoplasmic serine threonine kinase (STK)
4) Xa21: extracellular LRR and cytoplasmic protein kinase,
5) RPW8: putative signal anchor at the N terminus.

1.2.6 Productivity and fruit quality traits of papaya

In addition to disease resistance, papaya faces another problem of consumer acceptance, especially in the Australian market. This is mainly due to the unpleasant taste of papaya fruit that was sold on the Australian market. Consumer choice is generally influenced by appearance and sensory response to fruits. Therefore, improvement in eating and skin quality are the keys to increased consumption of papaya. Furthermore, production efficiency and high marketable yield are also important from growers' perspectives. Thus, breeding for these traits is essential.

Papaya is a polygamous species. A cross can populate either dioecious (either male or female) plants or gynodioecious (either female or hermaphrodite) plants. These forms exist due to human interference and deliberate selection against non-productive male trees (Storey 1969). The advantage of dioecious populations is the uniformity of fruit size, shape and appearance because female flowers do not display instability in sex expression of flowers compared to hermaphrodites (Storey 1976; Ying 2008). Papaya sex expression was complicated and influenced by environment variables including temperature, humidity, and soil nutrients which may modify functional gender of a plant when it flowers (OECD 2005). In hot and dry conditions (temperature greater than 35°C), hermaphrodite flowers became functional male with a poorly developed female reproductive system (Watson 1997; Nakasone and Paull 1998). Conversely, at low temperatures (less than 20°C) the flowers may become female only. The distortion in fruit shape suggested to cause by stamens resembled to carpels (OEDC 2005).

A papaya plant has a single stem which provides structural support under its leaf canopy. The stem is responsible for most of the rigidity, body mass, storage capacity, defence substances, height and competitive ability. It carries a bidirectional flow of water, nutrients, various organic compounds, and chemical and physical signals that regulate root and shoot relations (Reis et al. 2006). Diameters of mature plants could be related to yield (Francisco et al. 2007). Morton (1987) demonstrated that a wide and thick base of the tree mechanically supported the entire weight of the plant. Height to first flower indicated the potential ability of bearing fruit early and tolerance to fruit dropping (Anh 2011). O'Hare (1993) noted that ideally, plants should start fruit set at as low as possible, however, this can be achieved by maximising leaf growth in young trees, cultural practices and propagation techniques.

Several researchers reported the effect of environments toward phenotypic expressions that eventually affected in yield. Dioecious outcrossed varieties generally produced higher yields than gynodioecious varieties (Drew et al. 1998b; Chay-prove et al. 2000). Change of temperature caused hermaphrodite trees to reverse sex and reduced yields (OECD 2005). Papaya Seed Australia (2007) observed that carpelloid fruit were produced abnormally by the fusion of ovary and stamens during unfavourable weather conditions. It resulted in production of deformed and unmarketable fruit. Some varieties produce higher numbers of carpellodic fruit. 'Solo" varieties produced 100% carpellody fruits when minimum temperature was less than 17°C (Nakasone and Paull 1998). Yield and fruit quality varied by location, variety and season as well as agronomic practice (Elder et al. 2000a; 2000b). Age of tree also affected yield as trees yielded well for the first two years, but after that production declined (Benson and Poffley 1998).

Yield gap represents inconsistency of fruit production on a tree and a section of stem where

no fruit sets between two harvesting times. It varied considerably and was affected by cross-pollination and other factors such as environmental conditions, floral characteristics, and flower receptivity (OEDC 2005). Papaya fruit production in Australia varied in seasons due to several factors including low pollen viability and absence of suitable pollinators (Pollination Aware Report, 2008). Pollen of papaya can be produced all year round, but varied by seasons and varieties (Magdalita et al. 1998). In general, there was a trend of decrease in quantity of pollen during winter and early spring, while the receptivity of papaya stigmas remained high throughout the year even in winter (Garrett 1995). Ninety percent of freshly dispersed pollen grains were viable in winter, but some lines presented only 45% of pollen availability and it was as low as 4.5% in other lines. Allan (1963) reported that high humidity reduced the storage life of papaya pollen.

Fruit shape is a sex-linked characteristic. A female flower usually develops a round, spherical or ovoid fruit shape, whereas, a hermaphrodite flower develops a pyriform or elongate one. The standard of fruit size, shape and quality are dependent on market preferences which is different from country to country. Small fruits are more preferred in European, USA and Chinese markets, while the medium fruits are preferred in Malaysia. In Australia, yellow-fleshed papaya is preferred to be round female fruit from dioecious varieties and red-fleshed papaya is preferred from elongated hermaphrodite fruit (Ying 2008).

There are a number of papaya varieties that have been developed worldwide to meet a range of expectations including disease tolerance, improved fruit quality and yield, such as 'Solo' from Hawaii, 'Tainung' from Taiwan, 'Eksotika I, II and III' from Malaysia, Maradol from Cuba and Hortus Gold from South Africa (Fitch 2005; OECD 2005). In the Australian market, the majority of papaya fruit for commercial production were from varieties hybrid 1B (approximately 60%) and 'Sunrise Solo' (approximately 30%) (Hansen 2005). Even through these two varieties accounted for some desirable characteristics compared to other varieties available in the market, they both have some poor characteristics. Hybrid 1B fruit has good appearance, but lacks flavour, while 'Sunrise Solo' has good flavour, but low yields and the fruit has poor shape, thin flesh and blemished skin (Drew 2005). The current commercial varieties in North Queensland are Hybrid 1B and Hybrid 13 for yellow-flesh papaw; and Hybrid RB1, Hybrid RB2 and Hybrid RB4 for red-flesh papaya (Kath, personal communication).

Breeding programs to improve productivity and fruit quality traits have been attempted in many fruit crops during the last century. However, genetic improvement for productivity and fruit quality traits has some limitations from low diversity and unknown inheritability of traits. That led to insufficient genetic variability in gene pools for use in conventional breeding (Grandillo et al. 1999; Ulrich and Olbricht 2011). Moreover, fruit quality traits such as yield, size, shape, colour, texture, sweetness, flavour, appearance, and shelf life are complex because there are many factors involved including genetic and environmental factors. Some traits are controlled by multiple genes and express in relation to environmental factors (Fernandez-Trujillo 2011). Consequently, breeding for productivity and fruit quality requires improvement of breeding and the establishment of efficient methods for selection.

Several breeding methods and techniques have been employed to develop superior varieties for commercial production. There was a report of using cycles of random pollination to break linkage between high sugar content and susceptibility to winter spot (Hansen 2005). *In vitro* propagation to propagate promising emerging lines is ideal to speed up breeding programs. An efficient protocol for papaya micropropagation was published by Drew (1992). As the

result of advances in genome studies in papaya, molecular breeding is an alternative and effective way to improve papaya genotypes. Details of the development of molecular markers in papayas was reviewed by Kanchana-udomkan et al. (2014) and is presented in Section 1.1.

Appendix 2

Chapter 4 of Ph.D. thesis of Dr. Chat Kanchana-udomkan

This chapter provides complete experimental details of research associated with the breeding trial as described in this report

Chapter 4:

Development of Papaya Breeding Populations and Analysis of Productivity and Fruit Quality Traits and Phenotypes

INTRODUCTION:

Even though PRSV-P devastates the papaya industry worldwide and causes economic losses for producers, the most important character trait that affects demand by consumers is fruit quality. In Australia, the papaya industry is relatively small in comparison to other countries, and has an estimated yield of 10,000 -13,000 tons per year worth AUD18-25 million (Diczbalis et al. 2012). The industry has potential to expand. One of the biggest challenges for the industry is to produce a uniform cultivar with consistently good eating quality all year-round. The traditional flavour of the yellow fleshed papaya is an acquired taste that is less popular among the younger generation and red-fleshed varieties are gaining in popularity. Consumers in Australia have come to associate yellow flesh with round fruit and red flesh with elongate fruit. Therefore, a breeding program for papaya is needed to improve the quality of cultivated varieties within Australia.

Papaya is the only member of the *Carica* genus in the family Caricaceae (Badillo 2000). Its ploidy level is 2x, it is generally dioecious ensuring a high degree of heterogeneity, and can be inbred and cross-pollinated with relative ease making it subject to a range of plant improvement methods (Simmonds 1979). Papaya germplasm shows much phenotypic variation for many important traits (Kim et al. 2002). These include fruit quality and production traits such as yield, pattern of fruit production, peduncle length for ease of harvesting, fruit size, shape, flesh colour, flavour and sweetness.

There are few cultivars grown commercially in Australia, therefore, it is a necessity to explore papaya germplasm to evaluate and select potential parental lines for breeding programs. Most Australian papaya breeding efforts in the past have moved gene frequency for various traits with relative ease by selecting in segregating plant populations. The implication is that some traits of commercial importance are quantitatively inherited and controlled mainly by additive gene action with quite high heritability. Nevertheless, within a narrow base of cultivars grown in Queensland, traits do vary significantly between environments including different crop management practices (Elder et al. 2000a; 2000b; 2002), implying heritability may be lower than thought. The main purpose of research in this chapter was to evaluate different lines of papaya for fruit quality and productivity traits, and then to select good trees to be parents in a breeding program with commercial varieties to improve fruit quality and tree productivity in a major producing region. In addition, other parent trees were selected and crossed so that DNA markers could later be identified to facilitate marker assisted breeding.

After consulting with reference groups of papaya growers in north Queensland, important traits that must be considered in applied plant breeding were identified. They include those relating to a tree's productivity: total yield, consistent set of fruit over time (i.e. no yield gap), consistent fruit size, ease of harvest and disease resistance and also to fruit quality traits: appearance and appeal, shape, flesh colour, sweetness and flavour. There is wide range in these traits observed in germplasm. In fact, most of these traits are quantitatively assessed (IBPGR 1988). Therefore

it is important to applied papaya breeding that proper assessment be made of the variability in commercially acceptable papaya in an Australian production area (north Queensland), and to produce segregating populations of plants by controlled plant breeding. Some of the many traits may be correlated. If so, fewer time-consuming assessments of trees might be possible for a population of segregating plants. This is vitally important to applied plant breeding with inherent high costs of land and labour.

RESEARCH AIMS:

1. Identify important productivity and fruit quality traits to be improved by applied payaya breeding.

2. Describe the phenotypes 27 papaya lines and choose lines and plants within lines for use as parents in a breeding program in north Queensland.

3. Establish breeding and segregating populations of plants for selecting the required traits for both commercial acceptability and DNA-marker analysis.

MATERIALS AND METHODS:

4.0 Overviews

Lines of papaya were grown for evaluation leading to selection of parental lines for two main purposes; firstly to improve eating-quality in commercial varieties and secondly to establish segregating populations to use for identifying DNA markers for other traits. The steps of work for this chapter are outlined graphically in Figure 4.0.

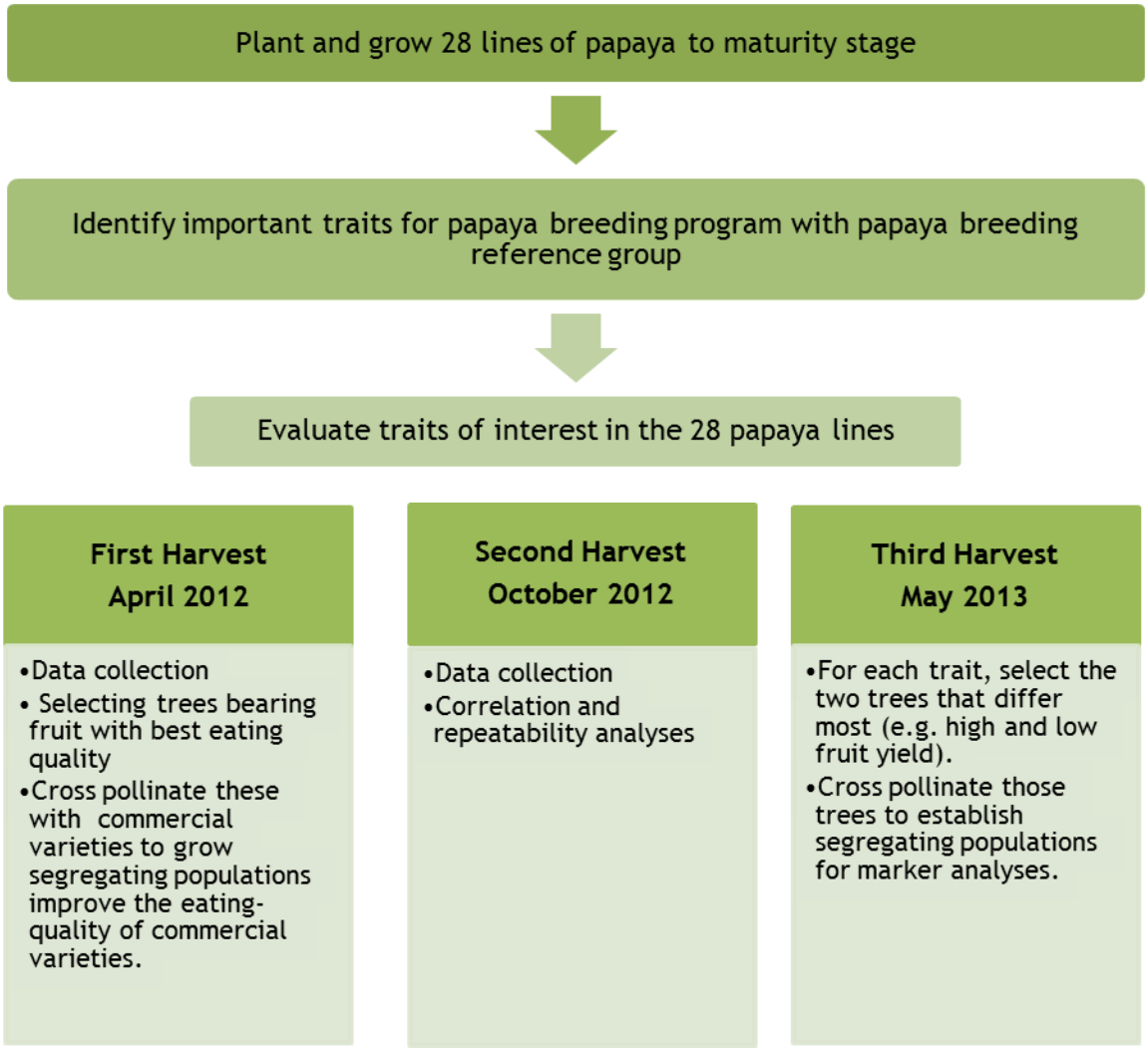

Figure 4.0: Steps of work for developing papaya breeding population and analysis of productivity and fruit quality traits and phenotypes

4.1 Plant materials

Twenty-eight different lines of papaya were obtained from three sources: Australian commercial varieties, crosses between Australian commercial varieties and selected lines from Department of Agriculture, Fisheries and Forestry, Queensland (QDAFF), and from collections held in Queensland but originally from Hawaii, Malaysia, Vietnam and Thailand. The latter collections are in the possession of Professor Rod Drew, Griffith University. Crosses between commercial varieties and QDAFF lines were made by Narenda Singh (QDAFF) in the industry-funded breeding project in 2010. The 28 lines include yellow- and red-fleshed papaya types and are detailed in Table 4.1. The trees were planted on a property owned by Lecker Farming, Mareeba, Queensland (latitude -16.96, longitude 145.34, average rainfall 918 mm/year).

4.2 Papaya seed preparation

Seeds of crosses between Australian commercial varieties and selected lines from DAFFQ were extracted from ripe fruits following cross-pollination. The fruits were cut in half and the seeds were collected then washed under tap water. The sarcotesta, which is the clear membrane covering the seed, was removed by gently rubbing the seeds between hessian fabrics. The seeds were then washed under running tap water eight times and placed on a sieve tray to dry in a cabinet that controlled temperature at 15ºC and relative humidity at 15%. Seeds of collections from Professor Rod Drew, which had been kept for more than five years, were first soaked in 2 mM gibberellic acid solution (Fermoz) for 15 minutes and then rinsed with tap water before sowing.

One hundred seeds of each line were sown in February 2011 in seed-raising mix (Searly, Australia) in 48-cell seedling trays. Two or three seeds per cell were placed on the mix before lightly covering with additional mix. Trays were held in a shade-house and were watered and treated according to the farm management practice at Lecker Farming in order to produce healthy seedlings ready for field-planting. Seedlings were field-planted three months after sowing. The number of seedlings field-planted per line of all but one line varied from 10 to 100 and reflected germination percentages.

Table 4.1: Identity, ancestry and source of 28 papaya lines planted at Lecker Farming, Mareeba, Queensland

Line number[1/]	Ancestry or variety name[2/]	Source of seed
R01	RB1 x 18-45	Cross of commercial red papaya RB1 and DAFFQ line #18-45
R02	RB1 x 24-29	Cross of commercial red papaya RB1 and DAFFQ line #24-29
R04	RB2 x 18-45	Cross of commercial red papaya RB2 and DAFFQ line #18-45
R06	RB2 x 25-5	Cross of commercial red papaya RB2 and DAFFQ line #25-5
R09	25-5 x RB1	Cross of DAFFQ line #25-5 and commercial red papaya RB1
Y11	24-29 x RB2	Cross of DAFFQ line #24-29 and commercial red papaya RB2
Y15	1B x 33-66	Cross of commercial yellow papaya 1B and DAFFQ line #33-66
Y16	7-82 x 1B	Cross of DAFFQ line #7-82 and commercial yellow papaya 1B
Y17	24-87 x 1B	Cross of DAFFQ line #24-87 and a commercial yellow papaya 1B
R19	24-29 Self	Self pollination of DAFFQ line #24-29
Y20	JC2	[3/]
R21	25-5 Self	Self pollination of a DAFFQ line #25-5
R22	TS2	[3/]
R23	Malaysian Red 1	[3/]
R24	Malaysian Red 2	[3/]
R25	Malaysian Red 3	[3/]
Y26	1B	A commercial yellow papaya
R27	RB2	A commercial red papaya
R28	RB4	A commercial red papaya
R29	Sunrise Solo	[3/]; it is a commercial red papaya in Hawaii
R30	Solo Linda	[3/]
R31	RD6 Self	[3/]
R33	Brazilian Solo	[3/]
Y34	2.54-14 self	[3/]
Y35	2.54-12 self	[3/]
R41	JC2 x Vietnam Red	[3/]
R42	TS2 Self	[3/]
R48	Red Lady, Taiwan	[3/]

[1/] The prefix R refers to red fleshed fruit; Y to yellow

[2/] Female parent is noted first in each cross.

[3/] From a collection of seed held by Professor Rod Drew, Griffith University, Brisbane, Queensland

Only one seed of R48 (Red Lady) germinated, therefore, this line has only one plant in the field. Trees were planted at 3.20 m intervals in rows 1.75 m apart and were treated according to the maintenance program of Lecker Farming. Fertiliser was applied by addition of nutrients to drip irrigation and trees were regularly and routinely treated with fungicides. Weeds were controlled by herbicide application according to standard farm management practice. Replicating plots of trees of the lines was not used.

4.3 Evaluation of traits of interest

Important commercial traits were identified during discussions with local commercial papaya producers. Traits were grouped into those relating to tree productivity and those relating to fruit quality.

Ten fruit-bearing trees of each line were selected at random to evaluate traits. They were evaluated at three different harvesting times, April 2012, October 2012 and May 2013, to confirm that the data represented the genetics of the trees rather than environmental effects.

4.3.1 Productivity traits

Nine productivity traits that related to performance of the trees were identified and recorded. They were:

i. Sex type: dioecious (male and female flowers on separate trees) or gynodioecious (hermaphrodite flowers on the same tree).

ii. Height to the first flower was measured in centimetres from the ground.

iii. Height to the first mature fruit was measured at harvest in centimetres from the ground.

iv. Height to the first marketable fruit was measured in centimetres from the ground. Number of side shoots was measured by counting on each tree before the first fruit was harvested.

v. Peduncle length was recorded using a 1,3 and 5 rating scale; where

1 = short (estimation of the length less than 3 cm),

3 = medium (estimation of the length between 3 and 5 cm) and

5 = long (estimation of the length greater than 5 cm).

Figure 4.1: Measuring length of fruit peduncle

vi. Yield of fruit of saleable quality was estimated by an experienced grower at each harvest time. The total number of marketable fruits per tree were counted. This number of fruits covered the duration of six months harvest time (April to October 2012, approximately). Therefore, this saleable yield was the estimation of tree production over the entire time. Number of fruit per cartons and weight of marketable fruit per carton was estimated. Saleable yield for each tree was calculated using Formula 4.1.

$$\text{Saleable yield (kg)} = \frac{\text{Number of fruits per tree}}{\text{Number of fruit per carton}} \times \text{weight per carton (kg)}$$

Formula 4.1: Equation used to calculate yield of saleable fruit

vii. Yield gap, which was a space on a tree where fruit were not produced between two harvesting times, was rated between 1 and 9; where

1 = no gap was observed between two harvesting times
3 = less than 20% of space of fruit set was observed between two harvesting times
5 = less than 40% of space of fruit set was observed between two harvesting times
7 = less than 60% of space of fruit set was observed between two harvesting times
9 = greater than 60% of space of fruit set was observed between two harvesting times

viii. Number of carpelliod fruits was counted for each tree.

4.3.2 Fruit quality traits

Thirteen traits that related to fruit quality were identified and recorded. One fruit at similar fruit harvesting stage of each tree was evaluated. Most traits were evaluated by using the standards of the International Board for Plant Genetic Resources (IBPGR 1988) as detailed below.

i. **Fruit shape:** Fruits from each tree were scored in numeric system as detailed in Figure 4.2.

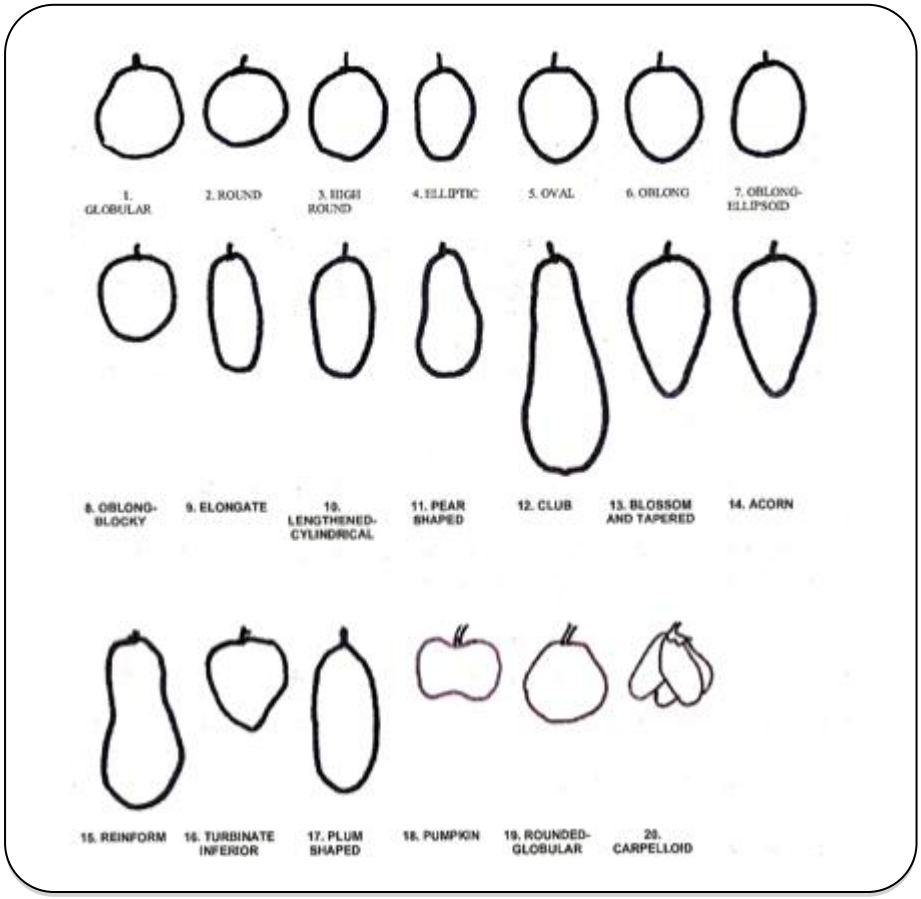

Figure 4.2: Fruit shape in papaya (IBPGR 1988)

ii. **Teat shape:** Teat of each fruit was scored in numeric system as detailed in Figure 4.3.

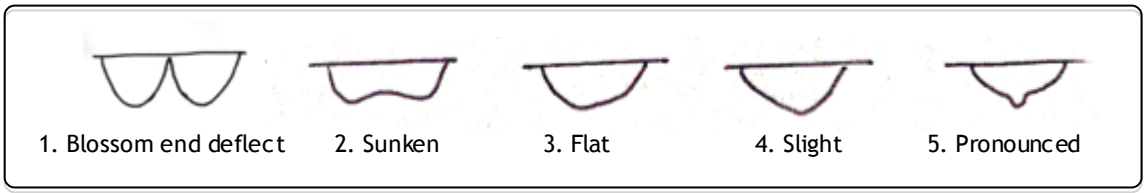

Figure 4.3: Teat shape in papaya (modified from IBPGR 1988)

iii. **Stalk insertion:** The insertion of stalk of each fruit was scored in a numeric system as detailed in Figure 4.4.

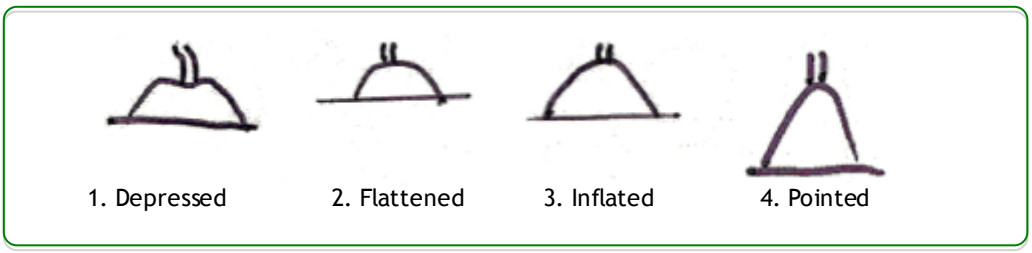

Figure 4.4: Stalk insertion in papaya (IBPGR 1988)

iv. **Skin quality:** Skin quality was scored using a rating system of 1 to 4 where

1 = poor, 2 = average, 3 = good, and 4 = excellent skin quality

v. **Skin freckle:** Skin freckle, which is skin blemish occurring on ripe fruit but not related to disease (Eloisa et al. 1994), were observed on mature fruits at the ripe full colour stage. The severity of skin freckle is recorded using a rating system of 0 to 4 rating as detailed in Figure 4.5.

Figure 4.5: Rating scale 0 to 4 for skin freckle in papaya; where 0 = skin freckle cover less than 1% of the surface, 1 = skin freckle cover 1% to 15%, 2 = skin freckle cover 16% to 30%, 3 = skin freckle cover 31% to 50%, and 4 = skin freckle cover more than 50%

vi. **Skin colour:** Skin colour was visually observed and recorded in a numeric rating system: 1 = Yellow, 3 = Yellow/Orange, 5 = Orange, 7 = Orange/Red and 9 = Red. All fruit rated 3 or less were phenotypically classed yellow, fruit rated >3 were classed red.

vii. **Cavity shape:** Each fruit was cross-sectioned laterally. The central cavity of each fruit was scored as detailed in Figure 4.6.

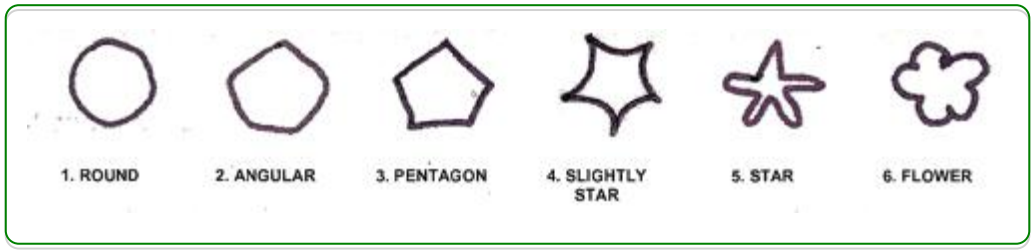

Figure 4.6: Shape of the central cavity of papaya fruit (IBPGR 1988)

viii. **Consistency in flesh colour**: Each fruit was cross-sectioned laterally in half and scored for consistency of flesh colour as detailed in Figure 4.7.

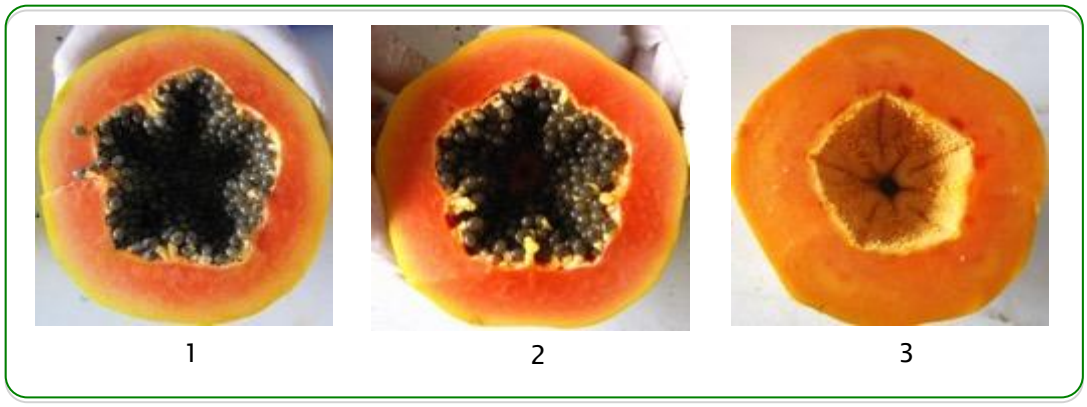

Figure 4.7: Numeric rating scale for consistency of flesh colour; where

1 = more than 50% colour inconsistency,
2 = colour inconsistency is between 50-75%, and
3 = flesh colour is 100% consistent

ix. **Flesh colour:** Each fruit was cross-sectioned in half laterally and scored for flesh colour as 1 = Yellow, 3 = Yellow/Orange, 5 = Orange, 7 = Orange/Red and 9 = Red.

Two flesh colours, yellow and red, were distinguished by using a cut-off value with scores of three and less representing for yellow flesh and above three for red flesh. Even though flesh colour is controlled by only a single dominant gene, quantitative expression can still be detected.

x. **Flesh firmness:** Flesh firmness was rated: 1, 3 or 5; where

1 = Soft, 3 = Intermediate and 5 = Firm.

xi. **Useable flesh thickness:** Flesh thickness was measured in millimetres from the skin to the seed cavity; one measurement per fruit.

xii. **Flesh sweetness:** Total soluble solids (TSS) were measured on ripe fruits by using a hand held refractometer. The measurement was recorded in ºT scale.

xiii. **Fruit flavour:** Each fruit was tasted at the ripe fruit stage. The details are described in Table 4.2.

Table 4.2: Papaya rating system for flesh flavour

Flavour rating system	Descriptions				
Type of flavour	Flavourless	Nasturtium	Solo	Khagdum	Musk
Overall taste	1 = Poor	2 = Average	3 = Good	4 = Excellent	
Strength of flavour	1 = Weak	2 = Intermediate	3 = Strong		

4.4 Data analysis

Analysis of Variance (ANOVA) was applied to data of the three harvests by use of XLSTAT software (Addinsoft, 2015). The harvest times were used as a fixed variable in ANOVA analysis. Duncan's multiple range test was used to calculate significant differences ($P<0.05$) between means.

Repeatability estimates (Falconer 1960) were calculated for each trait using the data from the first two harvests (Formula 4.2). If the data is consistent between assessments, it may not be a necessity to do more than one evaluation.

$$r = \frac{S_A^2}{S^2 + S_A^2} \qquad \textbf{Formula 4.2}$$

Where: r is repeatability, S^2_A is the between-groups variance component and S^2 is the within-group variance component.

These variance components are calculated from the mean squares in the analysis of variance using Formula 4.3 and Formula 4.4. Mean squares were calculated by the one-way analysis of variance in Microsoft Excel software.

$$S^2 = MS_{\text{within group}} \qquad \textbf{Formula 4.3}$$

$$S^2_A = \frac{MS_{\text{between group}} - MS_{\text{within group}}}{n_0} \qquad \textbf{Formula 4.4}$$

Where n_0 is a coefficient related to the sample size per group in the analysis of variance and in this case is equal to the group size = 2.

Correlation analysis was computed by XLSTAT software (Addinsoft, 2015) using data from first and second harvest. Clones were used as a fixed variable in ANOVA. Duncan's multiple

range test was used to calculate significant differences ($P<0.05$) between means.

4.5 Development of breeding populations to improve flesh flavour for commercial papayas

Fruits from each tree were tasted and scored according to Table 4.2 in April 2012. Trees that represented outstanding flavour and other eating quality traits were selected based on the result in the first harvest due to time limitation and the commitment to the project fund to produce crosses as soon as possible. Crosses were made from these selected trees to red and yellow fruited commercial papayas. Nevertheless, flavour characteristics of fruit from the selected trees were measured at all three harvesting times.

4.5.1 Controlled self- and cross-pollination

To self-pollinate hermaphrodite flowers, mature but tightly closed flowers were bagged with a paper bag to prevent out-crossing from other plants. The self-pollinated flower was labelled to record the variety and date of bagging.

Cross-pollination of selected trees was done by using either female or hermaphrodite flowers for the female parent, and either male or hermaphrodite flowers for the male parent. The tips of female flowers were carefully opened and the pollen from a male or a hermaphrodite parent was placed on stigmas. Hermaphrodite flowers were emasculated before pollen maturity. After three days, pollen from a selected male parent was placed on the stigma of the emasculated, hermaphrodite flower. Pollinated flowers were covered with cotton wool and bagged to prevent out-crossing from other plants. They were labelled as described above. The procedures of cross-pollination are detailed in Figure 4.8. The paper bag was removed when the fruit was set approximately 2 to 3 weeks after pollination. The fruit was tagged and covered with an orange mesh bag. It was then harvested at the maturing stage of 75% colour change, which was approximately 4 to 5 months after pollination, the seeds extracted and germinated to produce the second population of plants for selection.

4.6 Development of gene-mapping populations for papaya tree and fruit quality traits

Data from three harvesting times were used to calculate means of each trait for each line and to identify trees to be used as parents in cross-pollination. Only trees consistent in their traits across harvest times and which represented the widest variation in particular traits were selected for crossing. Trees were selected first on flesh colour (yellow and red). Crosses were made (detailed 4.5.1) between trees with poor and good traits in both yellow and red-fleshed types (Table 4.3). Reciprocal crosses were made where possible.

Figure 4.8: Cross-pollination process of papaya

i: A closed female flower at mature stage was selected. ii: The tip of the female flower was cut. iii: Petals of a selected male flower were peeled and anthesis was checked. iv: Pollen was gently tapped and brushed on the stigma. v: The flower of female parent was covered to protect it from out crossing by using cotton wool. vi: The female flower was covered again by using a paper bag and it was tagged using an aluminium tag which was recorded female and male parents, and date of pollination

Table 4.3: Traits selected for cross-pollination to produce hybrids for segregating populations for DNA marker analysis

Trait[1/]	Criteria to select parental lines for crossing for marker DNA analysis	
	Poor trait	**Good trait**
Fruit flavour	No Flavour	Solo
	No Flavour	Nasturtium
	No Flavour	Musk
	No Flavour	Khag Dum
Skin quality	Poor	Excellent
Skin colour	Light	Deep
Number of side shoots	High	Low
Height to first fruit	High	Low
Number of carpelloid fruit	High	Low
Peduncle length	Short	Long
Yield gap	High	Low
Flesh colour	Light	Deep
Flesh firmness	Soft	Firm
Flesh Thickness	Thin	Thick
TSS	Low	High
Flesh colour consistency	Inconsistent	Consistent

[1/] **Note:** Trees that had the required tree criteria of skin quality, skin colour, number of side shoots, height to first fruit, number of carpelloid fruit, peduncle length and yield gap were selected for crossing regardless of whether fruit were red- or yellow-fleshed.

However, trees with extremes of fruit quality traits (flesh colour, firmness, thickness, TSS and consistent flesh colour) were selected for each of red-and yellow-coloured fruit. Crosses were then made within each of the two fruit flesh colours.

RESULTS:

4.7 Difference of traits between harvest seasons

Means of maximum and minimum of temperature in Mareeba between 2011 and 2013 were obtained from Australian Bureau of Meteorology and show in Table 4.4. The means for each trait in each harvest time averaged across lines are presented in Table 4.5. They differed significantly between harvesting times for most traits, except numbers of carpelloid fruit that did not differ significantly in all harvest times. Yield of marketable fruit, peduncle length and flesh thickness were significantly less in the third harvest (May 2013) compared with the first two harvests, which did not differ significantly.

Table 4.4: Mean minimum and maximum temperature (°C) in Mareeba from 2011 to 2013 (Data from Australian Bureau of Meteorology)

Statistics	2011		2012		2013	
	Min[1/]	Max[2/]	Min[1/]	Max[2/]	Min[1/]	Max[2/]
January	21.2	30.2	21	31.4	21.2	32.2
February	21	29.5	21.4	30.7	21.3	31.9
March	21.2	29.4	21	29	20.8	29.8
April	19	28	18.2	28.5	18.8	28.6
May	14.9	26.4	17.1	25.9	17.8	26.6
June	12.6	25	13.6	25.2	15.6	25.9
July	12.9	24.5	14.5	24.4	15.1	24.7
August	13	25.9	13.3	25.8	12.8	27.1
September	14.6	28.2	15.2	28.3	15.1	29.4
October	17.5	31	16.8	30	17.5	31.1
November	18.8	30.4	18.5	31.4	19.9	31.2
December	21.3	32	20	33.1	19.3	31.5
Annual	17.3	28.4	17.6	28.6	17.9	29.2

[1/] Mean minimum temperature (°C)

[2/] Mean maximum temperature (°C)

Traits related to skin quality and flesh firmness trended lower over time. Skin quality and flesh firmness were rated highest at the first harvest (April 2012), while TSS and consistency of flesh colour were highest in the last harvest (May 2013). Fruits from winter (October 2012 harvest) had the highest score of skin freckle, while flesh colour rating was lowest in winter fruit compared with the other two harvests.

Table 4.5: Means of fruit productivity and quality traits at each harvest

Trait	Harvest time		
	April 2012	October 2012	May 2013
Peduncle length rating	2.69 a	2.80 a	2.21 b
Number of carpelloid fruit	2.55 a	2.33 a	2.01 a
Yield of marketable fruit (kg; estimated)	35.48 a	36.62 a	27.41 b
Skin quality	2.49 a	2.28 b	2.07 c
Skin freckle	1.59 b	2.36 a	1.04 c
Flesh colour	5.36 a	4.45 c	4.73 b
Flesh colour consistency	2.08 c	2.41 b	2.53 a
Flesh firmness	3.82 a	3.03 b	2.29 c
Flesh thickness (mm)	18.32 a	18.93 a	15.70 b
Total soluble solid (% Brix)	11.02 c	11.42 b	12.13 a

* Means followed by the same letter in each row are not significantly different (P>0.05)

In general, yield means over the first two harvest times were not significantly different, but a 25% reduction in yield was observed in the third harvest (P<0.0001).

After the second harvest in October 2012, some lines were culled as a result of poor performance of their trees in the field and they were not used in further breeding program. These lines were Y34 and Y35 in the yellow lines and R19, R21, R22, R23 and R30 for the red lines.

4.8 Repeatability of traits related to fruit

Flesh firmness showed the most consistent among all the traits in the two harvest times (repeatability of 0.69; Table 4.6). In general, traits related to productivity (yield, number of carpelloid fruits, peduncle length and yield gap) were relatively consistent with the repeatability between 0.23 and 0.34. High repeatability was also relatively high for fruit TSS (0.29). Other fruit quality traits (fruit shape, teat shape, consistency in flesh colour and flesh thickness had repeatability lower than 0.2. There were high P-values (> 0.05) associated with repeatability of stalk insertion, skin quality, skin freckle, skin colour, cavity shape and flesh firmness ratings. Repeatabilities of those traits were low because the variance among lines was small relative to within-lines.

Table 4.6: Repeatability of all traits between the first and the second harvest time in April and October 2012

Traits	Repeatability	P-Value
Yield	0.37	9.33E-13
Number of carpelloid fruits	0.26	6.41E-07
Peduncle length	0.23	8.54E-06
Yield gap	0.34	1.14E-07
Fruit shape	0.17	1.44E-03
Teat shape	0.14	7.60E-03
Stalk insertion	-0.11	0.9788
Skin quality	-0.03	0.7257
Skin freckle	-0.03	0.6751
Skin colour	-0.15	0.9965
Cavity shape	0.08	0.0697
Consistency in flesh colour	0.18	8.53E-04
Flesh colour	0.62	4.77E-33
Flesh firmness	-0.07	0.8884
Flesh thickness	0.16	2.09E-03
TSS	0.29	9.89E-08

4.9 Correlation analysis among traits related to fruit

Most traits showed moderate to low repeatability, so correlations between traits were calculated for each of two harvest times (Tables 4.7 and 4.8). Correlations between traits in most scenarios were significantly ($P < 0.05$) but at a low level (r^2 less than 0.5). The highest correlations were between skin freckle and skin quality (r^2 -0.543 and -0.522 in the first and second harvest, respectively); that is, the higher the rating for skin quality the lower the rating for skin freckle. Flesh colour in both harvests was comparatively highly related to skin colour and consistency in flesh colour ($r^2 > 0.3$); that is, the redder the flesh, the lower the rating for flesh colour consistency but the higher the rating for skin colour. Data from the second harvest revealed yield was then more highly correlated with number of carpelloid fruits, peduncle length and yield gap than at the first harvest.

Table 4.7: Correlation matrix (r^2 values) of Pearson's correlation coefficient of the first harvest in April 2012

Variables	Number of Carpelloid fruits	Peduncle length	Yield gap	Skin quality	Skin freckle	Skin colour	Consistent in flesh colour	Flesh colour	Flesh firmness	Flesh thickness	TSS
Yield	**-0.227**	**0.176**	**-0.136**	-0.012	-0.005	-0.008	-0.053	0.099	0.032	**0.211**	**-0.204**
Number of carpelloid fruit	1	**0.121**	0.001	-0.063	0.104	**0.267**	-0.064	0.105	**-0.110**	**-0.300**	**0.214**
Peduncle Length		1	-0.062	0.033	-0.023	0.059	0.044	**-0.158**	0.055	0.106	-0.022
Yield gap			1	0.060	**-0.119**	**-0.119**	0.040	-0.107	0.053	**0.144**	-0.037
Skin quality				1	**-0.543**	0.040	-0.039	**-0.140**	0.091	0.054	0.049
Skin freckle					1	0.098	0.048	**0.117**	-0.060	0.019	0.090
Skin colour						1	-0.051	**0.353**	-0.081	**-0.123**	**0.267**
Consistent in flesh colour							1	**-0.339**	**-0.108**	**0.159**	0.009
Flesh colour								1	-0.023	**-0.210**	**0.227**
Flesh firmness									1	**0.203**	**-0.130**
Flesh thickness										1	**-0.287**

Note: Values in bold are different from 0 with a significance level 0.05 (P > 0.05)

Table 4.8: Correlation matrix (r^2 values) of Pearson's correlation coefficient of the second harvest in October 2012

Variables	Number of Carpelloid fruits	Peduncle length	Yield gap	Skin quality	Skin freckle	Skin colour	Consistent in flesh colour	Flesh colour	Flesh firmness	Flesh thickness	TSS
Yield	**-0.315**	**0.344**	**-0.381**	**0.217**	**-0.185**	-0.004	-0.048	-0.110	-0.038	-0.038	**-0.230**
Number of carpelloid fruit	1	0.022	-0.008	-0.062	-0.021	0.057	0.015	0.042	**0.163**	**-0.162**	0.097
Peduncle Length		1	-0.050	**0.258**	**-0.225**	-0.107	0.065	**-0.317**	**0.256**	0.096	**-0.260**
Yield gap			1	-0.092	0.099	0.046	-0.120	0.051	0.031	**0.204**	**-0.191**
Skin quality				1	**-0.522**	0.035	-0.085	-0.025	**0.131**	**0.256**	**-0.147**
Skin freckle					1	0.011	0.023	**0.190**	-0.100	**0.175**	0.015
Skin colour						1	**0.119**	**0.321**	0.054	-0.023	**0.269**
Consistent in flesh colour							1	**-0.318**	0.056	**-0.150**	**0.200**
Flesh colour								1	-0.043	-0.030	**0.201**
Flesh firmness									1	**0.182**	**-0.182**
Flesh thickness										1	**-0.241**

Note: Values in bold are different from 0 with a significance level 0.05 (P > 0.05)

4.10 Analysis of tree productivity traits

The information on sex type, which can be found in Appendix 2, was recorded for future reference when selecting parental lines. Heights to the first flower, first fruit and first marketable fruit were highly, positively correlated (r^2 >0.65, p<0.001; Figure 4.9). The scatter plots of individual values and coefficients of determination (R^2) are detailed in Figure 4.9.

Mean values of each tree productivity trait of each line are presented in Table 4.9. Number of side shoots varied from zero to more than 20 (Figure 4.10). In general, yellow papaya had fewer side shoots than the red lines. The line with fewest side shoots was 1B, a commercial yellow papaya (Y26, Table 4.9), while the highest number was recorded in Solo type papayas (all red-fleshed): Solo Linda (R30), Sunrise Solo (R29), and Brazilian Solo (R33). Peduncle length varied from short to long (Figure 4.11); the result suggested the older the tree, the shorter the peduncle.

Total saleable yield of yellow lines was, in general, higher than that of red line (Figure 4.12). Yield of marketable fruits of yellow lines differed insignificantly over the three harvesting times, while that of most red papayas decreased significantly over time.

The yield gap ratings were between 10% and 60% of the space available for fruit set on the tree (Figure 4.13). The result was regardless of flesh colour.

The highest numbers of carpelloid fruits was observed in R22 (24.5 fruits per tree) and R41 (9.3 fruits per tree). The difference between number in R22 and all other lines was highly significant ($p < 0.0001$). All other lines had none to three carpelloid fruit per tree and their means did not differ significantly.

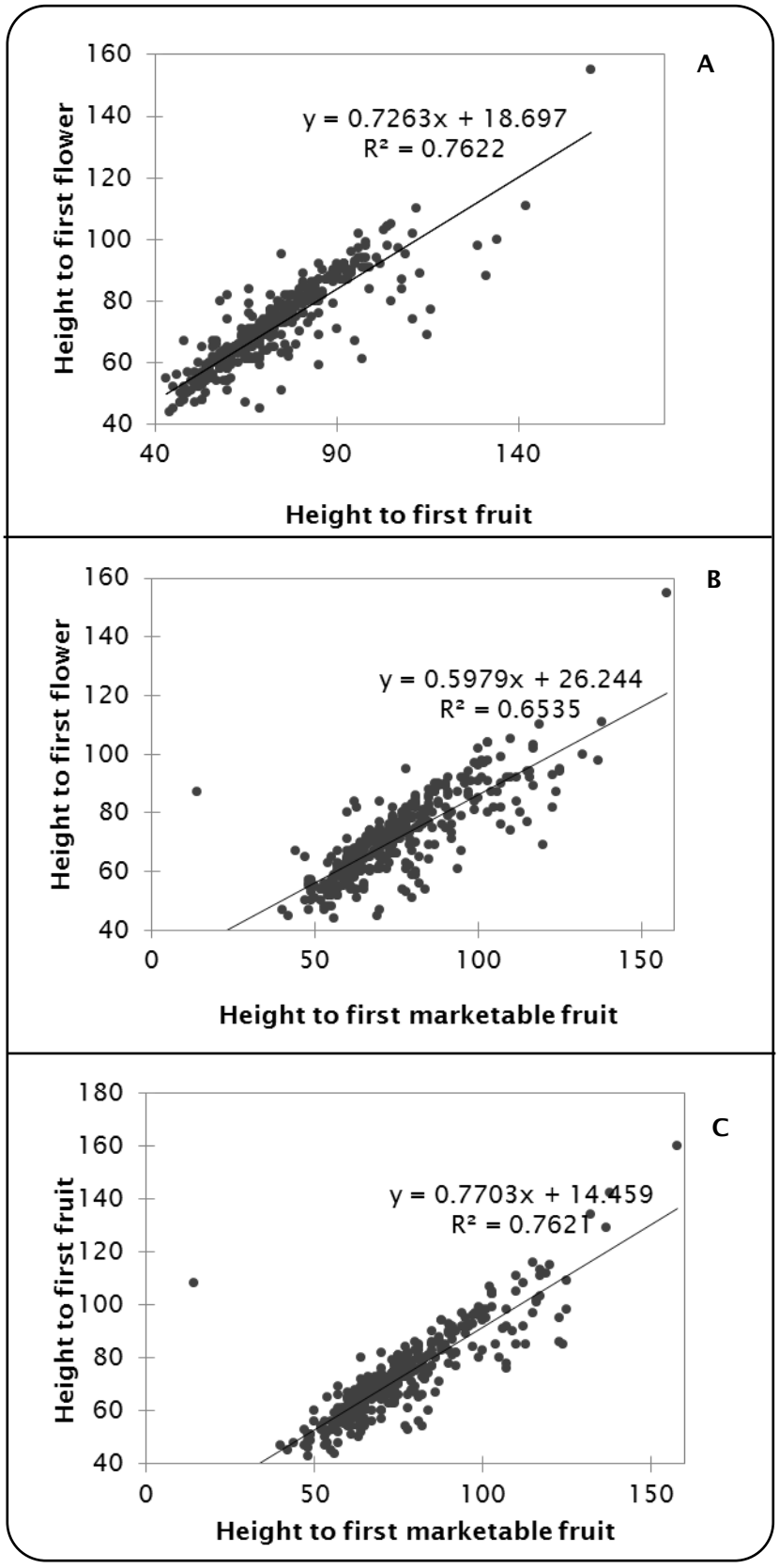

Figure 4.9 Scatter plots of A: height to first flower and height to first fruit, B: height to first flower and height to first marketable fruit, and C: height to first fruit and height to first marketable fruit

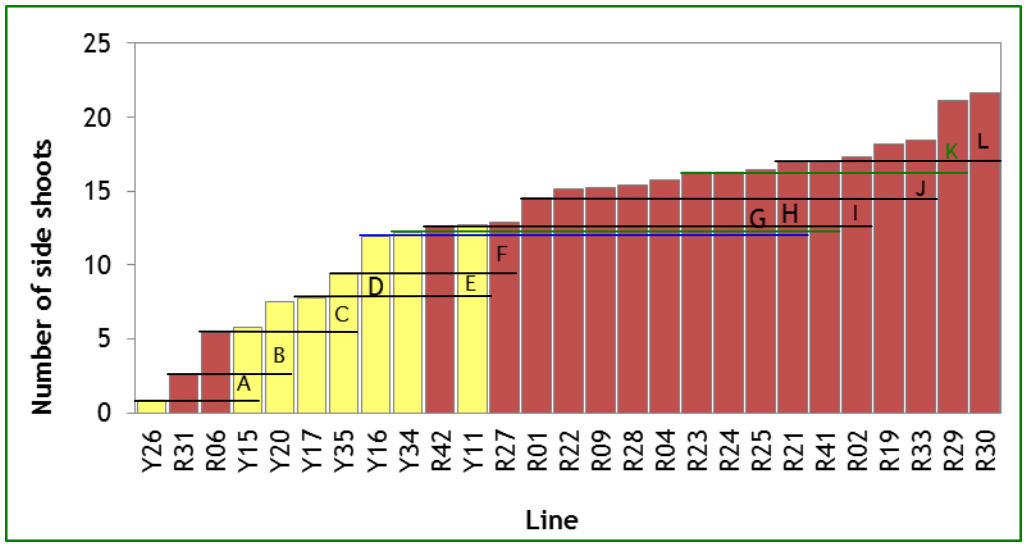

Figure 4.10: Number of side shoots of 27 lines papaya with yellow (yellow bars) and red (red bars) fruit. Means under the same line and letter are not significantly different from each other (P > 0.05)

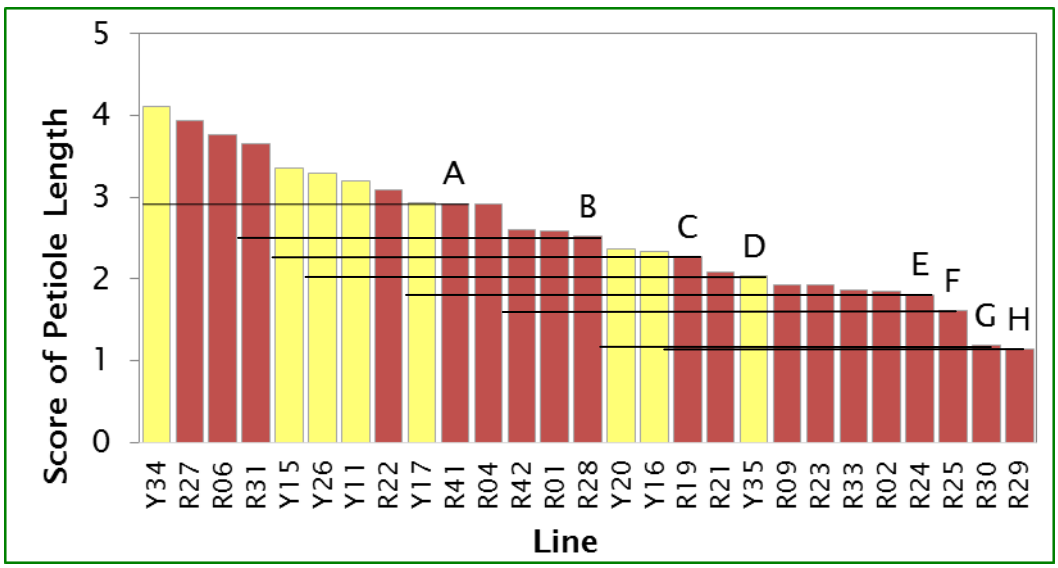

Figure 4.11: Rating of peduncle length of 27 lines of yellow (yellow bars) and red (red bars) papayas. Means under the same line and letter are not significantly different from each other (P > 0.05)

Table 4.9: Means of productivity traits for each line averaged across assessment times

Line	Height to 1st flower (cm)	#Side Shoot	Peduncle length	Saleable yield (kg/tree)	Yield gap	#Carpelloid fruits
R01	77.800	14.533	2.583	38.423	2.721	1.630
R02	67.200	17.333	1.848	34.621	4.149	1.164
R04	63.600	15.733	2.914	37.559	2.796	2.842
R06	79.267	5.467	3.764	**57.926**	2.865	0.131
R09	68.600	15.267	1.933	35.356	4.133	1.222
R19	56.267	18.133	2.267	25.552	2.921	1.644
R21	69.600	15.133	3.088	**6.907**	1.054	**24.857**
R22	70.867	16.267	1.926	23.947	4.206	0.336
R23	79.467	16.267	1.808	23.694	3.601	0.692
R24	70.400	16.400	1.615	35.783	4.220	1.071
R25	59.200	12.867	3.933	47.029	2.200	1.089
R27	68.786	15.429	2.518	35.939	2.538	1.244
R28	90.214	21.143	**1.143**	22.835	2.114	2.381
R29	**91.800**	**21.600**	1.182	20.734	**0.921**	2.028
R30	**54.867**	2.600	3.654	43.224	1.587	**0.000**
R31	64.533	18.467	1.869	23.816	1.968	2.296
R33	70.667	17.000	2.919	37.566	1.087	9.313
R41	64.600	12.600	2.596	43.058	3.692	2.387
R42	73.267	5.800	3.350	32.598	3.434	1.653
Y11	68.467	12.733	3.205	46.331	2.385	1.385
Y15	71.933	11.933	2.336	33.923	3.963	1.302
Y16	65.867	7.733	2.937	36.919	4.364	1.110
Y17	70.533	7.467	2.361	35.824	1.993	0.123
Y20	71.571	16.933	2.082	24.177	3.998	0.446
Y26	85.933	**0.800**	3.298	37.259	1.528	**0.000**
Y34	77.733	12.200	**4.107**	19.610	**5.830**	0.004
Y35	61.643	9.400	2.036	35.023	3.103	**0.000**
STD*	14.218	6.362	1.520	18.394	2.545	6.382

Note: Highlighted and bold figures are the highest and the lowest figures in each trait

* STD standard error of means for each data set

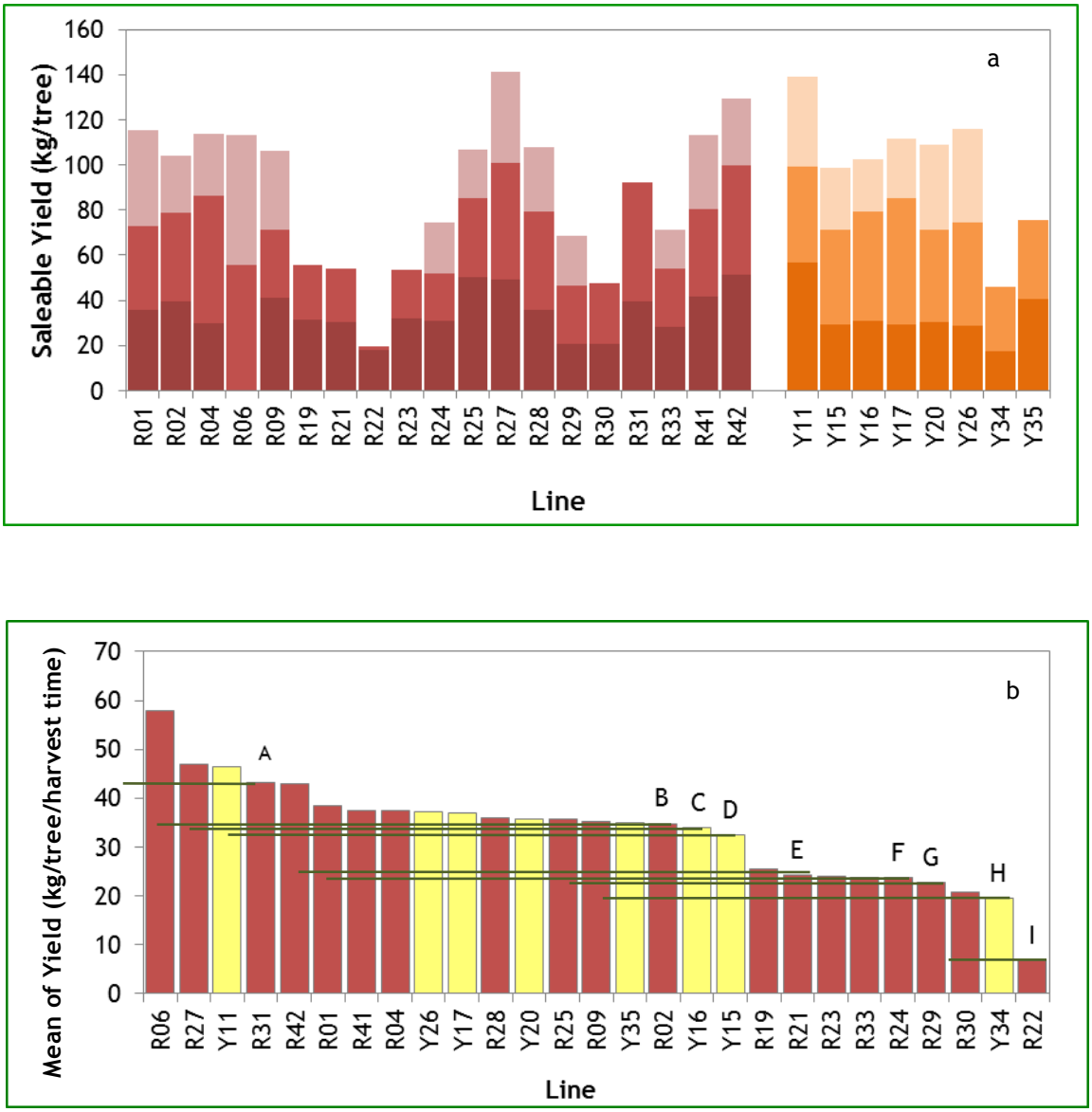

Figure 4.12: Saleable yield of 27 lines of papaya. a) Saleable yield over three harvest dates (1st harvest of red and yellow fruit, respectively, 2nd harvest and 3rd harvest). b) Mean of saleable yield of 27 lines of yellow (yellow orange bars) and red papaya (red bars). Means under the same line and letter are not significantly different from each other ($P > 0.05$)

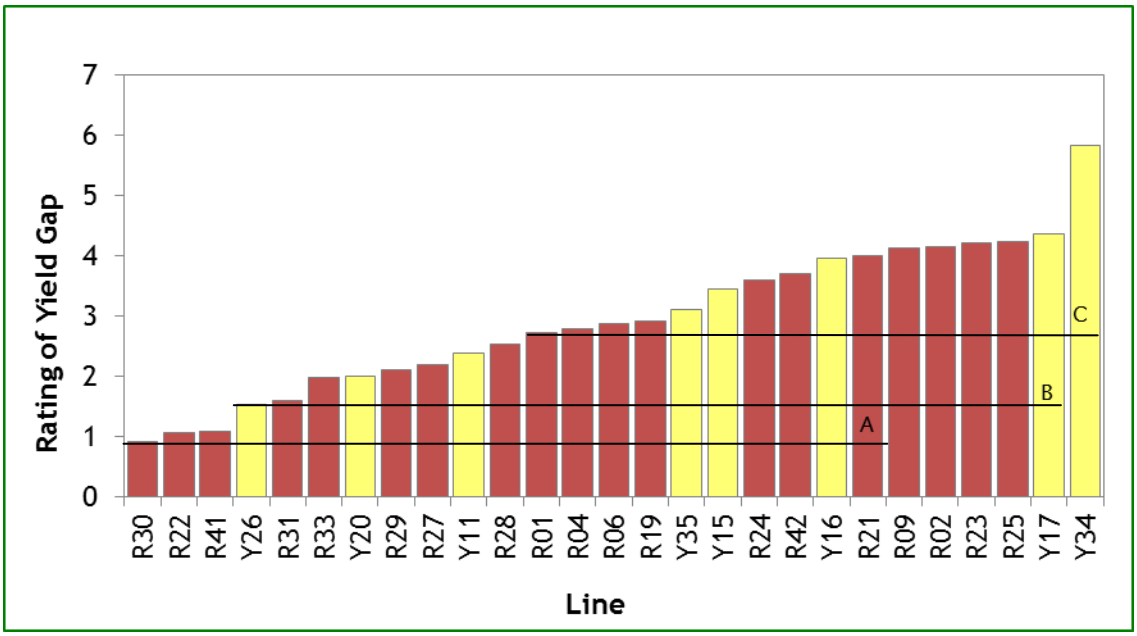

Figure 4.13: Rating of yield gap of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

4.11 Analysis of fruit quality traits

The data for shape of fruit, teat, stalk insertion and cavity is detailed in Appendix 2. This information was used as a reference when selecting parental trees. Histograms of the traits mention above are presented in Figure 4.14. The frequencies of each trait in each harvest season were different as shown in different patterns of distribution. Most traits showed continuous segregation except stalk insertion that the majority of fruits scored 2 (flattened).

Mean values of all the traits related to fruit quality for each line are shown in Table 4.10. Skin quality rating varied among lines in this study (Figure 4.15) and ranged between 1.5 and 3. The score of skin freckle varied among lines in this study (Figure 4.16) with red-fleshed fruits showing greater susceptibility to skin freckle than yellow.

The variation of skin colour in this population is reported in Figure 4.17. The chart showed yellow papaya tended to have lighter skin than the red flesh fruits. Line R06 and Y15 were the best two lines for skin traits, while R29 and R30 were the two lines with the lowest scores for both skin quality and skin freckle.

More than twice as many red fleshed lines than yellow fleshed lines were grown in this study (Figure 4.19). Lines with yellow flesh showed more consistency in flesh colour than those with red flesh (Figure 4.18). Flesh firmness and thickness varied among all the lines without any pattern (Figure 4.20 and 4.21). The measurement of total soluble solids in the 27 lines varied from eight to 15 (Figure 4.22). The highest TSS was detected in Solo type fruit (Linda and Sunrise Solo). However, the lowest TSS was found in an Australian commercial yellow papaya (1B). Fruit flavour was recorded to assist selection for parent lines to use in the next cycle of breeding program.

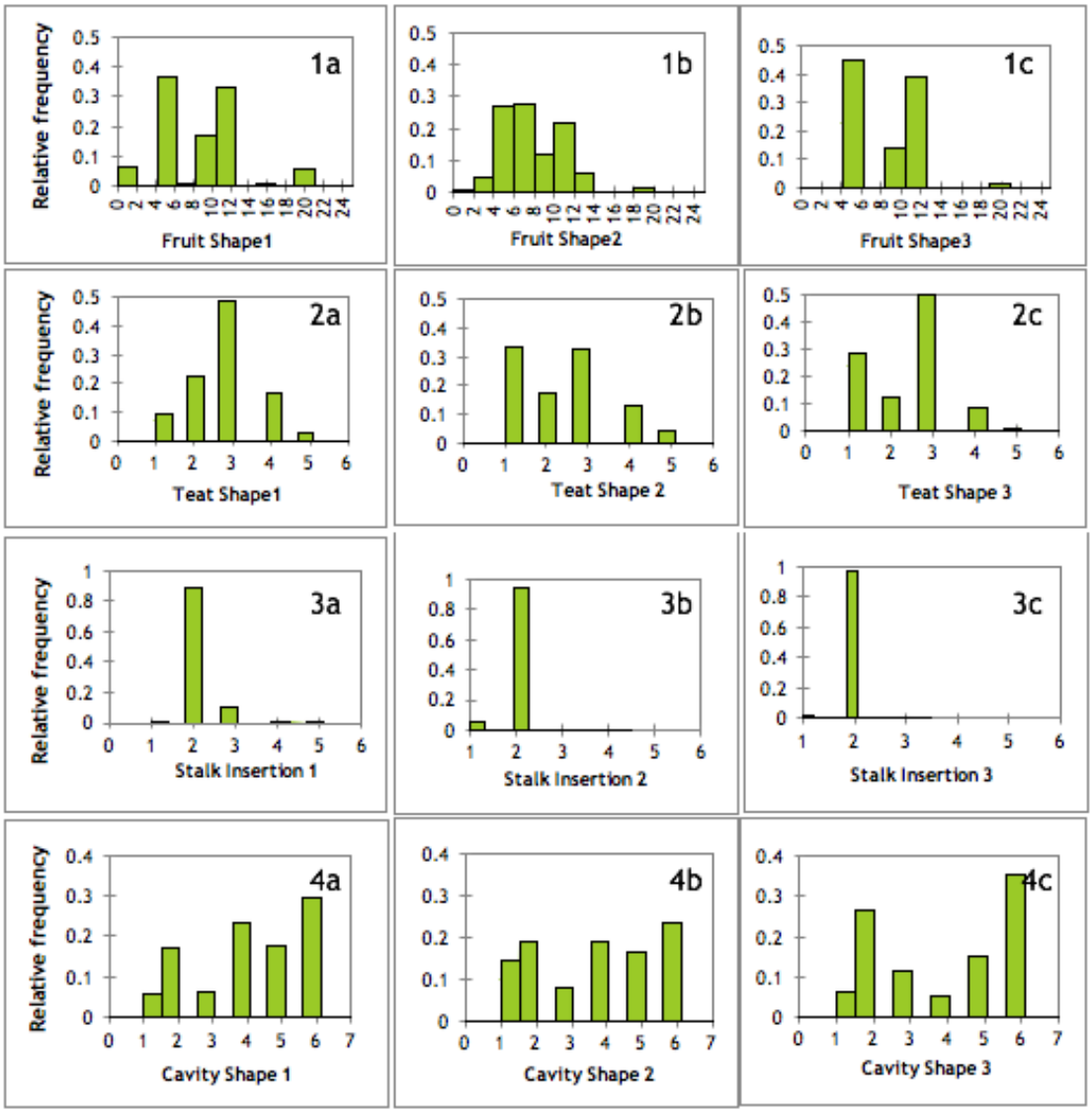

Figure 4.14: Histogram of papaya plants for 1) fruit shape, 2) teat shape, 3) stalk insertion and 4) cavity shape in three harvest times (a: first harvest in April 2012, b: second harvest in October 2012, and c: third harvest in May 2013)

Table 4.10: Means of fruit quality traits of each line

Line	Skin quality	Skin freckle	Skin colour	CFC[1/]	Flesh colour	Flesh firmness	UFT[2/] (mm)	TSS [3/] (ºBrix)
R01	2.215	1.731	3.148	1.833	6.733	3.370	18.630	10.948
R02	1.924	2.526	3.538	2.107	6.357	3.154	19.920	11.184
R04	2.694	1.048	3.368	2.000	6.378	3.368	18.432	11.274
R06	2.968	0.489	3.654	1.800	5.600	3.240	19.040	10.544
R09	2.119	2.024	4.143	1.800	5.689	3.571	18.762	11.571
R19	1.924	1.949	3.385	2.393	7.214	4.040	17.423	12.165
R21	2.508	1.148	3.650	2.036	5.310	2.850	19.375	11.946
R22	2.145	1.584	4.351	2.344	4.489	4.081	13.486	12.603
R23	2.161	1.774	4.040	1.643	6.286	4.040	19.760	12.228
R24	2.445	1.404	3.850	2.133	5.200	2.500	19.500	12.118
R25	2.339	1.644	4.250	2.356	6.200	2.950	18.000	11.168
R27	2.683	1.600	3.634	2.044	6.467	3.341	19.317	10.956
R28	2.366	2.070	3.857	2.262	6.000	2.943	16.514	11.780
R29	1.580	2.641	4.100	2.655	6.452	2.500	13.875	13.082
R30	1.490	2.821	3.476	2.511	5.333	2.100	12.897	13.295
R31	2.268	1.563	3.500	2.077	6.625	4.500	19.458	9.699
R33	1.886	2.198	3.864	2.422	6.000	2.318	16.455	12.675
R41	2.175	2.055	5.000	2.233	5.489	3.529	13.529	12.824
R42	2.100	2.001	3.857	2.100	6.067	2.464	20.143	11.514
Y11	2.338	1.433	3.080	2.786	2.000	4.680	19.960	10.295
Y15	2.728	0.950	2.571	2.833	1.286	3.286	18.286	11.744
Y16	2.622	1.479	2.588	2.905	1.738	3.000	17.971	10.913
Y17	2.093	1.688	3.300	2.458	1.250	3.800	19.700	9.853
Y20	2.348	1.143	3.000	3.000	1.308	2.545	17.409	11.349
Y26	2.105	1.583	2.905	3.000	1.182	2.333	22.619	9.238
Y34	2.647	1.333	2.941	2.795	1.154	3.059	19.500	12.865
Y35	2.673	0.996	2.667	2.125	3.444	3.333	20.708	11.251
STD*	0.764	1.137	1.300	0.726	2.380	1.516	4.329	1.710

Note: Highlighted and bold figures are the highest and the lowest figures in each trait

* STD standard error of means for the data set

1/ CFC means consistency in flesh colour

2/ UFT means useable flesh thickness

3/ TSS means total soluble solid for flesh sweetness

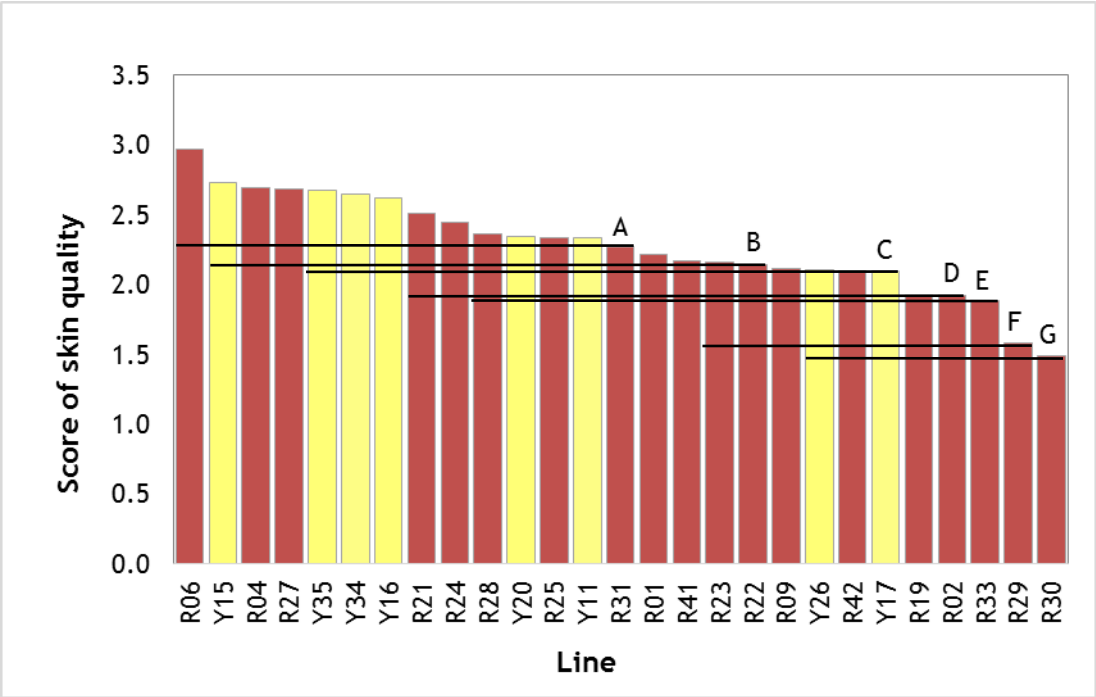

Figure 4.15: Score of skin quality of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

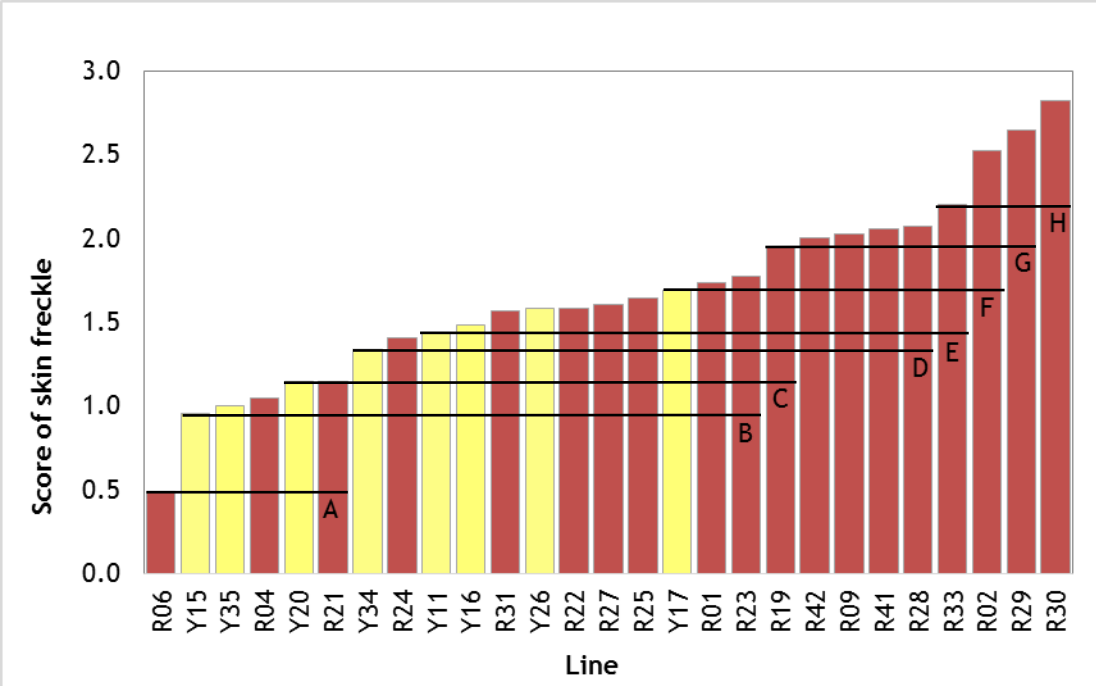

Figure 4.16: Score of skin freckle of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

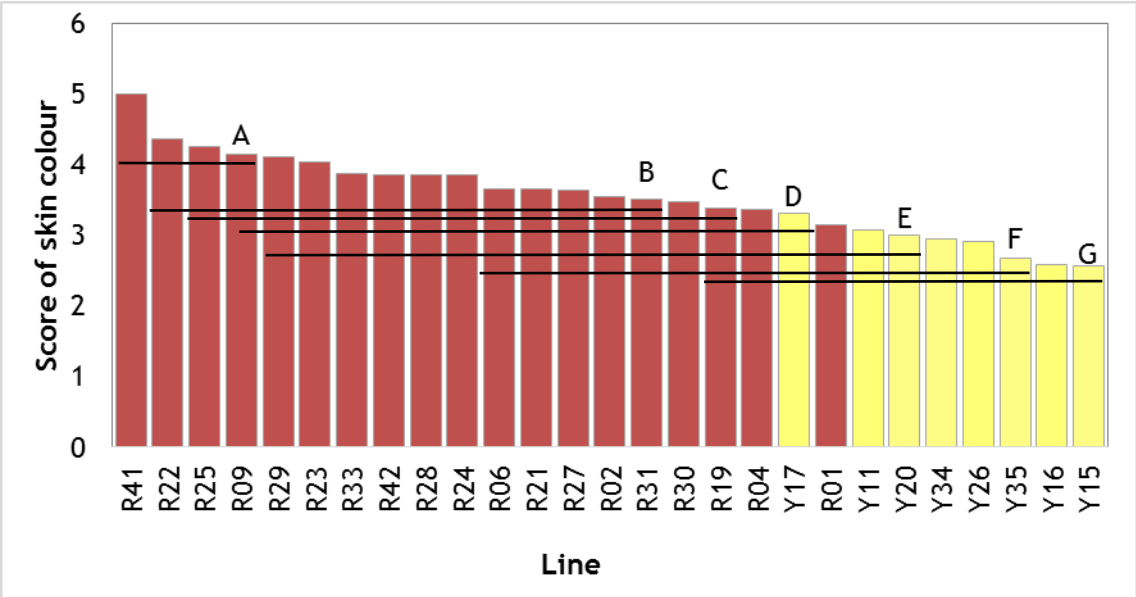

Figure 4.17: Score of skin colour of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

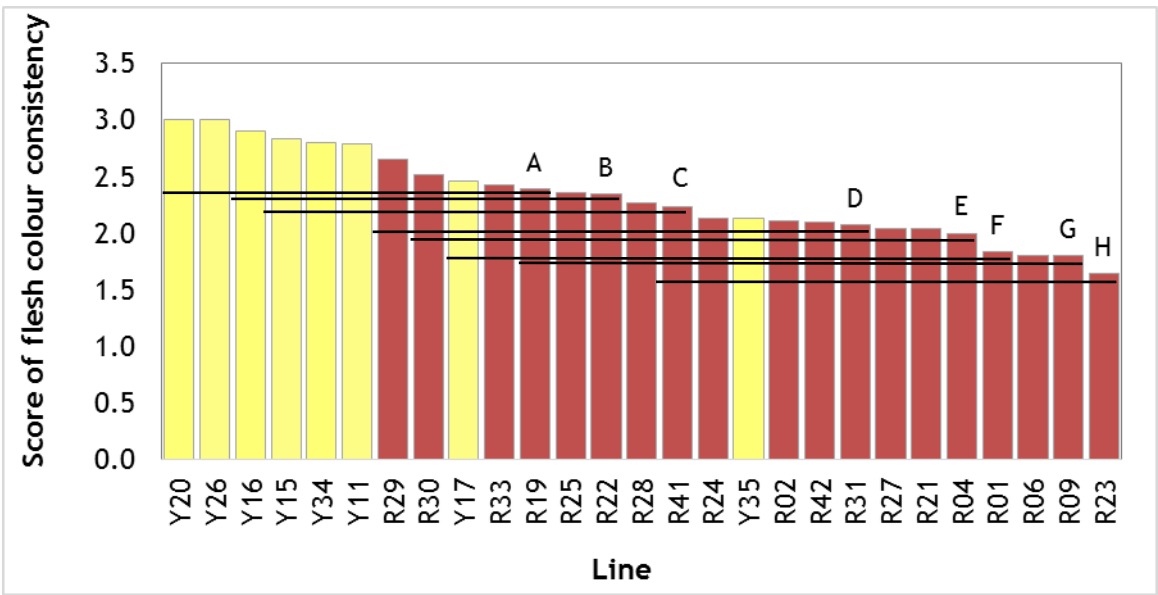

Figure 4.18: Score of consistency in flesh colour of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

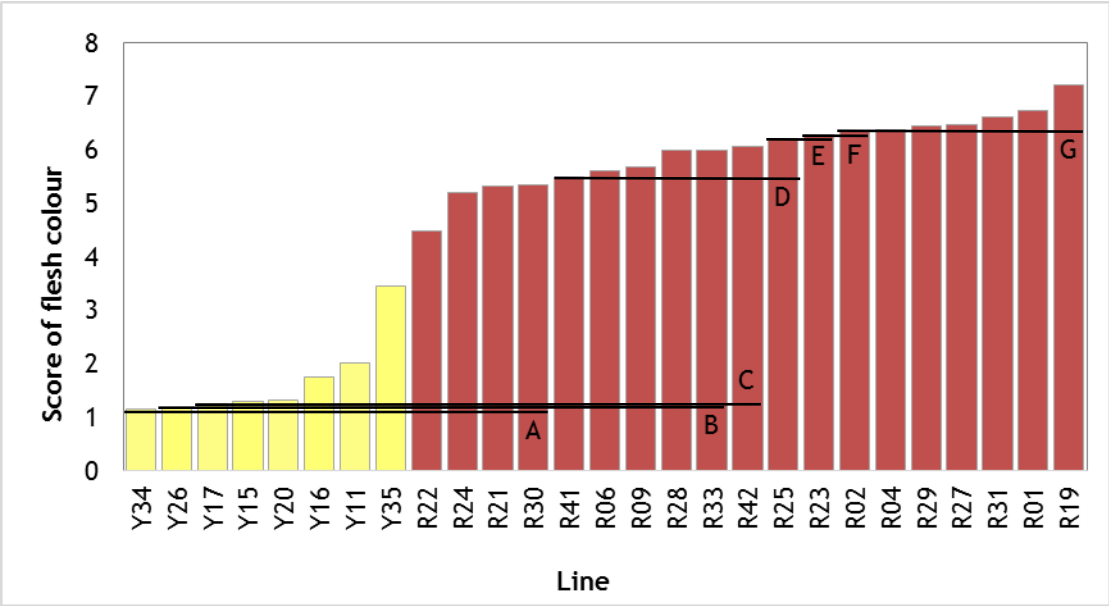

Figure 4.19: Score of flesh colour of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

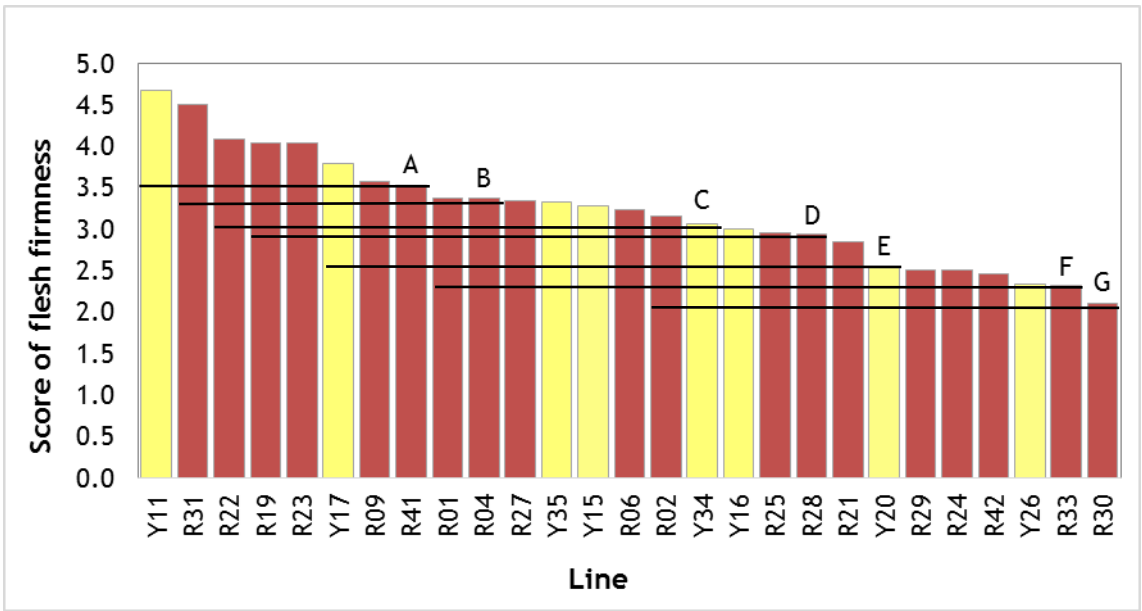

Figure 4.20: Score of flesh firmness of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

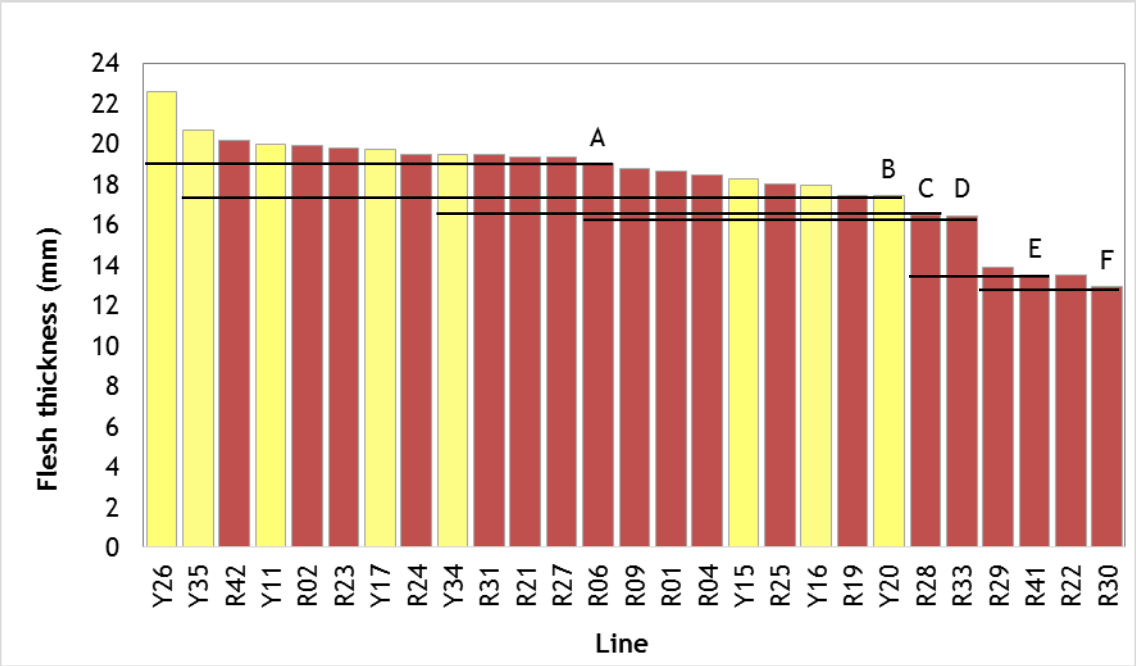

Figure 4.21: Score of flesh thickness of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

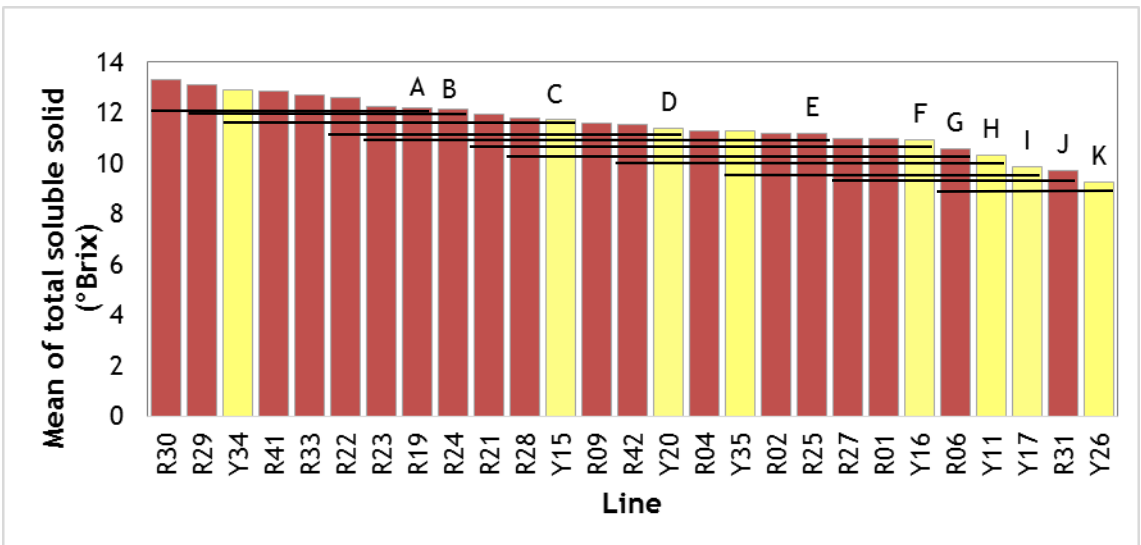

Figure 4.22: Means of total soluble solid (TSS) of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

4.12 Progress of breeding program to improve Australian commercial lines

The best three lines that were selected on the first evaluation (April 2012) were TS2, Malaysian Red 2 and Sunrise Solo. They exhibited excellent fruit eating quality (flesh flavour, firmness and thickness) and were selected to cross with Australian commercial lines 1B, RB1 and RB2. The data from all three harvests are presented in Table 4.11. Variation of the same trait in the same tree can be detected in different harvesting times, a fact noted (above) by the relatively low repeatability estimates. Sixteen crosses were established for the second phase of the breeding program (Table 4.12). F_2 populations of QDAFF lines and Australian commercial lines were also produced.

Table 4.11: Fruit eating quality in three harvest time of the selected trees to improve Australian commercial lines

Line	Harvest time	Flesh firmness	TSS (ºBrix)	Flavour Score	Flavour
Y22-7	1	5	12	12	Khagdum
(TS2)	2	5	14	9	Solo
	3	5	14	6	Solo
	Mean	5	13	9	
R24-2	1	5	13	12	Musk
(Malaysian Red 2)	2	3	11	4	Nasturtium
	3	1	13	9	Musk
	Mean	3	12	8.3	
R29-9	1	3	13.5	12	Solo
(Sunrise Solo)	2	3	14	6	Solo
	3	1	15	12	Solo
	Mean	2.33	14	10	

Table 4.12: Crosses used to produce progeny populations in the second cycle of applied breeding

Code	Female x Male	Line details
#50	Y22 x Y26	TS2 x 1B
#51	Y15 self (tree #7)	F_2 (1B x 33-66)
#52	Y15 self (tree #8)	
#53	Y16 self (tree #4)	F_2 (7-82 x 1B)
#54	R04 self (tree #7)	F_2 (RB2 x 18-45)
#56	RB1 x R24 (tree #2)	RB1 x Malaysian Red
#57	RB1 x R24 (tree #2)	
#62	R27 (tree #5) x R24 (tree #2)	RB2 x Malaysian Red
#63	RB2 x R24 (tree #2)	
#65	RB2 x R24 (tree #2)	
#64	RB2 x R29 (tree #13)	RB2 x Sunrise Solo
#67	RB2 x R29 (tree #13)	
#68	Y26 (tree #8) x R24 (tree #2)	1B x Malaysian Red
#69	Y26 (tree #6) x R24 (tree #2)	
#70	Y26 (tree #4) x R24 (tree #2)	
#71	R41 self (tree #1)	F2 (JC2 x Vietnam Red)

Of these 16 crosses from the second breeding population, seven trees, two and five of yellow and red papaya, respectively, were selected for their flavour and overall yield. These trees were used as parental lines to backcross to commercial varieties 1B, RB1 and RB2 and to sib cross to produce new hybrids. The selected trees are detailed in Table 4.13. An evaluation of this population has not been completed. Details of futures direction are described in Chapter 6.

Table 4.13: Parent lines selected for improvement flavour of commercial papayas

Flesh colour	Line	Flower sex
Yellow Papaya	#50-1	Female
	#50-male	Male
	#68-7	Hermaphrodite
Red Papaya	#62-5	Female
	#63-5	Hermaphrodite
	#64-2	Hermaphrodite
	#56-2	Hermaphrodite

	#56-5	Female

4.13 Progress of segregating population for development of fruit quality traits in papaya

Five categories of fruit and tree attributes (flavour, skin attributes, eating quality attributes of both yellow and red-fleshed types and tree productivity traits) were identified from the 22 traits that were evaluated in order to produce wide crosses segregating populations for the identification of DNA markers. Twenty-three trees were selected from 270 because they were the most consistent in expressing each trait in the three evaluation times. The details of the five categories and selected lines are detailed in Table 4.14. Trees were crossed and progeny populations were produced but evaluating these progeny was not completed within the time frame of this chapter. Future directions of this work are described in Chapter 6.

Table 4.14 : Five categories of traits selected to produce segregating populations for the development of DNA markers

Categories	Tree selected	Details
1. Flavour		
	R29-13 (Sunrise Solo)	Solo flavour
	Y15-6 (1Bx33-66)	Nasturtium flavour
	R24-2 (Malaysian Red 2)	Musk favour
	R41-8 (JC2 x Vietnam Red)	Khag Dum flavour
	R27-5 (RB2)	No flavour
2. Skin traits:		
skin quality and skin freckle	R30-5 (Solo Linda)	Poor skin quality
	R04-2 (RB2x18-45)	Good skin quality
Skin colour	Y34-11 (2.54-14 self)	Light yellow skin colour
	R41-6 (JC2 x Vietnam Red)	Deep orange skin colour
3. Eating quality: Yellow papaya		
Flesh colour, flesh firmness, flesh thickness and TSS content	Y16-1 (7-82 x 1B)	Light yellow flesh colour, soft, thin and low TSS
	Y35-12 (2.54-12 self)	Orange skin colour, firm, thick and high TSS
Flesh colour consistency	Y17-15 (24-87 x 1B) and Y35-12 (2.54-12 self)	< 50% flesh colour consistency
	Y16-1	100% flesh colour consistency
4. Eating quality: Red Papaya		
Flesh colour	R24-1 (25-5 self)	Light orange flesh colour
	R27-5 (RB2) R4-10 (RB2 x 18-45)	Deep red flesh colour
Flesh firmness and thickness	R30-9 (Solo Linda)	Soft and thin flesh
	R27-1 (RB2)	Firm and thick flesh
TSS content	R27-2 (RB2)	Low TSS
	R29-9 (Sunrise Solo)	High TSS

Flesh colour consistency	R09-12 (25-5 x RB1)	< 50% colour consistency
	R29-9 (Sunrise Solo)	100% colour consistency
5. Production traits		
Number of side shoots	26-13 (1B)	0 side shoot
	R30-9 (Solo Linda)	High number of side shoot
Height to first marketable fruit	R27-5 (RB2)	Low
	R29-9 (Sunrise Solo)	High
Number of carpeloid fruit (Hermaphrodite tree)	R42-11 (TS2 self) or R02-13 (RB1x24-29)	Less (0 fruit)
	R04-10 (RB2x18-45)	More than 20 fruits
Peduncle length	R30-10 (Solo Linda) R09-14 (25-5 x RB1) Y17-12 (24-87x1B)	Short
	R04-10 (RB2x18-45) R27-7 (RB2) R27-11 (RB2)	Long
Yield gap:	R29-9 (Sunrise solo)	No yield gap
	Y17-15 (24-87x1B)	High yield gap

DISCUSSION:

There was a wide range in the phenotypic variations observed in these populations. Similar variations were reported in the descriptors provided by IBPGR 1988. The IBPGR work showed high phenotypic variation in leaf, flower and fruit attributes and reaction to pests and diseases. The differences of most traits between lines were quantitative and did not allow for grouping of individual varieties. This might be due to many fruit quality traits being controlled by multiple genes and low heritability.

4.14 Effect of harvesting time to fruit productivity and quality traits

Evaluation of performance of trees in different harvest times is necessary as the result showed relatively low repeatability in most traits and some seasonal effects of on fruit and tree development can be observed in this study.

Fruit productivity traits expressed relatively consistently in the first two harvesting times, but varied more in in the last of three harvests. A similar result was found in the report of Benson and Poffley (1998). In general, yield of yellow papaya was higher than red papaya. This could be because of the nature of dioecious outcrossed plants that can produce higher yields than selfed hermaphrodites which may be exhibiting inbreeding depression (Drew et al. 1998a; 1998b; Chay-Prove et al. 2000). Interestingly, some traits such as number of carpelloid fruits were observed by growers to be affected by season, but this study showed no significantly different in this trait between the three harvesting times at the one location. This suggests that environmental effects on the expression of this trait were not as important as gene expression. Yield gap varied with lines. Magdalita et al. (1998) and the The Organisation for Economic Co-operation and Development (OEDC 2005) suggested yield and yield gap varied considerably and was affected by cross pollination, environmental condition, floral character and flower

receptivity. Female sterility in hermaphrodite trees can be exacerbated in stress conditions such as high temperatures and water and nitrogen shortages (Awada and Ikeda 1957; Arkle and Nakasone 1984; Amleida et al. 2003). In Australia, variable fruit production could be due to low pollen viability and absence of pollinators especially in cooler, winter seasons. In subtropical climates, fruit set declines or may even cease during the colder winter months (Garret 1995; Allan 2002). Further study into factors affecting pollen and flower receptivity could be recommended because of the magnitude of the gaps noted in this study (up to 60% or more).

Fruit quality traits are complex and could be controlled by quantitative loci. The quality of fruits varied according to location, variety and season as well as farm practice (Elder et al. 2000a). In South Africa, fruits harvested from more mature trees, giving better quality fruit, and in particular, a higher sugar content (Department of Agriculture, Forestry and Fisheries, South Africa 2009). Uniformity of fruit size, shape and appearance of dioecious fruits were better than those from hermaphrodites (Storey 1976; Ying 2008). Fruit shape and size were controlled by 14 QTL with phenotypic effects; they were identified across six LGs with clusters of two or more QTL on LG2, LG3, LG7 and LG9 (Blas et al. 2012). These loci contain homologs to the tomato fruit QTL ovate, sun and *fw2.2* regulating fruit size and shape (Van der Knaap and Tanksley 2001).

One of the traits affected by temperature or season of harvest was flesh colour. A single gene controls colour wherein yellow is dominant over red flesh (Ying 2008; Blas et al. 2010). In the present study, lines and plants within lines were either yellow or red-fleshed, and repeatability of the attribute was relatively high. Nevertheless, colour intensity varied with the season being lowest in fruits developed in winter 2012 (the October 2012 harvest). This could be related to ripening process of papaya because fruit ripen satisfactorily between 20 and 25ºC (Akamine 1966; Broughton et al. 1977) but do not ripen at 10 and 15ºC (Nazeeb and Broughton 1978). The result of temperature to the development of flesh colour is noted in other fruit, for example in tomato so that that higher temperatures affect red color development and softening (Hall 1964).

The season when fruit developed also affected skin freckle, which is one of the important traits for fruit appearance. Eloisa and Paull (1994) suggested freckles resulted from aberrant physiology associated with a rapid growth late in the fruits' development. Both low and high temperatures in the two months before harvest increased freckle occurrence. This was supported the result of fruits developed in winter (second harvest in October 2012) had higher score of skin freckle than the other two harvest.

4.15 Correlation of traits

Even though some traits in this study were significantly related, most of them showed low degree of correlation. Only the measurement of height to first flower, first fruit and first marketable fruit were highly (positively) correlated, therefore, height to first flower can be used to determined the height at which fruit will set on the tree. Yield, number of carpelloid fruits and yield gap can be negatively correlated. However, peduncle length tended to positively associated with yield. These factors were previously predicted by the growers of north Queensland to directly affect yield (Kath, personal communication).

Interestingly, flesh colour score was positively correlated with skin colour but negatively with consistency of flesh colour. This effect was also noted by growers: viz. the darker of the skin colour, the deeper of the flesh colour. The positive relationship between skin colour and flesh

colour is also reported in other fruit, for example apricot (Ruiz and Egea 2008).

One of the important traits to improve in commercial papayas in Australia is level of TSS which is commonly refer to Brix (Papaya breeding reference group and growers, personal communication). The TSS content is usually associated with sweetness and reports of TSS range from 8% to 16% or more (Singh 1990). In the present study, TSS was generally in the range of 10 to 12%. There was no correlation between TSS and other traits in this study, which was different from the report of Hansen (2005) who reported linkage between high sugar content and skin freckle, that is, the greater the TSS the worse the skin freckle. The TSS is controlled by additive gene effects and has a high heritability. Papaya fruit lack starch reserves for post harvest conversion to soluble sugars and final sugar content is determined by the quality and quantity of translocatable sugar in the fruit at time of harvest (Chan et al. 1979; Manshardt 1992).

4.16 Applied plant breeding

To generate new varieties, expeditious evaluation and prompt decision-making are part of effective plant breeding. In the present study, parental lines used to improve eating quality in commercial Australian papayas were selected and crossing was done after the first harvest. Despite relatively low repeatability of measurements of most traits across harvest times in these plants, plants were noted that did consistently perform at high (or low) levels. It should be noted, too, that the time required to establish the subsequent generation of plants following cross-pollination did allow for repeated observation of the selected parent plants. It is likely that repeatability of measurements will improve as gene frequencies are moved by selection, re-crossing and focus on the most important traits. The wide heterogeneity within groups (see Formula 4.2) is likely to progressively decline with selection and re-crossing.

Another aim of this research was to establish segregating populations for applied plant breeding to develop DNA markers for quantitative traits. Parents for this work were selected on consistent expression of each trait in three harvests. For this parent material, trait expression was quite repeatable. Discussion here regards some concerns about future breeding plans.

As noted in the introduction to this chapter, the Australian market associates yellow flesh with round fruit and red flesh with elongate fruit. Fruit shape is determined by the sex of the plant (Storey 1938; Ming et al. 2007). The Australian consumers' association of flesh colour with fruit shape is not a fixed genetic linkage. Plant sex type also influences flesh thickness and fruit uniformity (Anh et al. 2011). Papaya has three basic sex types: female, male and hermaphrodite. Genetic control of sex of papaya has been studied since 1938 (Hofmeyr 1938; Storey 1938). It was hypothesised that papaya has three distinct sex chromosomes (X, Y and Y^h) with a lethal factor in the male- and hermaphrodite-specific region (Storey 1941). Males (XY) and hermaphrodites (XY^h) are heteromorphic and the expression of sex is controlled by Y for male and Y^h for hermaphrodite, while females (XX) are homomorphic. Any combination of YY, Y^hY or Y^hY^h lines is lethal (Ming et al. 2007). Therefore the segregation ratio of 1:1 of female to either male or hermaphrodite can be found in female crosses to either male or hermaphrodite, respectively. Selfed hermaphrodite or sib crosses between hermaphrodite flowers express 1:2 of female to hermaphrodite plants. Thus the selection of females and males to be used as parental lines for yellow papaw is ideal to meet the requirement of the market in terms of fruit shape. But for red papaya it is preferable to self or sib-cross hermaphrodite flowers to achieve a greater number of hermaphrodite fruits for evaluation and selection.

Flavour is a very complicated trait and it needs further study to be able to classify flavour scientifically. There are a number of reports in the literature discussing the volatile chemicals in papaya fruits (Flath and Forrey 1977; Idstein et al. 1985; Pino et al. 2003, Almora et al. 2004). Papaya possesses a characteristic aroma, which is due to several volatile components, such as alcohols, esters, aldehydes, and sulphur compounds (Marostica and Pastore 2007). Almost 400 volatiles profiles of various papaya cultivars were identified in more than 40 years of intensive work (Pino et al. 2003).

CONCLUSION:

The result from this study led to the selection of three lines, which were TS2, Sunrise Solo and Malaysian Red 2, to use in a breeding program to improve flavour of commercial varieties in Australia. They exhibited excellent fruit eating quality (flesh flavour, firmness and thickness) were used to pollinate Australian papaya commercial lines, 1B, RB1 and RB2.

Of the 22 evaluated traits, five groups of traits which deal with fruit flavour, skin quality, eating quality of red papaya, eating quality of yellow papaya and yield were identified for the production of segregating populations to be used for subsequently developing DNA markers for those traits. Twenty-three representative trees were selected as parental lines because they were consistent in expression of each trait of interest over the three evaluation times. Seventeen crosses were made for a future breeding program to develop improved commercial lines and for a program of MAS.

Appendix 4

Chapter 5 of Ph.D. thesis of Dr. Chat Kanchana-udomkan

This chapter provides complete experimental details of research associated with the development of molecular markers as described in this report

Chapter 5:

Application of DNA Markers to Select for Fruit Flesh Colour in Papaya

INTRODUCTION:

The majority of papaya fruits are consumed fresh as ripe fruit or when green as a component in salads. In Australia, the market clearly distinguishes types of papaya according to flesh colour, as yellow papaw or red papaya. Flesh colour of papaya is an indicator of antioxidant activity in red flesh and a source of high vitamin A in yellow flesh (World Health Organization 2007; Blas et al. 2010). In general, red papaya has better and more acceptable flavour to consumers than yellow papaya. Cross pollination between red and yellow papaya is a potential approach toward improving the flavour of yellow papaya. Yellow flesh colour was observed to be dominant over the red flesh colour in a simple Mendelian ratio (Ying 2008). Cross pollination of heterozygous yellow flesh papaya to either another heterozygous yellow or red-fleshed papaya can result in segregation of flesh colour in later progenies. However, a mixture of yellow and red flesh coloured fruit within the same plantation area is a farm management issue related to time of harvesting and fruit sorting. This may lead to a mixture of red- and yellow-flesh fruits in one sale unit (one carton) and mislabelling such that consumers may not get the fruit that they desire. This factor has resulted in loss of consumer confidence.

A breeding program to improve eating quality of both fruit types has been funded by Horticulture Innovation Australia (known as Horticulture Australia Limited) since 2005 (Hansen 2005; Drew 2005). The selection process is the most crucial procedure for a breeding program. Conventional breeding relies on phenotypic expression, in this case, flesh colour, which takes time to express and is likely greatly influenced by the environment. Fruit flesh colour is easily to identify when trees are mature and produce fruit, which can take approximately 12 months after planting. However, to identify this trait in seedling can be very useful for growers in terms of farm management. Especially, in breeding lines where red papaya may cross pollinate with yellow papaya and following generations may present mix phenotype. Subsequently, DNA markers, that select for the major genetic components of such a trait have become routinely used as an efficient tool. DNA marker assisted selection can be done at any stage of plant development, without having to wait for the desired traits to be expressed. A gene that controls yellow flesh colour, *CpCYC-b* was identified and DNA markers linked to this trait were identified (Blas et al. 2010). It is, therefore, an opportune time to take this genomic information and use it to apply to the breeding programs, to demonstrate marker assisted selection (MAS) for improving the efficiency of papaya breeding.

Australia has limited papaya genetic resources (Hansen 2005), therefore papaya germplasm from various sources was obtained and evaluated as detailed in Chapter 4. Over 300 trees of 27 lines were evaluated for traits related to production and fruit quality. Fruit flesh colour is an important trait for papaya marketing in Australia and as an indicator of nutritional benefit leading to consumer preference. It is, therefore, of great interest to breeders to be able to accurately select for flesh colour throughout the breeding process. Flesh colour in papaya is a result of the accumulation of □-carotenoids in yellow flesh or lycopene in red flesh in fruit cell chromoplast (Yamamoto 1964; Blas et al. 2009).

The location of genes controlling fruit flesh colour, *CpCYC-b*, and genes related to PRSV-P resistance on genetic maps is very important in the development of markers, for future breeding, and for an understanding of gene function. They were reported to be mapped on the same linkage group by Chen et al. (2007). They reported that the gene controlling fruit flesh colour was mapped on LG5 of a high density genetic map of papaya, which was the same linkage group where *prsv-1* was located (Dillon 2006b; 2006c; Razean Haireen 2013). Another report was that the flesh colour gene (Fcolor) was identified on LG7 of the high density AFLP map and the transgenic PRSV-P coat protein gene (PRSVCO) was also mapped to the same LG7 (Ma et al. 2004). Moreover, there were two large indels of 1,805 and 2,556 bp between the two BAC clones, one from red flesh SunUp (SH18O09) and the other from yellow flesh AU9 (DM105M02). The insertion of two Ts in red flesh papaya produced a frame-shift mutation and result in a premature stop codon in red fleshed papaya (Blas et al. 2010). Three SCAR markers were developed from the variation between the two BAC clones (Blas et al. 2010). The presence of the fragments was surprisingly found only in nontransgenic AU9 (yellow flesh) but absent in transgenic SunUp which is a red flesh papaya (Blas et al. 2010). Thus, it is possible to hypothesise that *CpCYC-b* and gene(s) related to PRSV-P resistance may locate on the same linkage group.

This approach would be to identify sequences that code for the genetic components that condition the colour. This may include components such as carotenoid, a precursor for the biosynthesis of Abscisic acid (ABA) (Cunningham and Gantt 1998; Walton and Yi 1995; Zeevaart and Creelman 1988). Carotenoid is also involved in plant defence (Ton et al. 2009; Mauch-Mani and Mauch 2005; Anderson et al. 2004).

RESEARCH AIMS:

1. To determine the transferability of published flesh colour markers of papaya to the current lines of the breeding population

2. To determine if these and other markers on the supercontig 28 on linkage group 5 are accurate in flesh colour selection within a wide population

MATERIALS AND METHODS:

5.1 Plant materials

Between 10 and 15 individuals of each of the 28 lines (330 plants in total) were used from Chapter 4 (Table 4.1). A 1 to 9 scale was used for assessing flesh colour, where 1 was yellow and 9 was red. The segregation of flesh colour score among all individuals assessed is shown in Figure 5.1. Based on the scale of flesh colour, trees were grouped as yellow flesh with a score ≤ 3 and as red flesh with a score >3. The details of the scoring system are described in Chapter 4, 4.1.1.

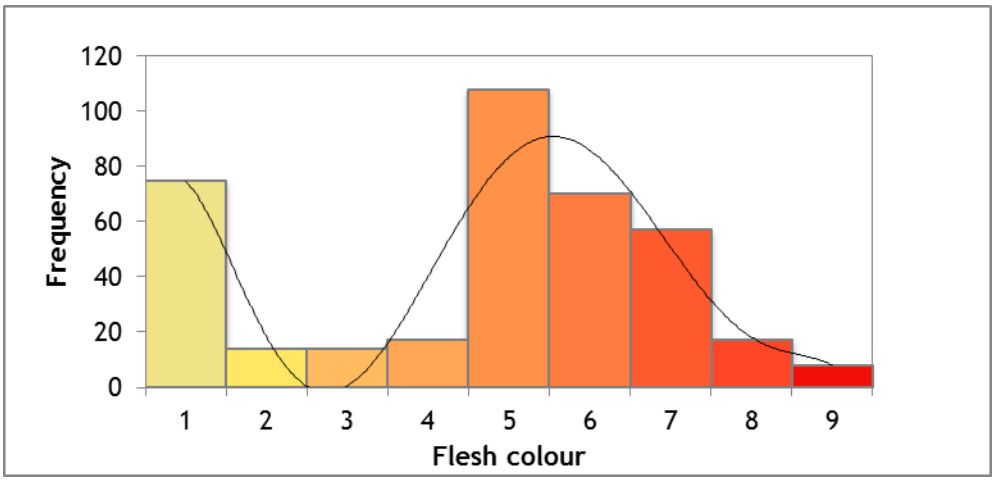

Figure 5.1: Segregation of flesh colour of all of the individuals used in this study. The scale of 1 to 9 represented the variation from yellow to red flesh

5.2 Bulked segregant analysis

Total genomic DNA was extracted separately from a leaf sample each individual using protocols described in Chapter 2, 2.1.1. DNA quantity and quality were evaluated by spectrophotometry (detailed in Chapter 2, 2.2.1). DNA samples were diluted to a 25 ng/µl working concentration and kept at -20ºC. The DNA samples were sorted according to flesh colour. Ten µl (250 ng) of DNA from each of 11 trees with a score of 1 were bulked as 'Yellow' and 10 µl (250 ng) of DNA from each of 11 trees with the score of ≥8 were bulked as 'Red' (Table 5.1). The bulked DNAs were then used for optimisation of PCR reactions and identification of candidate markers potentially associated to flesh colour based on their segregation within the population and statistical association with flesh colour.

Table 5.1: Individual plants (genotypes), their flesh colour score and grouping into 'Yellow' or 'Red' bulk

Line 'Yellow'	Tree Number	Flesh score	Line 'Red'	Tree Number	Flesh score
27-29 x RB2	Y11-5	1	RB1 x 18-45	R1-9	9
1B x 33-66	Y15-1	1	RB1 x 24-29	R2-3	9
	Y15-10	1	Malaysian Red 1	R23-7	8
7-82 x 1B	Y16-5	1		R23-8	9
	Y16-12	1		R23-14	9
24-87 x 1B	Y17-10	1	Malaysian Red 3	R25-10	8
JC2	Y20-1	1	RB4	R28-12	9
	Y20-9	1	RD6 self	R31-13	8
1B	Y26-11	1	2.54-12 self	R35-7	9
2.54-14 self	Y34-9	1	TS2 self	R42-11	8
	Y34-13	1		R42-14	9

5.3 PCR analysis

5.3.1 Published DNA markers for papaya flesh colour

Sequence characterised amplified region (SCAR) primers (Table 5.2), which were developed by Blas et al. (2010) for differentiation of flesh colour in papaya, were synthesised (Sigma Aldrich, Australia) and applied to amplify a product in both bulked DNAs. Primers that amplified a clear and reproducible size polymorphisms among the 'Red' and 'Yellow' bulk were further investigated for segregation among all of the individual 330 trees.

Table 5.2: SCAR primers used to differentiate papaya flesh colour (Blas et al. 2010)

Primer name	Primer sequence (5' → 3')	Flesh colour detected	Expected size
CPFC1	Forward: GACGTGTTAGTGTCCGACAA	Yellow/Red	500
	Reverse: GACCAGGAAGCAAATTTTGTAA		
CPFC2	Forward: GGACCACAGGAGCTGATTAG	Yellow	600
	Reverse: TATCTCTGCCACATGCAACC		
CPFC3	Forward: TGCAAAGAAATGGAGGGTTT	Yellow/Red	450
	Reverse: TGAAATCCTTCTGAGCCAAA		

5.3.2 The development of novel SSR and EST markers in SC28

Three SSR primer pairs, SSR28.50, SSR28.106 and SSR28.103-104) were designed on SC28 by Dr Cameron Peace (personal communication). These primers targeted microsatellite sequences as follows:

- SSR28.50 targeted a 247 bp length of just Cs and Ts in gene 28.50.
- SSR28.106 targeted a $(TTTTC)_5$ microsatellite that is in the promoter of 28.106, 387 bp upstream from that gene's start codon.
- SSR28.103-104 targeted a $(AG)_{10}$ repeatas 11.7 kb upstream of 28.105, placing it between genes 28.103 and 28.104.

Further primer pairs were designed to the putative PRSV-P diseases resistance gene LRR28.12 on SC28 using Primer 3 software (http://frodo.wi.mit.edu/ primer3; Rozen and Skaletsky 2000). Optimum primer length was 20 nucleotides, optimum melting temperature (Tm) of primers was 60ºC, optimum primer GC content was 50% and maximum self-complementarity at the 3'-end was 3.00. The primer pairs were then tested again for their self-complementarity using the Oligo Analysis Tool Kit (http://www.operon.com/tools/oligo-analysis-tool.aspx). Details of primers are shown in Table 5.3.

Table 5.3: Primers on supercontig 28 used in the flesh colour study

Primer name	Primer Sequences	Primer Tm (ºC)
SSR28.50	Forward: GCGTGCAACACTTTTCTCC	63.4
	Reverse: AAAAACTGCGTGAGATGTCG	62.8
SSR28.106	Forward: ACACCATTGTCAGCTCAACG	63.8
	Reverse: TGGTATTGGTTTCAGCATGG	63.3
SSR28.103-104	Forward: GTTGGACGCATCTACTCACG	63.3
	Reverse: CCTCTTCGACTACGCACACC	64.8
LRR28.12.1	Forward: TTCCTCCTCTTCCTCCTCCT	59.37
	Reverse: TCATTTGGTATTTCGCCAGA	59.11
LRR28.12.2	Forward: CAATCCCTCCTTTCAACCAA	59.90
	Reverse: TATTCATCTCCCGCATCCTC	60.00
LRR28.12.3	Forward: CAACAGGAGAGGGACACACA	59.70
	Reverse: TTGGGATGAACCAGAGGAAG	60.04

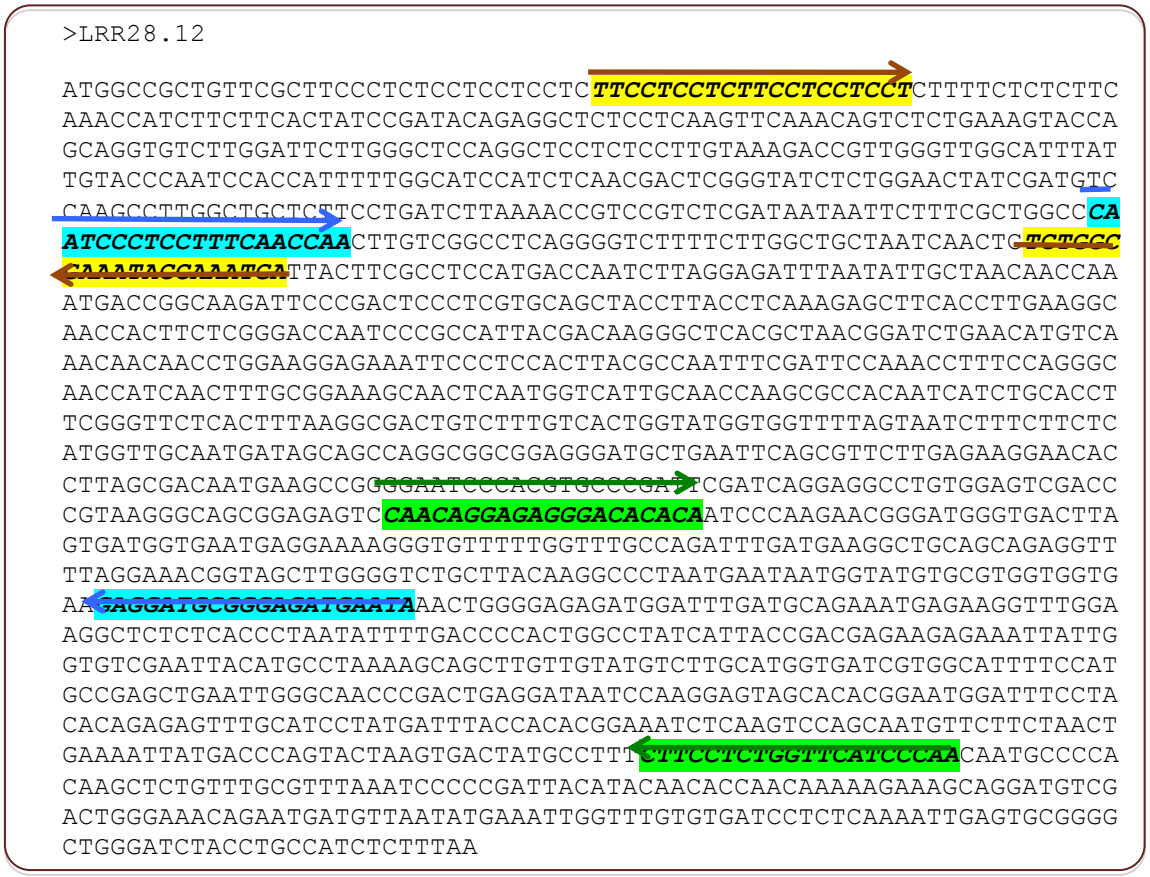

Figure 5.2: Nucleotide sequence of LRR28.12 (Zhu, personal communication) and priming site of primer LRR28.12-1 (highlighted in yellow), LRR28.12-2 (highlighted in blue) and LRR28.12-3 (highlighted in green). The arrows indicated direction of amplification for each primer, where → were forward primers and ← were reverse primers

5.3.3 PCR cycle optimisation

PCR reactions were optimised by using pooled DNA of 'Yellow' and 'Red' as the DNA templates. The components for each reaction and the PCR conditions are detailed in Chapter 2, 2.2.3. The reactions were optimised at eight different annealing temperatures using gradient function between 50 and 60ºC in MyCycler PCR machine (BioRad). The 2-step PCR cycle (detailed in Chapter 2, Figure 2.4) was applied to primer CPFC2 because it produced a dominant marker only in 'Yellow' but no amplification was detected in 'Red'.

5.3.4 DNA sequencing

The PCR products that were amplified from primers pairs were amplified in triplicate then purified and sequenced using protocols described in Chapter 2, 2.4.

5.4 Fragments and gene analysis

DNA sequences of forward and reserves strands were analysed and trimmed at Phred scores higher than 30 using 4peak software version 1.7.1 (Griekspoor and Groothuis, www.mekentosj.com). The sequences were initially aligned using the alignment tool within the NCBI database, and then by using CLUSTALW to obtain a longer sequence. Sequences for each set of primers for both flesh colours were aligned using CLUSTALW to search for dissimilarity between the alleles from the two flesh colours. The sequences were then BLASTed to NCBI and papaya databases on www.phytozome.net to search for similarity to sequences from papaya and other related crops.

Correlation between marker and flesh colour were analysed and calculated for percentage of association using Equation 5.1.

$$\text{Percentage of association (\%)} = 100 - \frac{\text{number of mismatchs between marker and expression x 100}}{\text{total number of plants}}$$

Equation 5.1: Calculation of percentage of association between marker and trait

RESULTS:

5.5 DNA polymorphism between yellow and red bulked DNAs

5.5.1 Amplicon size differentiation using published DNA markers linked to flesh colour

Three primers successfully amplified products from both bulked DNAs. Fragments of 399 bp, 566 bp and 316 bp were amplified from primer pairs CPFC1, CPFC2 and CPFC3, respectively (Figure 5.3). The CPFC2 marker was dominant, present in the yellow bulk and absent in the red bulk. The other two markers, CPFC1 and CPFC3, were dominant but present in both bulks.

When the 2-step PCR cycle was applied to the samples with the CPFC2 primers, two distinct alleles appeared at this locus of 600 bp and 900 bp, which were correlated with yellow and red flesh, respectively (Figure 5.4).

5.5.2 Sequence polymorphism among amplicons

Sequence comparision of the CPFC1 fragment amplified from the 'Yellow' and 'Red' bulks uncovered base substitutions at nucleotides 182 and 193 (Figure 5.5). A similar result was found comparing the CPFC3 sequence among the bulks. Base substitutions were observed at nucleotides 124, 131, 154, 219 and 256. Additionally, a single base insertion was present at 214 bp in the yellow bulk DNA (Figure 5.6).

5.5.3 Sequence alignment of amplified markers

The BLAST results showed a 98-100% identity for each of the three loci-generated sequences among the bulks to two *C. papaya* BAC clones, DM105M02 (accession number GQ478572) and SH18O09 (accession number GQ478573) (Table 5.4). The yellow flesh related sequences were most similar to DM105M02, and the red flesh associated sequences were most similar to SH18O09. All of the sequences queried covered only 0% of the target sequences, but of these, 98-100% were similar to the database sequence fragments.

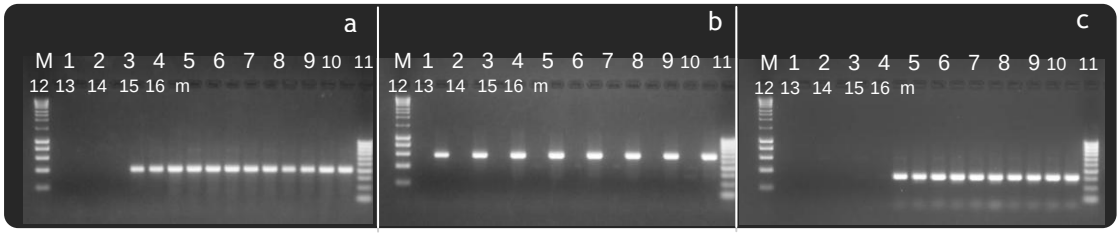

Figure 5.3: Optimisation of PCR using published DNA markers linked to flesh colour (a-CPFC1; b-CPFC2; and c-CPFC3) in bulk DNAs of 'Red' (lanes which were indicated in odd number) and 'Yellow' (lane which were indicated in even number). The reactions were performed at eight different annealing temperatures

M and m are DNA molecular weight markers, HyperLadder I and IV (Bioline), respectively. Lane 1, 2 at 65ºC; lane 3,4 at 64ºC; lane 5,6 at 62ºC; lane 7,8 at 59ºC; lane 9, 10 at 55ºC; lane 11, 12 at 53ºC; lane 13, 14 at 51ºC, lane 15, 16 at 50ºC

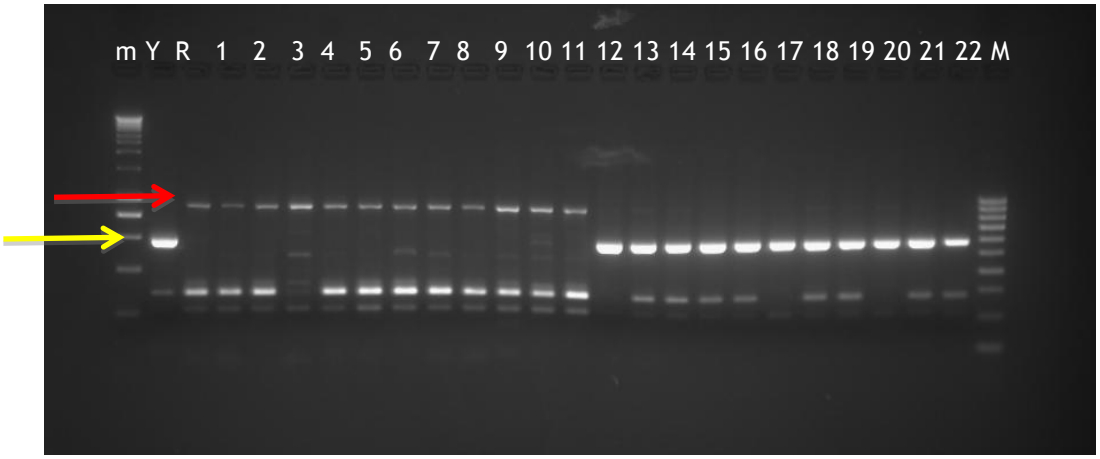

Figure 5.4: DNA markers associated with yellow (yellow arrow) and red (red arrow) flesh colour in individuals of each bulked DNA. Lane Y and R are bulked DNA of yellow and red fleshed fruit, respectively. Lane 1-11 are individual DNAs of red flesh colour used in 'Red Bulk'. Lane 12-22 are individual DNAs of yellow flesh colour used in 'Yellow Bulk'. Lane m amd M are DNA molecular weight marker HyperLadder I and IV (Bioline), respectively

```
CPFC1-Yellow      GTCCGACAAAAAAATTTAAATTTTACAAATTGATTAAGTGTCTAATTAAATATTTTATTA    60
CPFC1-Red         GTCCGACAAAAAAATTTAAATTTTACAAATTGATTAAGTGTCTAATTAAATATTTTATTA    60
                  ************************************************************

CPFC1-Yellow      AATTAAAAAATTTATTAAACGTTTGATTAGATATTTCGTTTCGTTCATTAAAACTACATA    120
CPFC1-Red         AATTAAAAAATTTATTAAACGTTTGATTAGATATTTCGTTTCGTTCATTAAAACTACATA    120
```

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                  ************************************************************

CPFC1-Yellow      ATTAAAAATGACGATATCACAATTACGCCAACTAAAAATTTTATTGAAATTATGTTGTAA    180
CPFC1-Red         ATTAAAAATGACGATATCACAATTACGCCAACTAAAAATTTTATTGAAATTATGTTGTAA    180
                  ************************************************************

CPFC1-Yellow      ACAACTTATTGAGCATCTAACTAATTAAAATTTCATAATTAAAAATTCAAACAATTATTT    240
CPFC1-Red         ATAACTTATTGAACATCTAACTAATTAAAATTTCATAATTAAAAATTCAAACAATTATTT    240
                  * ********** ********************************************

CPFC1-Yellow      GAGTCACTGGAGAAATAAAATAAAATATGTCTTATATTAAGTGTTAAAATAAAAAATTTA    300
CPFC1-Red         GAGTCACTGGAGAAATAAAATAAAATATGTCTTATATTAAGTGTTAAAATAAAAAATTTA    300
                  ************************************************************

CPFC1-Yellow      ATAAAAATATAATTAATTATATAACCTGTACTAGATTCGAACTGGAAATGATGTTATATT    360
CPFC1-Red         ATAAAAATATAATTAATTATATAACCTGTACTAGATTCGAACTGGAAATGATGTTATATT    360
                  ************************************************************

CPFC1-Yellow      ATGTCATAAAAATTTAAAGTTTAAATTATTACAAAATTT   399
CPFC1-Red         ATGTCATAAAAATTTAAAGTTTAAATTATTACAAAATTT   399
                  ***************************************
```

Figure 5.5: Alignment of DNA sequences of marker CPCF1 in bulked DNA of yellow (CPFC1-Yellow) and red (CPFC1-Red)

```
CPFC3-Yellow      TTGCAAAGAAAGGAGGGTTTCTATAACATCATCAGGGTCTTTGTTTGTGACACATAATGA    60
CPFC3-Red         TTGCAAAGAAAGGAGGGTTTCTATAACATCATCAGGGTCTTTGTTTGTGACACATAATGA    60
                  ************************************************************

CPFC3-Yellow      ATTGTATCATCTAGTTGACTGACAGAAGCAATTCCCATGGACCATGCAGGTTATGATGTT    120
CPFC3-Red         ATTGTATCATCTAGTTGACTGACAGAAGCAATTCCCATGGACCATGCAGGTTATGATGTT    120
                  ************************************************************

CPFC3-Yellow      TTCCATGTTTGTTTCTGCAAAGAACATTGTTATGCATTGACTGGCATTTACTTGACTACA    180
CPFC3-Red         TTCTATGTTTATTTCTGCAAAGAACATTGTTATACATTGACTGGCATTTACTTGACTACA    180
                  *** ****** ********************** ************************

CPFC3-Yellow      TGTTATACTTAACTGTCCTGGTGATCTTTTAGGTATTAGGGCATTTGATGCAAGATTAAT    240
CPFC3-Red         TGTTATACTTAACTGTCCTGGTGATCTTTTAGG-ATTACGGCATTTGATGCAAGATTAAT    239
                  ******************************** **** ********************

CPFC3-Yellow      CATCTTTCCTCTTCAGGATATTTCTTTTCTCATCAACCCAACAAGAAGTTAATTTGTTTG    300
CPFC3-Red         CATCTTTCCTCTTCAAGATATTTCTTTTCTCATCAACCCAACAAGAAGTTAATTTGTTTG    299
                  *************** ******************************************

CPFC3-Yellow      GCTCAGAAGGATTTCA    316
CPFC3-Red         GCTCAGAAGGATTTCA    315
```

Figure 5.6: Alignment of DNA sequences of marker CPCF3 in bulked DNA of yellow (CPFC3-Yellow) and red (CPFC3-Red)

Table 5.4: BLAST result of nucleotide sequences for CPFC1, CPFC2 and CPFC3 markers in bulked DNA of yellow and red fleshed papaya on NCBI database

Marker	*C. papaya* clone BAC DM105M02, complete sequence Accession #GQ478572.2				*C. papaya* clone BAC SH18O09, complete sequence, Accession #GQ478573.2			
	Max score	Total score	E value	Ident	Max score	Total score	E value	Ident
CPFC1-Yellow	739	739	0.0	100%	728	728	0.0	99%
CPFC1-Red	726	726	0.0	99%	737	737	0.0	100%
CPFC2-Yellow	1046	1046	0.0	100%	520	520	7e-150	99%
CPFC3-Yellow	425	478	2e-121	99%	545	545	1e-157	98%
CPFC3-Red	409	462	2e-116	98%	573	573	5e-166	99%

The alignment of BAC clones DM105M02, SH18O09 and all the sequences from the CPFC primer set as listed on Table 5.2 were aligned to each other by using DM105M02 as the template. The query of SH18O09 covered 89% of DM105M02. The BAC clones showed 99% identity with an E value of 0.00. Each CPFC fragment aligned to each BAC clones at a different place. CPCF2-Yellow aligned 100% to DM105M02, but it was located at the insertion/deletion between the sequence alignments of the two BAC clones.

5.5.4 Amplification using novel markers from SC28

All primers as listed in Table 5.3 were successfully amplified in bulked DNA of both red and yellow-fleshed papaya. Base substitutions were found in SSR-28.50 but the other two SSR markers did not contain any sequence polymorphisms between the two bulks at those loci (Figure 5.7, 5.8 and 5.9).

The set of primers for 28.12, which targeted gene LRR28.12, successfully amplified a fragment in both bulk DNAs that were 100% identical (Figure 5.10, 5.11 and 5.12). Due to the overlapping of each primer pair (Figure 5.2), the nucleotide sequences of 28.12.1, 28.12.2 and 28.12.3 were aligned and of the contigs 28.12-Red for the 'Red' bulk DNA and 28.12-Yellow for the 'Yellow' bulk DNA were assembled and confirmed to be 100% identical. Interestingly, a 137 bp insertion, from position 1257 to 1394 bp was detected in this sequence when compared to the sequence of LRR28.12 sourced from the papaya genome database deduced amino acids remained the same due to the insertion of intron 1 was detected (Figure 5.13).

```
28.50-YELLOW      ------CTCTTTTTTCGGTCATGCACCATACTCACTTCCTCCTCCTCCTCCTCCTTCTTC    54
28.50-RED         ACACAACTCTTTTTTCGGTCATGCACCATACTCACTTCCTCCTCCTCCTCCTCCTTCTTC    60
                        **************************************************
```

```
28.50-YELLOW      TTTTTCTTCTTCTTCTCCTTCTCCTTCTTTTTCTTCTTCTTCTTCTCCTTCTCCTTTTTT    114
28.50-RED         TTTTTCTTCTTCTTCTCCTTCTCCTTCTTTTTCTTCTTCTTCTTCTCCTTCTCCTTTTTT    120
                  ************************************************************

28.50-YELLOW      TCTTCTTCTTCTTCTCCTTTTTTTTCTTCTTCTTCTTTTCCTTCTTTTCATCTTCATCTT    174
28.50-RED         TCTTCTTCTTCTTCTCCTTTTTTTTCTTCTTCTTCTTTTCCTTCTTTTCATCTTCATCTT    180
                  ************************************************************

28.50-YELLOW      CTTCTTCTCCTTCTTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTTTTCTTCTCTTCT    234
28.50-RED         CTTCTTCTCCTTCTTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTTTTCTTTCTTCT    240
                  ************************************************** ******

28.50-YELLOW      TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT------------------          273
28.50-RED         TCTTCTTCTTCTTCTTCTTTTTCTTCTTCTTCTTCTTCTTGGCGGCAGCGCGTGCCG      298
                  ****************** ******************
```

Figure 5.7: Alignment of DNA sequences of marker 28.50 in bulked DNA of yellow (28.50-Yellow) and red (28.50-Red) papayas. Blue colour fonts show the target microsatellite sequences

```
28.103-YELLOW     GATCGGGATTGAGAGGACATCTTTGAATGGTCTGGATCCCTTCTCACAATGGACGACCAC    60
28.103-Red        --TCGGGATTGAGAGGACATCTTTGAATGGTCTGGATCCCTTCTCACAATGGACGACCAC    58
                    ************************************************************

28.103-YELLOW     GATTTAATTTCACTTATATATAAAAAATTATGCTTTTACAAAAAAGAGAGGACAAGAAAT    120
28.103-Red        GATTTAATTTCACTTATATATAAAAAATTATGCTTTTACAAAAAAGAGAGGACAAGAAAT    118
                  ************************************************************

28.103-YELLOW     CCTAAGAGAGAGAGAGAGAGAGA   143
28.103-Red        CCTAAGAGAGAGAGAGAGAG-     140
                  **********************
```

Figure 5.8: Alignment of DNA sequences of marker 28.103 in bulked DNA of yellow (28.103-Yellow) and red (28.103-Red) papayas. Blue colour fonts show the target microsatellite sequences

```
28.106-Yellow     TTCAATGCAGTGTACGACAGAATTACCCACAAGACAAGATGGACAAATCTTTCATTGTTG
```

```
28.106-Red          TTCAATGCAGTGTACGACAGAATTACCCACAAGACAAGATGGACAAATCTTTCATTGTTG
                    ************************************************************

28.106-Yellow       CTGACTTGACAATAGTGTATGTATAAGCAGAAAAACATGGTGAATGATCATTTTCTTTTC
28.106-Red          CTGACTTGACAATAGTGTATGTATAAGCAGAAAAACATGGTGAATGATCATTTTCTTTTC
                    ************************************************************

28.106-Yellow       TTTTCTTTTCTTTTCTTCTTATTTTCGTTTTTCAGAGCTGGACCCTGAAGGTATTAAAGT
28.106-Red          TTTTCTTTTCTTTTCTTCTTATTTTCGTTTTTCAGAGCTGGACCCTGAAGGTATTAAAGT
                    ************************************************************

28.106-Yellow       ATTGGTTTTCACCTGTACTAAGATTAGAAGTAGATCATCCATGCTGAAACCAATACCA
28.106-Red          ATTGGTTTTCACCTGTACTAAGATTAGAAGTAGATCATCCATGCTGAAACCAATACCA
                    ************************************************************
```

Figure 5.9: Alignment of DNA sequences of marker SSR28.106 in bulked DNA of yellow (28.106-Yellow) and red (28.106-Red) papayas. Blue colour fonts show the target microsatellite sequences

```
28.12.1-Red         ----------CCTCCTCCTCTTTTCTCTCTTCAAACCATCTTCTTCACTATCCGATACA   49
28.12.1-Yellow      TTCCTCCTCTTCCTCCTCCTCTTTTCTCTCTTCAAACCATCTTCTTCACTATCCGATACA   60
                              *********************************************

28.12.1-Red         GAGGCTCTCCTCAAGTTCAAACAGTCTCTGAAAGTACCAGCAGGTGTCTTGGATTCTTGG   109
28.12.1-Yellow      GAGGCTCTCCTCAAGTTCAAACAGTCTCTGAAAGTACCAGCAGGTGTCTTGGATTCTTGG   120
                    ************************************************************

28.12.1-Red         GCTCCAGGCTCCTCTCCTTGTAAAGACCGTTGGGTTGGCATTTATTGTACCCAATCCACC   169
28.12.1-Yellow      GCTCCAGGCTCCTCTCCTTGTAAAGACCGTTGGGTTGGCATTTATTGTACCCAATCCACC   180
                    ************************************************************

28.12.1-Red         ATTTTTGGCATCCATCTCAACGACTCGGGTATCTCTGGAACTATCGATGTCCAAGCCTTG   229
28.12.1-Yellow      ATTTTTGGCATCCATCTCAACGACTCGGGTATCTCTGGAACTATCGATGTCCAAGCCTTG   240
                    ************************************************************

28.12.1-Red         GCTGCTCTTCCTGATCTTAAAACCGTCCGTCTCGATAATAATTCTTTCGCTGGCCCAATC   289
28.12.1-Yellow      GCTGCTCTTCCTGATCTTAAAACCGTCCGTCTCGATAATAATTCTTTCGCTGGCCCAATC   300
                    ************************************************************

28.12.1-Red         CCTCCTTTCAACCAACTTGTCGGCCTCAGGGGTCTTTTCTTGGCTGCTAATCAACTCTCT   349
28.12.1-Yellow      CCTCCTTTCAACCAACTTGTCGGCCTCAGGGGTCTTTTCTTGGCTGCTAATCAACTCTCT   360
                    ************************************************************

28.12.1-Red         GGCGAAATACCAAATGA 366
28.12.1-Yellow      GGCGAAATACCAAATGA 377
                    *****************
```

Figure 5.10: Alignment of DNA sequences of marker 28.12.1 in bulked DNA of red (28.12.1-Red) and yellow fleshed (28.12.1-Yellow) papayas

```
28.12.2-Red         ----------------------------------------------------------AC   2
28.12.2-Yellow      CAATCCCTCCTTTCAACCAACTTGTCGGCCTCAGGGGTCTTTTCTTGGCTGCTAATCAAC   60
                                                                             **

28.12.2-Red         TCTCTGGCGAAATACCAAATGATTACTTCGCCTCCATGACCAATCTTAGGAGATTTAATA   62
28.12.2-Yellow      TCTCTGGCGAAATACCAAATGATTACTTCGCCTCCATGACCAATCTTAGGAGATTTAATA   120
                    ************************************************************

28.12.2-Red         TTGCTAACAACCAAATGACCGGCAAGATTCCCGACTCCCTCGTGCAGCTACCTTACCTCA   122
28.12.2-Yellow      TTGCTAACAACCAAATGACCGGCAAGATTCCCGACTCCCTCGTGCAGCTACCTTACCTCA   180
                    ************************************************************

28.12.2-Red         AAGAGCTTCACCTTGAAGGCAACCACTTCTCGGGACCAATCCCGCCATTACGACAAGGGC   182
28.12.2-Yellow      AAGAGCTTCACCTTGAAGGCAACCACTTCTCGGGACCAATCCCGCCATTACGACAAGGGC   240
                    ************************************************************

28.12.2-Red         TCACGCTAACGGATCTGAACATGTCAAACAACAACCTGGAAGGAGAAATTCCCTCCACTT   242
28.12.2-Yellow      TCACGCTAACGGATCTGAACATGTCAAACAACAACCTGGAAGGAGAAATTCCCTCCACTT   300
                    ************************************************************

28.12.2-Red         ACGCCAATTTCGATTCCAAACCTTTCCAGGGCAACCATCAACTTTGCGGAAAGCAACTCA   302
28.12.2-Yellow      ACGCCAATTTCGATTCCAAACCTTTCCAGGGCAACCATCAACTTTGCGGAAAGCAACTCA   360
                    ************************************************************

28.12.2-Red         ATGGTCATTGCAACCAAGCGCCACAATCATCTGCACCTTCGGGTTCTCACTTTAAGGCGA   362
28.12.2-Yellow      ATGGTCATTGCAACCAAGCGCCACAATCATCTGCACCTTCGGGTTCTCACTTTAAGGCGA   420
                    ************************************************************

28.12.2-Red         CTGTCTTTGTCACTGGTATGGTGGTTTTAGTAATCTTTCTTCTCATGGTTGCAATGATAG   422
28.12.2-Yellow      CTGTCTTTGTCACTGGTATGGTGGTTTTAGTAATCTTTCTTCTCATGGTTGCAATGATAG   480
                    ************************************************************

28.12.2-Red         CAGCCAGGCGGCGGAGGGATGCTGAATTCAGCGTTCTTGAGAAGGAACACCTTAGCGACA   482
28.12.2-Yellow      CAGCCAGGCGGCGGAGGGATGCTGAATTCAGCGTTCTTGAGAAGGAACACCTTAGCGACA   540
                    ************************************************************

28.12.2-Red         ATGAAGCCGGGGAATCCCACGTGCCCGATTCGATCAGGAGGCCTGTGGAGTCGACCCGTA   542
28.12.2-Yellow      ATGAAGCCGGGGAATCCCACGTGCCCGATTCGATCAGGAGGCCTGTGGAGTCGACCCGTA   600
                    ************************************************************

28.12.2-Red         AGGGCAGCGGAGAGTCCAACAGGAGAGGGACACACAATCCCAAGAACGGGATGGGTGACT   602
28.12.2-Yellow      AGGGCAGCGGAGAGTCCAACAGGAGAGGGACACACAATCCCAAGAACGGGATGGGTGACT   660
                    ************************************************************

28.12.2-Red         TAGTGATGGTGAATGAGGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAG   662
28.12.2-Yellow      TAGTGATGGTGAATGAGGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAG   720
                    ************************************************************

28.12.2-Red         CAGAGGTTTTAGGAAACGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTA   722
28.12.2-Yellow      CAGAGGTTTTAGGAAACGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTA   780
                    ************************************************************

28.12.2-Red         TGTGCGTGGTGGTGAAGAGG------      742
28.12.2-Yellow      TGTGCGTGGTGGTGAAGAGGATGCGG     806
                    ********************
```

Figure 5.11: Alignment of DNA sequences of marker 28.12.2 in bulked DNA of red (28.12.2-Red) and yellow fleshed (28.12.2-Yellow) papayas

```
28.12.3-Red         -------------------------AAGAACGGGATGGGTGACTTAGTGATGGTGAATGA   35
28.12.3-Yellow      CAACAGGAGAGGGACACACAATCCCAAGAACGGGATGGGTGACTTAGTGATGGTGAATGA   60
                                             **********************************

28.12.3-Red         GGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAGCAGAGGTTTTAGGAAA   95
28.12.3-Yellow      GGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAGCAGAGGTTTTAGGAAA   120
                    ************************************************************

28.12.3-Red         CGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTATGTGCGTGGTGGTGAA   155
28.12.3-Yellow      CGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTATGTGCGTGGTGGTGAA   180
                    ************************************************************

28.12.3-Red         GAGGATGCGGGAGATGAATAAACTGGGGAGAGATGGATTTGATGCAGAAATGAGAAGGTT   215
28.12.3-Yellow      GAGGATGCGGGAGATGAATAAACTGGGGAGAGATGGATTTGATGCAGAAATGAGAAGGTT   240
                    ************************************************************

28.12.3-Red         TGGAAGGCTCTCTCACCCTAATATTTTGACCCCACTGGCCTATCATTACCGACGAGAAGA   275
28.12.3-Yellow      TGGAAGGCTCTCTCACCCTAATATTTTGACCCCACTGGCCTATCATTACCGACGAGAAGA   300
                    ************************************************************

28.12.3-Red         GAAATTATTGGTGTCGAATTACATGCCTAAAAGCAGCTTGTTGTATGTCTTGCATGGTAG   335
28.12.3-Yellow      GAAATTATTGGTGTCGAATTACATGCCTAAAAGCAGCTTGTTGTATGTCTTGCATGGTAG   360
                    ************************************************************

28.12.3-Red         GAAACACTCTCTTCATTTTTCCATTATATACAAATATTTTTCATTATATAGGATGTCTTT   395
28.12.3-Yellow      GAAACACTCTCTTCATTTTTCCATTATATACAAATATTTTTCATTATATAGGATGTCTTT   420
                    ************************************************************

28.12.3-Red         AAACTGTCTGAATCGGTGAGCGTTTCTCTCATCAAGACAATACGGAATTTAGATGGTTTG   455
28.12.3-Yellow      AAACTGTCTGAATCGGTGAGCGTTTCTCTCATCAAGACAATACGGAATTTAGATGGTTTG   480
                    ************************************************************

28.12.3-Red         TTTATGATCTTCAGGTGATCGTGGCATTTTCCATGCCGAGCTGAATTGGGCAACCCGACT   515
28.12.3-Yellow      TTTATGATCTTCAGGTGATCGTGGCATTTTCCATGCCGAGCTGAATTGGGCAACCCGACT   540
                    ************************************************************

28.12.3-Red         GAGGATAATCCAAGGAGTAGCACACGGAATGGATTTCCTACACAGAGAGTTTGCATCCTA   575
28.12.3-Yellow      GAGGATAATCCAAGGAGTAGCACACGGAATGGATTTCCTACACAGAGAGTTTGCATCCTA   600
                    ************************************************************

28.12.3-Red         TGATTTACCACACGGAAATCTCAAGTCCAGCAATGTTCTTCTAACTGAAAATTATGACCC   635
28.12.3-Yellow      TGATTTACCACACGGAAATCTCAAGTCCAGCAATGTTCTTCTAACTGAAAATTATGACCC   660
                    ************************************************************

28.12.3-Red         AGTACTAAGTGACTATGCCTTTCTTCCTCTGGTTCATCCCAA      677
28.12.3-Yellow      AGTACTAAGTGACTATGCCTTTCTTCCTCT-----------      690
                    ****************************
```

Figure 5.12: Alignment of DNA sequences of marker 28.12.3 in bulked DNA of red (28.12.3-Red) and yellow fleshed (28.12.3-Yellow) papayas

```
LRR28.12          16 LLLFSLFKPSSSLSDTEALLKFKQSLKVPAGVLDSWAPGSSPCKDRWVG
                     LLLFSLFKPSSSLSDTEALLKFKQSLKVPAGVLDSWAPGSSPCKDRWVG
                     LLLFSLFKPSSSLSDTEALLKFKQSLKVPAGVLDSWAPGSSPCKDRWVG
28.12-Red          2 cccttctactttctgaggccatactcagcgggtgttgcgttctagctgg
                     ttttcttacccctcacacttataactatccgttacgccgcccgaaggtg
                     cccttccaattaactagtccgcagtgaaaatcgttgtacctttactgtc

LRR28.12          65 IYCTQSTIFGIHLNDSGISGTIDVQALAALPDLKTVRLDNNSFAGPIPP
                     IYCTQSTIFGIHLNDSGISGTIDVQALAALPDLKTVRLDNNSFAGPIPP
                     IYCTQSTIFGIHLNDSGISGTIDVQALAALPDLKTVRLDNNSFAGPIPP
28.12-Red        149 attactaatgaccagtgatgaaggcgtggccgcaagccgaattggcacc
                     tagcaccttgtataacgtcgctatactcctcatactgtaaactcgctcc
                     tttcaccttcctcccgtctatctcacgtttttacctcttttctcactt

LRR28.12          114 FNQLVGLRGLFLAANQLSGEIPNDYFASMTNLRRFNIANNQMTGKIPDS
                      FNQLVGLRGLFLAANQLSGEIPNDYFASMTNLRRFNIANNQMTGKIPDS
                      FNQLVGLRGLFLAANQLSGEIPNDYFASMTNLRRFNIANNQMTGKIPDS
28.12-Red         296 taccggcagcttggacctggacagttgtaaacaataagaacaagaacgt
                      taattgtggtttccaatcgatcaaatcctcatggtatcaaatcgatcac
                      ccatcccgttcgtttactcaaattccccgcttgattttccagccgtccc

LRR28.12          163 LVQLPYLKELHLEGNHFSGPIPPLRQGLTLTDLNMSNNNLEGEIPSTYA
                      LVQLPYLKELHLEGNHFSGPIPPLRQGLTLTDLNMSNNNLEGEIPSTYA
                      LVQLPYLKELHLEGNHFSGPIPPLRQGLTLTDLNMSNNNLEGEIPSTYA
28.12-Red         443 cgccctcagcccggacttgcacctccgcacagcaataaacgggactatg
                      ttatcataatatagaatcgctcctgagtctcatatcaaatagatcccac
                      cggatccagtctaccccgaacgaaaagcgagtgcgacccgaaatcctcc

LRR28.12          212 NFDSKPFQGNHQLCGKQLNGHCNQAPQSSAPSGSHFKATVFVTGMVVLV
                      NFDSKPFQGNHQLCGKQLNGHCNQAPQSSAPSGSHFKATVFVTGMVVLV
                      NFDSKPFQGNHQLCGKQLNGHCNQAPQSSAPSGSHFKATVFVTGMVVLV
28.12-Red         590 atgtactcgaccctgaccagctacgccttgctgtctagagtgagaggtg
                      atacactagaaatggaatagagaaccacccccgcatacctttcgttttt
                      tctcatcgcctatcagactttccagaaatatgttctggtctcttggtaa

LRR28.12          261 IFLLMVAMIAARRRRDAEFSVLEKEHLSDNEAGESHVPDSIRRPVESTR
                      IFLLMVAMIAARRRRDAEFSVLEKEHLSDNEAGESHVPDSIRRPVESTR
                      IFLLMVAMIAARRRRDAEFSVLEKEHLSDNEAGESHVPDSIRRPVESTR
28.12-Red         737 atccaggaaggaccagggtagcgagccagaggggtcgcgtaaacggtac
                      ttttttcttccggggacatgttaaaatgaaacgacatcactggctaccg
                      cttcgtagaacggggttaccttggactcctacgaccgctgcggtgggct

LRR28.12          310 KGSGESNRRGTHNPKNGMGDLVMVNEEKGVFGLPDLMKAAAEVLGNGSL
                      KGSGESNRRGTHNPKNGMGDLVMVNEEKGVFGLPDLMKAAAEVLGNGSL
                      KGSGESNRRGTHNPKNGMGDLVMVNEEKGVFGLPDLMKAAAEVLGNGSL
28.12-Red         884 agaggtaaagacacaagaggtgagaggaggtgtcgtaaggggggtgagat
                      agggacagggcaacaagtgattttaaaagttgtcattacccattgaggt
                      gccagccgagactcgcggtcagggtgagttttgatgggtaagtaactcg

LRR28.12          359 GSAYKALMNNGMCVVVKRMREMNKLGRDGFDAEMRRFGRLSHPNILTPL
                      GSAYKALMNNGMCVVVKRMREMNKLGRDGFDAEMRRFGRLSHPNILTPL
                      GSAYKALMNNGMCVVVKRMREMNKLGRDGFDAEMRRFGRLSHPNILTPL
28.12-Red        1031 gtgtagcaaagatgggaaacgaaacgaggtgggaaatgactccaataccc
                      gccaacttaagtgtttagtgataatggagtacatggtggtcacattcct
                      gttcgcagtttgcgggggggggtaggatattaagagtagctctttgcag

LRR28.12          408 AYHYRREEKLLVSNYMPKSSLLYVLH
                      AYHYRREEKLLVSNYMPKSSLLYVLH
                      AYHYRREEKLLVSNYMPKSSLLYVLH
28.12-Red        1178 gtctccggattgtatacaaatttgtc
                      caaaggaaatttcaatcaggttatta
                      cttcaaagaagggtcgtaccggtcgt
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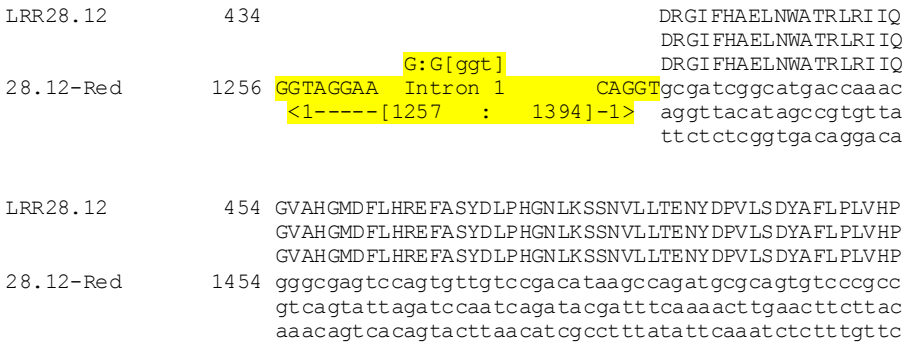

Figure 5.13: The result of Genewise comparison between the sequence of deduced amino acid of LRR28.12 and assembled DNA sequences of marker 28.12 of bulked DNA of red flesh (28.12-Red) papaya

5.6 Segregation of CPFC2 in population

Only marker CPFC2 was co-dominant and size polymorphic among the bulks, therefore, only this locus marker was investigated further in the current population of 28 lines. Figure 5.4 shows the newly developed markers at 900 and 600 bp, which were indicated by red and yellow arrows, respectively. The 900 bp marker was potentially linked to red flesh colour and was designated as CPCF2-R. The marker at 600 bp was a candidate to link to yellow flesh colour and was designated as CPCF2-Y. When screened for consistent association among phenotype (colour) and genotype (marker allele), there was a mismatch rate for CPCF2-R and CPCF2-Y of 2 and 6, repectively. This equated to a 97% and 93% association to red and yellow flesh, respectively.

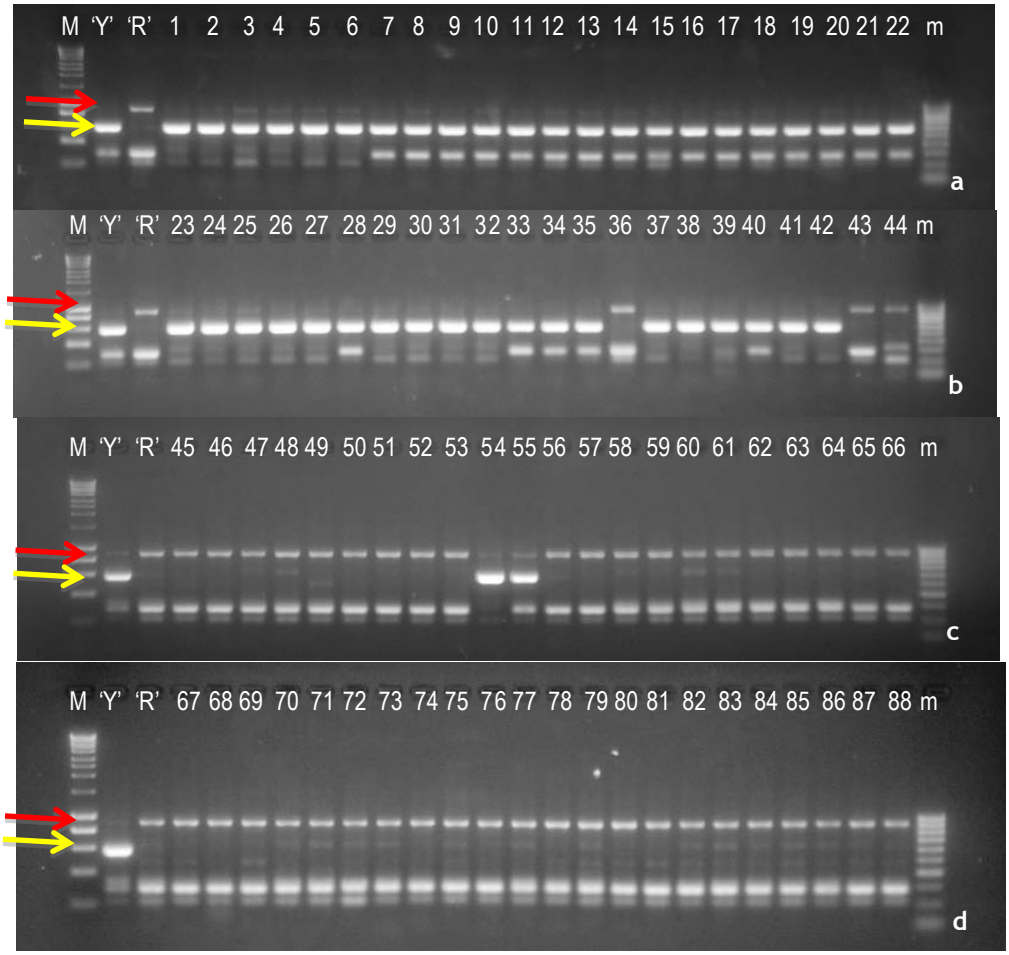

Figure 5.14: Segregation of CPCF2-R (→) and CPCF2-Y (→) in current breeding lines. Lane 'Y' and 'R' are bulked DNA of yellow and red fleshed papayas, respectively. Lane 1-44 represent individual DNAs of yellow fleshed papaya. Lane 45-88 are individual DNAs of red fleshed papayas. Lane m amd M are DNA molecular weight marker HyperLadder I and IV (Bioline), respectively. Lane 36, 43 and 44 were mismatches of the presence of marker CPCF2-R in yellow fleshed papayas. Lane 54 and 55 were mismatches of the presence of marker CPCF2-Y in red flesh papayas

DISCUSSION:

The current study demonstrated transferability of three SCAR markers developed by Blas et al. (2010) for papaya fruit flesh colour to wide range of papaya genotypes. CPFC1 and CPFC3 were reported to be co-dominant markers that can differentiate between yellow and red flesh colour. Although SCAR marker CPFC1 was reported to have approximately 98% identity to fruit flesh colour genes (Blas et al. 2010), this marker was non-polymorphic between the two colour bulk DNAs used in this study. Conversely, the CPFC2 was polymorphic and quite accurately discriminated between the yellow (93%) and red (97%) fruit bearing plants with. A small number of plants were misclassified with the CPFC2 marker most likely due to the chance of recombination and hence loss of linkage between the actual gene(s) conditioning the colour trait and the marker developed. CPFC1, which was reported to be tightly linked marker, located 580 bp away from the target gene, was still not 100% accurate due to the extremely high recombination rate (Blas et al. 2010). Data of F_2 mapping populations indicated the local recombination rate in the *CpCYC-b* region were more than 100-fold and 82-fold higher than the genome average when using KD × 2H94 (n = 219) and AU9 × SunUp (n = 54), respectively. High-density genetic mapping placed the flesh colour locus near the end of LG5 (Ma et al. 2004; Chen et al. 2007; Blas et al. 2009) indicating its position near the telomere, which is commonly associated with plant recombination hot spot (Mézard 2006).

Also, it is likely that colour is conditioned by multiple quantitative genes with varying functional influence. Therefore, it is possible that in some individuals, the additional genic component is not captured leading to a mismatch between trait and prediction based on the CPFC2 marker itself. Additionally, the colour trait is likely influenced greatly by environmental factors. There are several reports showing intensity of flesh colour of papaya varied in different temperature, which may relate to ripening process (Akamine 1966; Broughton et al. 1977; Nazeeb and Broughton 1978). This could be due to the expression of fruit flesh colour, which is affected by environment (result from Chapter 4). However, this hypothesis needs to be confirmed in a different segregating population when it becomes available.

Currently, there is no report of QTL study on flesh colour in papaya, however, there are reports of QTL to flesh colour in other fruit crops. The study of flesh colour in sweet cherry indicated the trait was controlled by one major gene with another two minor QTLs. The major QTL explained the attribute for 84.7% (Sooriyapathirana et al. 2010). Kinkade and Foolad (2013) reported two major QTLs, lyc7.1 and lyc12.1, involved in flesh colour in tomato. The lyc12.1 increased the lycopene content of ripe tomato fruits by 52-70%. Flesh colour in tomato is regulated by carotenoid biosynthesis pathway, which is the same pathway as flesh colour in papaya (Skelton et al. 2006).

Both the newly developed markers (SSR and EST) were located at the other end of LG5 from *CpCYC-b* locus. This could be a reason for the primers from SC28 to unable to detect variation of DNA between the two flesh colours in these regions, as they are located too far away from each other.

CONCLUSION:

To improve eating quality of papaya is the main goal of thepapaya breeding program. Fruit flesh colour is one of the important traits for breeders, consumers and the Australian market. Marker

assisted selection can speed up a breeding program by enabling selection for traits at the seedling stage. This research developed DNA markers linked to red flesh, CPFC2-R, and yellow flesh, CPFC2-Y. These markers identifed the trait at 95.75% in 330 breeding lines, where CPFC2-R and CPFC2-Y showed 96.64% and 93.48% homology to red and yellow fleshed plants, respectively. These markers will be validated in future research in a segregating population to confirm the linkage between the trait and the markers.

Marker Assisted Breeding of Papaya to Develop new Commercial lines

Project Leader: Professor Roderick Drew

Research Provider: Griffith University

Project Number: PP10005

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1 Chifley Square
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

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Contents

Summary

One of the challenges facing the papaya industry in Australia is to grow disease resistant varieties that produce fruit with superior eating quality all year round. This goal can only be achieved through the availability of disease resistant, high yielding and flavoursome varieties. Breeding to improve current commercial varieties of Australian papaya is a necessity. The research presented here had two major aims.

A challenge for the Australian papaya industry is to improve eating quality of commercial varieties of both yellow and red papayas, and to enhance uniformity and yield of red papayas. Twenty-seven lines and breeding selections of papaya were evaluated for 11 productivity traits and 13 characteristics related to fruit quality based on both grower and consumer preferences. Trees were evaluated at three different harvesting times, April 2012, October 2012 and May 2013, to determine the extent to which differences represented the genetics of the individuals rather than environmental effects. Selection of parents for the next phase of a breeding program to develop segregating populations was based on consistent expression of the attributes of interest over the three harvest seasons. Five groups of traits (flavour, skin quality, eating quality of red papaya, eating quality of yellow papaya and yield) were chosen for the production of segregating populations, which could be used for the development of DNA markers for those traits. Twenty-three representative trees were selected as parental lines and 17 crosses were made for the breeding programs to develop improved commercial lines and for a program of DNA marker-assisted selection.

To demonstrate the application of DNA markers to improve efficiency of the seedling selection process, flesh fruit colour was chosen, as it is one of the important traits for papaya production in Australia. DNA markers with diagnostic alleles associated with red flesh, CPFC2-R, and yellow flesh, CPFC2-Y were developed by using information from published DNA markers. These markers identified the attribute at 95.75% in 330 breeding lines, where CPFC2-R and CPFC2-Y showed 97% and 93% association with red- and yellow-fleshed plants, respectively.

Information from this research can be applied to plant improvement through conventional breeding, marker assisted breeding and genetic modification. In addition, segregation populations have been developed for future breeding work and for the development of molecular markers for a wide range of character traits. This will facilitate future research and enhance selection of new commercial varieties.

Work is progressing for the development of PRSV-P resistant genotypes that can be used to cross this virus resistance into elite papaya lines. Resistance genes from V. pubescens have been crossed into susceptible species V. parviflora. The resistant V. parviflora has been crossed top papaya and plants containing the R genes have been identified in vitro by a DNA marker after embryo rescue of hybrid embryos. These plants will be tested for fertility in the next phase of the project.

Keywords

Papaw, papaya, DNA markers, MAS, breeding, papaya ringspot virus, micropropagation, plant tissue culture

Introduction

Breeding programs in other major papaya producing countries have produced commercially named varieties (e.g. Solo, Tainung, Exotica), and promising breeding lines. However, Australia industry funded breeding programs undertaken by Queensland Government researchers for the last 20 years have not produced any new commercial or useful breeding lines. The commercial lines that are grown in Queensland were produced in a private breeding program. Currently, the commercial lines preferred by growers are 1B (yellow) and RB1, RB2 and RB4 (red). These lines are high yielding and produce fruit with clean skins, with good shape, and weight. This breeding program used a different approach to previous methods in Queensland. We aimed at maintaining the good characteristics of B1, RB1, RB2 and RB4 but incorporating some of the better traits of other available lines such as improved flavour, flesh texture, higher brix levels, longer shelf life, antracnose and phytophthora resistance. HAL projects FR99018 and PP04004 micropropagated and evaluated breeding lines that were produced in previous programs. These micropropagation systems were also employed in this project to maintain and multiply all parent lines, F1 plants and others that showed potential, and elite trees from grower properties. Although superior lines can be multiplied in tissue culture, the long term goal of this project is to produce hybrid lines that can be grown from seed OR micropropagated by tissue culture.

Rod Drew has had comprehensive breeding programs to develop PRSV-P resistance in papaya (papaw) genotypes for 20 years and recently achieved the first successful transfer of PRSV-P resistance from a *Vasconcellea* species (V. quercifolia) to papaya *(Carica papaya* L.). There are conflicting reports on PRSV-P resistance of *V. quercifolia*. It has been reported to be resistant to PRSV-P in Florida (Conover, 1964), Hawaii (Manshardt and Wenslaff, 1989b), and Australia (Drew et al., 2006a), but susceptible to PRSV-P in Venezuela (Horovitz and Jimenez, 1967). However, *V. pubescens* has been reported to be resistant against all strains of PRSV-P in all countries for more than 60 years. Therefore in this project, we continued a breeding program aimed at transferring PRSV-P resistance from *V. pubescens* to *papaya* using *V. parviflora* as a bridging species.

Previously at Griffith University, a genetic map of two *Vasconcellea* species was generated using Randomly Amplified DNA (RAF) polymorphisms (Dillon et al., 2005). DNA markers linked to a single dominant PRSV-P resistant gene locus (*prsv-1*) in *V. pubescens* were identified (Dillon et al., 2005). A SCAR (Sequence Characterised Amplified Region) marker and a codominant CAPS (Cleavage Amplified Polymorphic Sequence) marker were developed, are diagnostic for PRSV-P resistance in *V. pubescens* and its progeny (Dillon et al., 2006) and were used in subsequent breeding programs (O'Brien and Drew, 2009). In this project, a PhD student (Chutchamas Kanchana-udomkan**)** enrolled at Griffith University and worked on continued development of molecular markers for disease resistance and other useful agronomic traits that could be used in marker assisted selection.

Methodology

Breeding for New Commercial Lines

Parent lines were obtained from collections (at GU and from growers in Australia) and lines that were available in Queensland from Philippines, Malaysia, Taiwan, Thailand, Vietnam, Hawaii, Brazil and commercial lines in Queensland. Breeding lines produced in previous breeding trials were used as parents.

Lines of papaya were grown for evaluation leading to selection of parental lines for two main purposes; firstly to improve eating-quality in commercial varieties and secondly to establish segregating populations to use for identifying DNA markers for other traits.

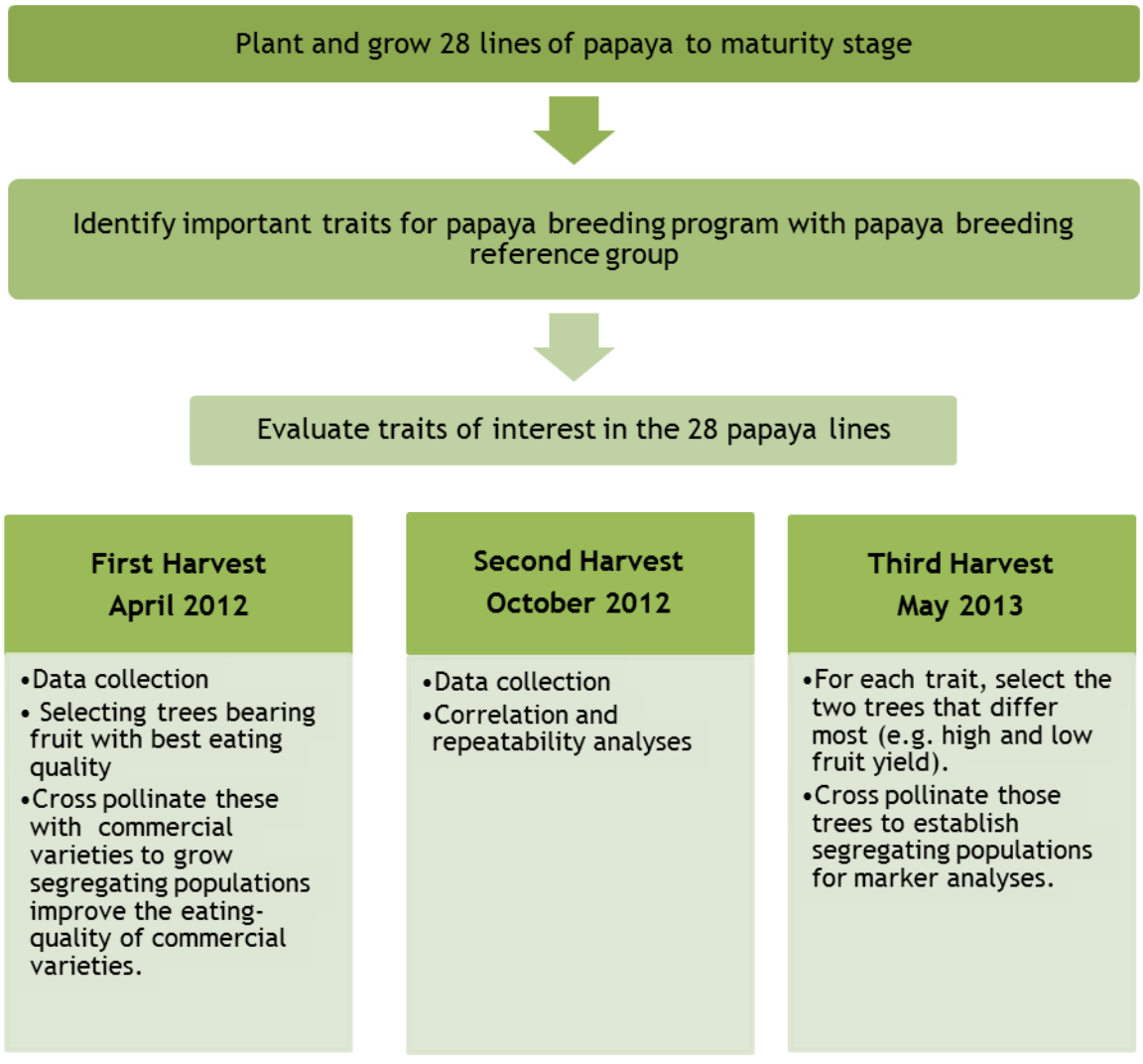

Figure 1: Steps of work for developing papaya breeding population and analysis of productivity and fruit quality traits and phenotypes

Table1: Identity, ancestry and source of 28 papaya lines planted at Lecker Farming, Mareeba, Queensland

Line number[1/]	Ancestry or variety name[2/]	Source of seed
R01	RB1 x 18-45	Cross of commercial red papaya RB1 and DAFFQ line #18-45
R02	RB1 x 24-29	Cross of commercial red papaya RB1 and DAFFQ line #24-29
R04	RB2 x 18-45	Cross of commercial red papaya RB2 and DAFFQ line #18-45
R06	RB2 x 25-5	Cross of commercial red papaya RB2 and DAFFQ line #25-5
R09	25-5 x RB1	Cross of DAFFQ line #25-5 and commercial red papaya RB1
Y11	24-29 x RB2	Cross of DAFFQ line #24-29 and commercial red papaya RB2
Y15	1B x 33-66	Cross of commercial yellow papaya 1B and DAFFQ line #33-66
Y16	7-82 x 1B	Cross of DAFFQ line #7-82 and commercial yellow papaya 1B
Y17	24-87 x 1B	Cross of DAFFQ line #24-87 and a commercial yellow papaya 1B
R19	24-29 Self	Self pollination of DAFFQ line #24-29
Y20	JC2	[3/]
R21	25-5 Self	Self pollination of a DAFFQ line #25-5
R22	TS2	[3/]
R23	Malaysian Red 1	[3/]
R24	Malaysian Red 2	[3/]
R25	Malaysian Red 3	[3/]
Y26	1B	A commercial yellow papaya
R27	RB2	A commercial red papaya
R28	RB4	A commercial red papaya
R29	Sunrise Solo	[3/]; it is a commercial red papaya in Hawaii
R30	Solo Linda	[3/]
R31	RD6 Self	[3/]
R33	Brazilian Solo	[3/]
Y34	2.54-14 self	[3/]
Y35	2.54-12 self	[3/]
R41	JC2 x Vietnam Red	[3/]
R42	TS2 Self	[3/]
R48	Red Lady, Taiwan	[3/]

[1/] The prefix R refers to red fleshed fruit; Y to yellow

[2/] Female parent is noted first in each cross.

[3/] From a collection of seed held by Professor Rod Drew, Griffith University, Brisbane.

Important commercial traits were identified during discussions with local commercial papaya producers. Traits were grouped into those relating to tree productivity and those relating to fruit quality. Ten fruit-bearing trees of each line were selected at random to evaluate traits. They were evaluated at three different harvesting times, April 2012, October 2012 and May 2013, to confirm that the data represented the genetics of the trees rather than environmental effects.

Nine productivity traits that related to performance of the trees were identified and recorded. They were:

i. Sex type: Height to the first flower
ii. Height to the first mature fruit
iii. Height to the first marketable fruit
iv. Peduncle length was recorded using a 1,3 and 5 rating scale; where
v. Yield of fruit of marketable fruit
vi. Yield gap
vii. Number of carpelliod fruits was counted for each tree.

Thirteen traits that related to fruit quality were identified and recorded:

i. Fruit shape
ii. Teat shape
iii. Stalk insertion
iv. Skin quality
v. Skin freckle
vi. Skin colour
vii. Cavity shape
viii. Consistency in flesh colour
ix. Flesh colour
x. Flesh firmness
xi. Useable flesh thickness.
xii. Flesh sweetness
xiii. Fruit flavour

Trees that good flavour flavour and other eating quality traits were selected as based on the result in the first harvest due to time limitation and the commitment to the project fund to produce crosses as soon as possible. Crosses were made from these selected trees to red and yellow fruited commercial papayas. The best three lines that were selected on the first evaluation (April 2012) were TS2, Malaysian Red 2 and Sunrise Solo. They exhibited excellent fruit eating quality (flesh flavour, firmness and thickness) and were selected to cross with Australian commercial lines 1B, RB1 and RB2. Of these 16 crosses from the second breeding population, seven trees, two and five of yellow and red papaya, respectively, were selected for their flavour and overall yield. These trees were used as parental lines to backcross to commercial varieties 1B, RB1 and RB2 and to sib cross to produce new hybrids.

A full description of materials and methods is presented in appendix 2.

Breeding for Papaya Ringspot Resistance (PRSV-P)

BC_4 (*V. parviflora* x *V. pubescens*) plants were produced by backcrossoing a BC_3 (*V. parviflora* x *V. pubescens*) plant to *V. pubescens*. They were evaluated for PRSV-P resistance and fertility. One line (clone 113) was selected as it produced a high yield of viable pollen and was PRSV-P resistant. Embryos were rescued from a cross of a BC_4 (*V. parviflora* x *V. pubescens* clone 113) X papaya to breed for PRSV-P resistance by introgression of the resistance from *V. pubescens*. They have been micropropagated and will be evaluated for fertility and resistance to PRSV-P. These plants could be included in the next phase of the breeding program. Molecular markers for PRSV-P resistance have been developed and refined and are available for use in evaluating these lines and future progeny.

Development of Molecular Markers

A segregating population was evaluated both for commercial traits and MAS. Selected parental trees for each trait including flavour, flesh quality, skin quality and yield, from the first breeding trial were cross-pollinated to produce F_1 plants, Seventeen crosses have been made. Seeds have been collected and cleaned.

DNAs of approximately 350 trees of the first breeding trial were extracted; and, a DNA marker linked to flesh fruit colour was selected to validate in these plant material. Co-dominant markers were developed and they showed 93% and 99% association to yellow and red flesh colour, respectively. The linkage between the markers and trait will be analysed in a F2 segregating population. This marker will be useful to aid selection for flesh colour when crossing unknown genotypes of red flesh and yellow flesh. It can also be used to help plan for on farm plantings and management.

Micropropagation / Plant Tissue Culture

Apically dominant plants were dissected into nodal sections and sub-cultured in a multiplication medium for 4 weeks (Appendix 1). Shoots were removed from the nodes and transferred to a root induction medium for 3 days (Appendix 1). They were then transferred to a plant growth regulator-free medium (Appendix 1), also known as "single shoot medium" for 3-4 weeks. Then, the cycle of micropropagation was repeated by multiplication, root induction and shoot induction (Figure 2.1) to multiply the number of plants that were required. All plant sections were incubated under conditions of 16 hours light from fluorescent lamps and 8 hours dark at 25ºC ± 1ºC.

Rooted plants from shoot induction medium were acclimatized following the procedure of Drew (1988). Roots were washed under tap water to remove residual agar. Each plant was placed in a 25mm square by 150mm tall black tube containing steam-pasteurized potting mix (peat: perlite: polystyrene balls in the ratio of 1: 1: 1). Plants were gown initially at 90% humidity with a gradual decrease by 5% every day for 7 days or until the humidity in the cabinet reached ambient relative humidity.A liquid fertilizer (Aquasol® 23N: 4P: 18K) was applied to the plants initially at a quarter-strength and increased at weekly intervals to full strength after 3 weeks.

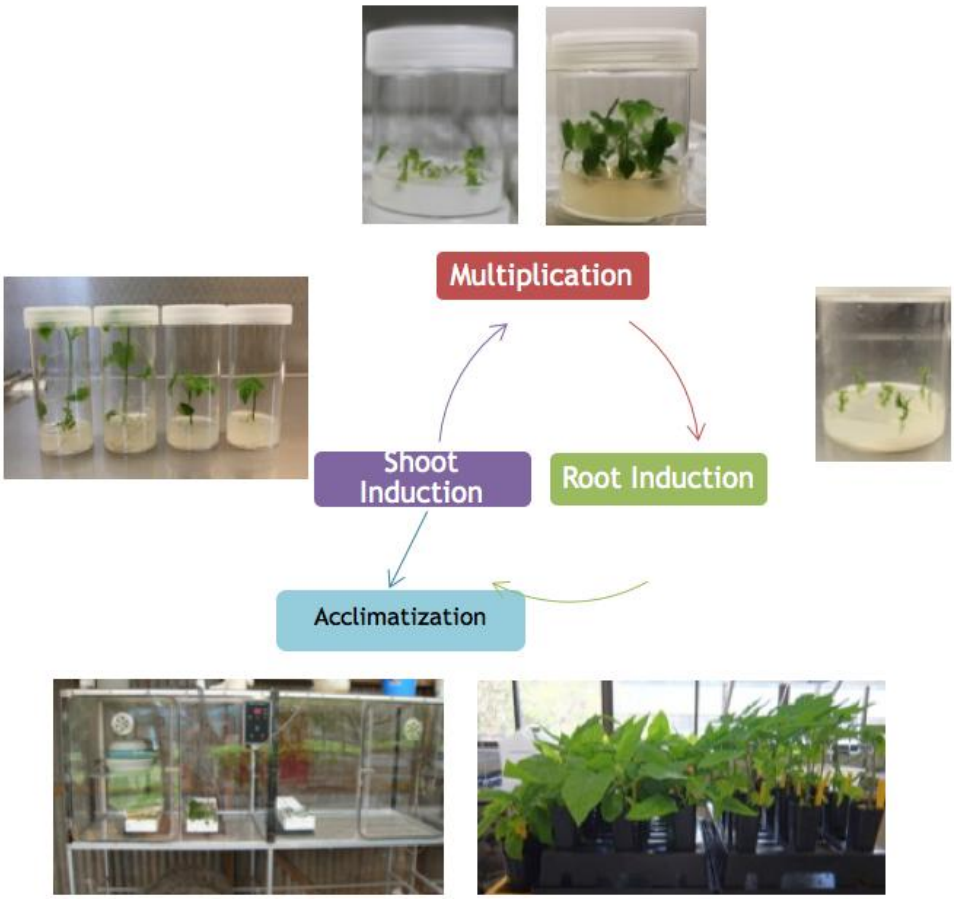

Figure 2: Procedure of *In vitro* propagation of *C. papaya* and *Vasconcellea spp.*

Outputs

1) Twenty-seven lines and breeding selections of papaya were evaluated for 11 productivity traits and 13 characteristics related to fruit quality based on both grower and consumer preferences. Results were used to determine selection of best parents to be used in the next stages of the breeding programs.
2) Establishment of potential parental lines, best F1 plants and selected plants from elite commercial lines in tissue culture.
3) Production and plantation of F_2 population of improved lines in two regions to be evaluated in the next phase of the project.
4) Evaluation of papaya germplasm and data was generated and stored in database system.
5) Development and validation of DNA markers linked to flesh colour and virus resistance.
6) Establishment of segregating populations of a wide range of traits.
7) Production of potential new line of papayas that contained PRSV-P resistance.
8) A comprehensive literature review has been produced – full details in appendix 1.

International Symposium on papaya

A papaya farm visit was organized in North Queensland after the International Papaya symposium so that researchers could meet growers. The event was held during last week of August aiming to exchange knowledge and build on papaya cultivation techniques.

A group of researchers from Hawaii and Australia were supported from a HNRN/HAL project to visit the main papaya-growing region in North QLD, Australia. The participants were Dr Maureen Fitch, Dr Judy Zhu and Ron Fitch from Hawaii; Dr Rebecca Ford, Chat Kanchana-udomkan and Mai Nantawan from Australia; and a grower from Mexico, Diego Urena.

The tour was organised to visit growing regions in Innisfail and Mareeba after the 4th Internatioanl Symposium on papaya which was held during IHC2014. There were three farm visits in Innisfail (growers: Mark Darveniza, Hayden Darveniza and Michael Oldano) on 24 August 2014 and a BBQ dinner at South Johnstone research station, which was supported by the Innisfail Papaw/Papaya Growers Association at an INC papaya meeting. Researchers from Hawaii presented their works in the topic of the current Hawaii papaya and GMO situation by Dr Maureen Fitch and papaya diseases in Hawaii by Dr Judy Zhu. Also, Diego, a grower from Maxico, showed papaya plantation and growing system in Mexico for export market to the USA. A discussion panel was held after the presentations between researcher and growers. It focused on many aspects of papaya production such as future of GMO papaya, disease and pest control, farm management and papaya marketing.

On 25 August 2014, two farms in Mareeba (Lecker Farming and Skybury Farmgate) were visited. Both farms showed their plantation and packing systems. They also presented their own breeding programs to improve flavour. At Lecker Farming, this group of researchers met with another group of researchers who took a North QLD tour which was organized by Yan Diczbalis, DAFFQ. Both groups visited plantation at Lecker Farming and a papaya tissue culture laboratory. Chat Kanchana-udomkan presented the Australian papaya breeding programs for PRSV-P resistance and improvement of fruit quality, and tissue culture of papaya for commercial scale.

Both growing regions showed their production systems from seed germination, planting, harvest to packing systems. Problems of papaya production in Innisfail are Phytophthora infection, black spots, fruit spotting bugs and use of suitable fallow crops. The same disease and insect problems are also found in Mareeba plantations. Other existing issues are an increase cost of production from chemical sprays to control pest and disease, labour costs, and seed price.

Figure 3: Group photo at Hayden Darveniza's farm, Innisfail, QLD.

Outcomes

Fruit flavour has been improved in both red and yellow fleshed papaya from the breeding program to improve flavour in commercial varieties. Trees with better flavour and high yield were crossed back to commercial varieties to keep improving the Australian genetics. A potential new variety of yellow papaya was established in tissue culture and can be field tested in different regions in the next phase of breeding program. Germplasm of 26 lines were evaluated and identified for potential parental lines for each trait of interest. Crosses to represent each trait were made and will be used for genetics study and development of DNA markers linked to each trait in the future. This represents a strong base for future papaya breeding programs.

DNA markers linked to fruit flesh colour, sex and virus resistance were developed and improved. They can be used to assist the selection at seedling stage in the next phase of breeding program and to improve farm management practices by planting red and yellow papaya of one sex in separate plantings.. The markers can also save time and cost in labour, evaluation and selection.

Crosses of the PRSV-P resistant line (clone 113) to cultivated papaya contained promising genetics of the resistant line as confirmed by DNA markers and morphology. These lines are being micropropagated in tissue culture and will be inoculated to screen for PRSV-P susceptibility/resistance and fertility.

Knowledge of techniques related to papaya breeding such as pollination, *in vitro* propagation, molecular techniques, gene expression analysis and bioinformatics, was transferred to new researchers. Dr Chat Kanchana-udomkan completed her Ph.D. under the support of this project. Local people on the Tableland learnt tissue culture techniques. This has stimulated new papaya research in Australia.

Evaluation and Discussion

The main purpose of this research was to evaluate germplasm in order to identify parents to develop populations for papaya breeding programs. The aim was to improve Australian commercial papaya varieties in terms of fruit quality and productivity. Phenotypic data for fruit quality and productivity traits were collected at three harvest times (April 2012, October 2012 and May 2013) to minimise environmental effects on the traits of interest. Some traits, such as number of carpelliod fruits, were believed to be affected by season (OCDE 2005), however this study showed no significant differences for this trait at the three harvest times. This suggests there should be a major gene involved in the expression of the trait, which opens up the possibility for improvement through breeding and selection. However, environmental factors remain important in trait expression as suggested by low repeatabilities in all traits and they need to be closely monitored. Fruit quality traits are complex traits and most of them are controlled by additive genes and quantitative trait loci. Parental lines were selected for traits of interest and crosses were made for crop improvement and establishment of segregating populations, to be used for the development of DNA markers to assist breeding selection.

A demonstration of the application of DNA markers to assist selection was applied and two DNA markers, CPFC-R and CPFC-Y, were developed to distinguish fruit flesh colour between red and yellow-fleshed varieties. The high percentage of association at 95.75% between trait and markers was presented in the germplasm representing a wide range of genetic backgrounds. In the future, these markers should be validated in segregating populations to calculate linkage distance between markers and the trait.

Full results and discussions are presented in appendix 2 and 3.

Recommendations

1. Genetic inheritance should be studied to enable design of suitable breeding strategies for each trait.
2. Bioassays for fruit quality and productivity traits should be standardised for future breeding research.
3. Commercial varieties from other countries should be included in germplasm evaluation to broaden the genetic base of papaya in Australia.
4. Chemical composition of flesh flavour of different flavours should be identified and evaluated for consumer perception and preference.
5. New varieties should be able to reproduced by seed production and micropropagation.
6. A second phase of the project should be started in 2016.

Scientific Refereed Publications

O'Brien, C.M. and Drew, R.A. 2009. Potential for using *Vasconcellea parviflora* as a bridging species in intergeneric hybridisation between *V. pubescens* and *Carica papaya.* Australian Journal of Botany 57:592-601.

O'Brien, C.M. and Drew, R.A. 2010. Marker-assisted hybridisation and backcrossing between *Vasconcellea* species and *Carica papaya* for PRSV-P resistance. Acta Horticulturae 859:361-368.

Scheldeman, X., Kyndt, T., Coppens d'Eeckenbrugge, G., Ming, R., Drew, R., van Droogenbroeck, B., van Damme, P. and Moore, P. 2011. Vasconcellea. In: Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits. (Cole, C. ed) p213-249. Springer Verlag, Berlin Heidelberg.

Siar, S.V., Beligan, G.A., Sajise, A.G.C., Villegas, V.N. and Drew, R.A. 2011. Papaya ringspot virus resistance in *Carica papaya* via introgression from *Vasconcellea quercifolia*. Euphytica 181:159-168.

Sair, S.V., Drew, R.A., Razali, R.M. and Villegas, V.N. 2012. Gene for PRSV-P Resistance in Vasconcellea species and Development of PRSV-P Resistant Papaya via Intergeneric Hybridisation. Acta Horticulturae: 929:335-342.

Razali, R.M. and Drew. R.A. 2013. A refined protocol for embryogenesis to transfer PRSV-P resistance genes from *Vasconcellea pubescens* to *Carica papaya*. Acta Horticulturae: 1022:47-53.

Kaity, A.; Drew, R.A.; Ashmore, S.E. 2013. Genetic and Epigenetic Integrity Assessment of Acclimatised Papaya Plants Regenerated Directly From Shoot-tips Following Short- and Long-term Cryopreservation. Plant Cell Tiss Organ Cult 112:75-86.

Alamery, S. and Drew. R.A. 2014. Studies on the genetics of PRSV-P resistance genes in intergeneric hybrids between Carica papaya and Vasconcellea quercifolia. Acta Horticulturae: 1022:55-62.

#Drew, R.A., 2014. The use of non-transgenic technologies for the development of Papaya Ringspot resistance in Carica papaya (L). Acta Horticulturae: 1022:23-30.

Razali, R.M. and Drew, R.A. 2014. Isolation and Characterisation of PRSV-P Resistance Genes in *Carica* and *Vasconcellea.* International Journal of Genomics: Volume 2014 (2014), Article ID 145403, 1-8.

Coppens d'Eeckenbrugge, G., Scheldeman, X., Drew, R.A. and Kyndt, T. 2014. *Vasconcellea* for papaya improvement. In: Genetics and Genomics of Papaya. (R. Ming and P. Moore. Eds) :47-80.

Razali, R.H.M. and Drew, R.A. 2014. A Review of PRSV-P Resistance Genes in Vasconcellea Species and Their Application to PRSV-P Resistance in Carica papaya. Acta Horticulturae: 1048:65-74.

Kanchana-udomkan, C., Ford, R. and Drew, R.A. 2014. Molecular Markers in Papaya. In: Genetics and Genomics of Papaya. (R. Ming and P. Moore. Eds) :355-376.

Razali, R.M. and Drew, R.A. 2015. Identification and Characterisation of PRSV-P Resistance Genes in Carica and Vasconcellea. Acta Horticulturae: in press

Kanchana-udomkan C., Razean M.R., Peace C. and Drew R. 2015. Developing DNA Markers for a Wild Source of Resistance to Papaya Ringspot Virus. Acta Horticulturae:in press.

Kanchana-udomkan C., Razean M.R., Peace C. and Drew R. 2015. Developing DNA Markers for a Wild Source of Resistance to Papaya Ringspot Virus. Acta Horticulturae: in press

Nantawan, U., Kanchana-udomkan C., and Drew R.2015. Progress in Marker Assisted Breeding of Papaya in Australia. Acta Horticulturae: in press.

Intellectual Property/Commercialisation

No commercial IP generated

References

References used in this report and the literature review in Appendix 1.

Afzal, I, Basra, SMA, Ashraf, M, Hameed, A & Farooq, M 2006, 'Physiological enhancements for alleviationof salt tolerance in spring wheat', *Pak. J. Bot*, vol. 38, pp. 1649–59.

Akamine, EK 1966, 'Respiration of fruits of papaya (*Carica papaya L.* var. Solo) with reference to effect of quarantine disinfestation treatments', *Proc. Amer. Soc. Hort. Sci*, vol. 89, pp. 231-6.

Albrecht, M & Takken, FLW 2006, 'Update on the domain architectures of NLRs and R proteins', *Biochemical and Biophysical Research Communications*, vol. 339, no. 2, pp. 459-62.

Allan, P 1963, 'Pollen studies in *Carica papaya*. II: Germination and storage of pollen', *South African Journal of Agricultural Science*, vol. 6, pp. 613-24.

Allan, P 2002, 'Carica papaya responses under cool subtropical growth conditions', Acta Horticulturae, no. 575, pp. 757-63.

Almeida, FT, Marinho, CS & Souza EF, GS 2003, 'Expressão sexual do mamoeiro sob diferentes lâminas de irrigação na Região Norte Fluminense', *Rev Brasil Fruticult*, vol. 25, pp. 383–5.

Almora, K, Pino, JA, Hernandez, M, Duarte, C, Gonzalez, J & Roncal, E 2004, 'Evaluation of volatiles from ripening papaya (*Carica papaya L.*, var. Maradolroja)', *Food Chemistry*, vol. 86, pp. 127-30.

Anderson, JP, Badruzsaufari, E, Schenk, PM, Manners, JM, Desmond, OJ, Ehlert, C, Maclean, DJ, Ebert, PR & Kazan, K 2004, 'Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis', *Plant Cell*, vol. 16, no. 12, pp. 3460-79.

Anh, NT, Trang, PN, Hong, NTB & Hoan, NV 2011, 'Evaluating agronomic characteristics of twelve local papaya (*Carica papaya L.*) varieties in Viet Nam', *Bull. Inst. Trop. Agr*, vol. 34, pp. 15-22.

Aradhya, MK, Manshardt, RM, Zee, F & Morden, CW 1999, 'A phylogenetic analysis of the genus *Carica L.* (Caricaceae) based on restriction fragment length variation in a cpDNA intergenic spacer region', *Genet. Resour. Crop Evol.*, vol. 46, no. 6, pp. 579-86.

Arkle, TD & Nakasone, HY 1984, 'Floral differentiation in the hermaphroditic papaya', *HORTSCIENCE*, vol. 19, pp. 832-4.

Artico, S, Nardeli, SM, Brilhante, O, Grossi-de-Sa, MF & Alves-Ferreira, M 2010, 'Identification and evaluation of new reference genes in Gossypium hirsutum for accurate normalisation of real-time quantitative RT-PCR data', *BMC Plant Biol*, vol. 10, no. 49, p. 49.

Arumuganathan, K & Earle, E 1991, 'Nuclear DNA content of some important plant species', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 9, no. 3, pp. 208-18.

Badillo, VM 1993, 'Caricaceae, segundo esquema.', *Revista de la Facultad de Agronomía de la Universidad de Venezuela*, vol. 43, pp. 1-111.

—— 2000, '*Carica* L. vs. *Vasconcellea* St.-Hil. (Caricaceae) con la rehabilitacion de este ultimo', *Ernstia*, vol. 10, no. 2, pp. 74-9.

Badillo, VM 2001, 'Nota correctiva *Vasconcellea* St. Hill. y no Vasconcella (Caricaceae). ', *Ernstia*, vol. 11, pp. 75-6.

Bateson, MF, Henderson, J, Chaleeprom, W, Gibbs, AJ & Dale, JL 1994, 'Papaya ringspot potyvirus-isolate variability and the origin of PRSV type P (Australia)', *Journal of General Virology*, vol. 75, pp. 3547-53.

Bau, H-J, Cheng, Y-H, Yu, T-A, Yang, J-S & Yeh, S-D 2003, 'Broad-Spectrum Resistance to Different Geographic Strains of Papaya ringspot virus in Coat Protein Gene Transgenic Papaya', Phytopathology, vol. 93, no. 1, pp. 112-20.

Bau, H-J, Cheng, Y-H, Yu, T-A, Yang, J-S, Liou, P-C, Hsiao, C-H, Lin, C-Y & Yeh, S-D 2004, 'Field Evaluation of Transgenic Papaya Lines Carrying the Coat Protein Gene of Papaya ringspot virus in Taiwan', Plant Disease, vol. 88, no. 6, pp. 594-9.

Benson, CW & Poffley, M 1998, *Growing pawpaws*, Northern Territory Government Department of Primary Industry Fisheries and Mines.

Blas, AL, Ming, R, Liu, Z, Veatch, OJ, Paull, RE, Moore, PH & Yu, Q 2010, 'Cloning of the papaya chromoplast-specific lycopene β-cyclase, CpCYC-b, controlling fruit flesh color reveals conserved microsynteny and a recombination hot spot', *Plant Physiology*, vol. 152, no. 4, pp. 2013-22.

Blas, AL, Yu, Q, Chen, C, Veatch, O, Moore, PH, Paull, RE & Ming, R 2009, 'Enrichment of a papaya high-density genetic map with AFLP markers', *Genome*, vol. 52, no. 8, pp. 716-25.

Blas, AL, Yu, Q, Veatch, QJ, Paull, RE, Moore, PH & Ming, R 2012, 'Genetic mapping of quantitative trait loci controlling fruit size and shape in papaya', *Mol Breed*, vol. 29, no. 801, pp. 457–66.

Broughton, WJ, Hashim, AM, Shen, TC & Tan, IKP 1977, 'Maturation of Malaysian fruits. I. Storage conditions and ripeningof papaya (*Carica papaya* L. CV. Sunrise Solo)', *Malaysian Agr. Res. & Dev. Inst. Res. Bul*, vol. 5, pp. 59-72.

Brown, PO & Botstein, D 1999, 'Exploring the new world of the genome with DNA microarrays', *Nat Genet*, vol. 21, no. 1 Suppl, pp. 33-7.

Bruening, G 2006, 'Resistance to infection', in G Loebenstein & JP Carr (eds), *Natural Resistance Mechanisms of Plants to Viruses*, Springer, Dordrecht, The Netherlands, pp. 211-40.

Bustin, SA, Benes, V, Garson, JA, Hellemans, J, Huggett, J, Kubista, M, Mueller, R, Nolan, T, Pfaffl, MW, Shipley, GL, Vandesompele, J & Wittwer, CT 2009, 'The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments', *Clin Chem.*, vol. 55, no. 4, pp. 611-22.

Cai, WQ, Gonsalves, C, Tennant, P, Fermin, G, Souza, M, Sarindu, N, Jan, FJ, Zhu, HY & Gonsalves, D 1999, 'A protocol for efficient transformation and regeneration of *Carica papaya* L.', *In Vitro Cellular & Developmental Biology-Plant*, vol. 35, no. 1, pp. 61-9.

Cao, J, Cao, Z & Wu, T 2007, 'Generation of antibodies against DMRT1 and DMRT4 of Oreochromis aurea and analysis of their expression profile in Oreochromis aurea tissues', *J Genet Genomics*, vol. 34, pp. 497 - 509.

Carrington, JC, Kasschau, KD, Mahajan, SK & Schaad, MC 1996, 'Cell-to-Cell and Long-Distance Transport of Viruses in Plants', *Plant Cell*, vol. 8, no. 10, pp. 1669-81.
Carvalho, FA & Renner, SS 2012, 'A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history', *Molecular Phylogenetics and Evolution*, vol. 65, no. 1, pp. 46-53.

Chan, HT, Kenneth, LH, Goo, T & Akamine, EK 1979, 'Sugar composition of papaya during fruit development', *HORTSCIENCE*, vol. 14, pp. 140–1.

Chaves-Bedoya, G & Nuñez, V 2007, 'A SCAR marker for the sex types determination in Colombian genotypes of *Carica papaya*', *Euphytica*, vol. 153, no. 1, pp. 215-20.

Chay-Prove, P, Ross, P, O'Hare, P, Macleod, N, Kernot, I, Evans, D, Grice, K, Vawdrey, L, Richards, N, Blair, A & Astridge, D 2000, *Your Growing Guide to Better Farming, Papaw Information Kit*, Queensland Horticulture Institute and Department of Primary Industries, Nambour, Qld.

Chen, C, Yu, Q, Hou, S, Li, Y, Eustice, M, Skelton, RL, Veatch, O, Herdes, RE, Diebold, L, Saw, J, Feng, Y, Qian, W, Bynum, L, Wang, L, Moore, PH, Paull, RE, Alam, M & Ming, R 2007, 'Construction of a sequence-tagged high-density genetic map of papaya for comparative structural and evolutionary genomics in brassicales', *Genetics*, vol. 177, no. 4, pp. 2481-91.

Clontech Laboratories Inc. 2012. Smarter™ RACE cDNA amplification kit user manual. Cat no:634923& 634924.

Conover, RA 1964, 'Mild mosaic and faint mottle ringspot, two papaya virus diseases of minor importance in Florida', *Proc. of the Fla. State Hort. Soc*, vol. 77, pp. 444-8.

Crute, IR & Pink, D 1996, 'Genetics and Utilization of Pathogen Resistance in Plants', *Plant Cell*, vol. 8, no. 10, pp. 1747-55.

Cunningham, FX & Gantt, E 1998, 'Genes and enzymes of carotenoid biosyntesis in plants', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 49, no. 1, pp. 557-83.

Czechowski, T, Stitt, M, Altmann, T, Udvardi, MK & Scheible, WR 2005, 'Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis', *Plant Physiol*, vol. 139, no. 1, pp. 5-17.

d'Eeckenbrugge, GC, Drew, RA, Kyndt, T & Scheldeman, X 2014, '*Vasconcellea* for Papaya Improvement', in R Ming & PH Moore (eds), *Genetics and Genomics of Papaya*, Springer New York, vol. 10, pp. 47-79.

Dangl, JL & Jones, JDG 2001, 'Plant pathogens and integrated defence responses to infection', *Nature*, vol. 411, no. 6839, pp. 826-33.

Dardick, C 2007, 'Comparative expression profiling of Nicotiana benthamiana leaves systemically infected with three fruit tree viruses', *Mol Plant Microbe Interact*, vol. 20, no. 8, pp. 1004-17.

de Oliveira, EJ, Amorim, VBO, Matos, ELS, Costa, JL, Castellen, MD, Padua, JG & Dantas, JLL 2010a, 'Polymorphism of Microsatellite Markers in Papaya (*Carica papaya* L.)', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 28, no. 3, pp. 519-30.

de Oliveira, EJ, Dantas, JLL, Castellen, MDS, de Lima, DS, Barbosa, HD & Motta, TBN 2007, 'Molecular markers for sex identification in papaya', *PESQUISA AGROPECUARIA BRASILEIRA*, vol. 42, no. 12, pp. 1747-54.

De Oliveira, EJ, Dantas, JLL, Castellen, MDS & Machado, MD 2008, 'Identification of microsatellites for papaya by DNA data bank exploration', *REVISTA BRASILEIRA DE FRUTICULTURA*, vol. 30, no. 3, pp. 841-5.

de Oliveira, EJ, dos Santos Silva, A, de Carvalho, FM, dos Santos, LF, Costa, JL, de Oliveira Amorim, VB & Dantas, JLL 2010b, 'Polymorphic microsatellite marker set for L. and its use in molecular-assisted selection', *Euphytica*, vol. 173, no. 2, pp. 279-87.

del Pozo, O, Pedley, KF & Martin, GB 2004, 'MAPKKKalpha is a positive regulator of cell death associated with both plant immunity and disease', *EMBO J*, vol. 23, no. 15, pp. 3072-82.

Dellaporta, SL, Wood, J & Hicks, JB 1983, 'A plant DNA minipreparation: Version II', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 1, no. 4, pp. 19-21.

Department of Agriculture, Forestry and Fisheries, South Africa, 2009 http://www.nda.agric.za/docs/Infopaks/papayas.pdf

Deputy, JC, Ming, R, Ma, H, Liu, Z, Fitch, MMM, Wang, M, Manshardt, R & Stiles, JI 2002, 'Molecular markers for sex determination in papaya (*Carica papaya* L.)', *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, vol. 106, no. 1, pp. 107-11.

DeYoung, BJ & Innes, RW 2006, 'Plant NBS-LRR proteins in pathogen sensing and host defense', *Nat Immunol*, vol. 7, no. 12, pp. 1243-9.

Dickinson, M 2003, *Molecular Plant Pathology*, Taylor & Francis.

Diczbalis, Y, Williams, B & Hickey, M 2012, *Papaya*, The Australian Papaya Industry, National Horticultural Research Network.

Dillon, S 2006a, 'Characterisation, genetic mapping and development of marker selection strategies for resistance to the Papaya ringspot virus type P (PRSV-P) in "highland papaya"', Ph.D. thesis, Griffith University.

Dillon, S, Ramage, C, Ashmore, S & Drew, RA 2006b, 'Development of a codominant CAPS marker linked to PRSV-P resistance in highland papaya', *Theor Appl Genet*, vol. 113, no. 6, pp. 1159-69.

Dillon, S, Ramage, C, Drew, R & Ashmore, S 2005, 'Genetic mapping of a PRSV-P resistance gene in "highland papaya" based on inheritance of RAF markers', *Euphytica*, vol. 145, no. 1-2, pp. 11-23.

Dillon, S, Ramage, C, O'Brien, CM & Drew, RA 2006c, 'Application of SCAR marker linked to a putative PRSV-P resistance locus for asissted breeding of resistant *C. papaya* cultivars', *Acta Hort. (ISHS)*, vol. 725, pp. 627-34.

Dixon, MS, Jones, DA, Keddie, JS, Thomas, CM, Harrison, K & Jones, JD 1996, 'The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins', *Cell*, vol. 84, no. 3, pp. 451-9.

Drew, RA 1988, 'Rapid clonal propagation of papaya *in vitro* from mature field-grown trees', *HORTSCIENCE*, vol. 23, no. 3, pp. 609-11.

—— 1992, 'Improved techniques for *in vitro* propagation and germplasm storage of papaya', *HORTSCIENCE*, vol. 27, pp. 1122-4.

—— 2005, *Development of new papaya varieties for Southeast and Central Queensland. Report No. FR02024*, Horticulture Australia Limited, Sydney, Australia.

Drew, RA, Godwin, ID, Magdalita, PM & Adkins, SW 1998a, 'Efficient interspecific hybridisation protocol for *Carica papaya* L. x *C. Cauliflora* Jacq', *Australian Journal of Experimental Agriculture (CSIRO)*, vol. 38, pp. 523-30.

Drew, RA, O'Brien, CM & Magdalita, PM 1998b, *Development of Carica interspecific hybrids*, International Society for Horticultural Science, Leuven, Belgique.

Drew, RA, Persley, DM, O'Brien, C & Bateson, MF 2005, 'Papaya ringspot virus in Australia and the development of virus resistant plant', *Acta Hort. (ISHS)*, vol. 692, pp. 101-6.

Drew, RA, Siar, SV, Dillon, S, Ramage, C, O'Brien, C & Sajise, AGC 2007, 'Intergeneric

hybridisation between *Carica papaya* and Wild *Vasconcellea* Species and Identification of a PRSV-P resistance Gene', *Proceedings of the International Symposium on Biotechnology of Temperate Fruit Crops and Tropical Species*, no. 738, pp. 165-9.

Drew, RA, Siar, SV, O'Brien, CM, Magdalita, PM & Sajise, AGC 2006a, 'Breeding for Papaya ringspot virus resistance in *Carica papaya* via hybridisation with *Vasconcellea quercifolia*', *Australian Journal of Experimental Agriculture*, vol.46, no. 3, pp.413-8.

Drew, RA, Siar, SV, O'Brien, CM & Sajise, AGC 2006b, 'Progress in backcrossing between *Carica papaya* × *Vasconcellea quercifolia* intergeneric hybrids and *C. papaya*', *Australian Journal of Experimental Agriculture*, vol. 46, no. 3, pp. 419-24.

Elder, RJ, Macleod, WNB, Bell, KL, Tyas, JA & Gillespie, RL 2000a, 'Growth, yield and phenology of 2 hybrid papayas (*Carica papaya* L.) as influenced by method of water application', *Australian Journal of Experimental Agriculture*, vol. 40, pp. 739-46.

Elder, RJ, Macleod, WNB, Reid, DJ & Gillespie, RL 2000b, 'Growth and yield of 3 hybrid papayas (*Carica papaya* L.) under mulched and bare ground conditions.', *Australian Journal of Experimental Agriculture*, vol. 40, pp. 747-54.

Elder, RJ, Reid, DJ, Macleod, WNB & Gillespie, RL 2002, 'Post-ratoon growth and yield of three hybrid papays (Carica papaya L.) under mulched and bare-ground conditions', Australian Journal of Experimental Agriculture, vol. 42, pp. 71-81.

Ellis, J & Jones, D 2003, 'Plant Disease Resistance Genes', in RA Ezekowitz & J Hoffmann (eds), *Innate Immunity*, Humana Press, pp. 27-45.

Eloisa, M, Reyes, Q & Paull, RE 1994, 'Skin freckles on solo papaya fruit', *SCIENTIA HORTICULTURAE*, vol. 58, no. 1, pp. 31-9.

Eustice, M, Yu, Q, Lai, CW, Hou, S, Thimmapuram, J, Liu, L, Alam, M, Moore, PH, Presting, GG & Ming, R 2008, 'Development and application of microsatellite markers for genomic analysis of papaya', *Tree Genetics & Genomes*, vol. 4, no. 2, pp. 333-41.

Fabi, JP, Seymour, G, Graham, N, Broadley, M, May, S, Lajolo, FM, Cordenunsi, BR & Oliveira do Nascimento, JR 2012, 'Analysis of ripening-related gene expression in papaya using an Arabidopsis-based microarray', *BMC Plant Biology*, vol. 12, no. 1, p. 242.

Falconer, DS 1960, *Introduction to Quantitative Genetics*, Oliver and Boyd, London.

Fan, M-J, Chen, S, Kung, Y-J, Cheng, Y-H, Bau, H-J, Su, T-T & Yeh, S-D 2009, 'Transgene-specific and event-specific molecular markers for characterization of transgenic papaya lines resistant to Papaya ringspot virus', *Transgenic research*, vol. 18, no. 6, pp. 971-86.

Fermin, G, Inglessis, V, Garboza, C, Rangle, S, Dagert, M & Gonsalves, D 2004, 'Engineered resistance against Papaya ringspot virus in Venezuelan transgenic papaya', *Plant Dis*, vol. 88, pp. 516-22.

Fernandez-Trujillo, JP, Picó, B, Garcia-Mas, J, M., ÁJ & Monforte, AJ 2011, 'Breeding for fruit quality in melon.', in MA Jenks & PJ Bebeli (eds), *Breeding for fruit quality*, John Wiley & Sons, pp. 261–78.

Fitch, MM, Manshardt, RM, Gonsalves, D, Slightom, JL & Sanford, JC 1990, 'Stable transformation of papaya via microprojectile bombardment', *Plant Cell Reports*, vol. 9, no. 4, pp. 189-94.

Fitch, MMM 2005, *'Carica papaya* Papaya', in RE Litz (ed.), *Biotechnology of Fruit and Nut Crops*, CABI Publishing, pp. 174-207.

Flath, RA & Forrey, RR 1977, 'Volatile components of papaya (*Carica papaya L.*, Solo variety)', *Journal of Agricultural and Food Chemistry*, vol. 25, no. 1, pp. 103-9.

Flor, HH 1971, 'Current status of the gene-for-gene concept', *Annu Rev Phytopathol*, vol. 9, pp. 275–96.

Francisco, FS, Messias, GP, Helaine, CCR, Pedro, CDJ, Telma, NSP & Carlos, DI 2007, 'Genotypic correlations of morpho-agronomic traits in papaya and implications for genetic breeding', *CROP BREEDING AND APPLIED BIOTECHNOLOGY*, vol. 7, pp. 345-52.

Fraser, RSS 1986, 'Genes for resistance to plant viruses', *CRC Crit. Rev. Plant Sci*, vol. 3, no. 6-15.

Fraser, RSS 1990, 'The genetics of resistance to plant viruses', *Annual Review of Phytopathology*, vol. 28, pp. 179-200.

Galván-Ampudia, CS & Offringa, R 2007, 'Plant evolution: AGC kinases tell the auxin tale', *Trends in Plant Science*, vol. 12, no. 12, pp. 541-7.

Gangopadhyay, G, Roy, SK, Ghose, K, Poddar, R, Bandyopadhyay, T, Basu, D & Mukherjee, KK 2007, 'Sex detection of *Carica papaya* and Cycas circinalis in pre-flowering stage by ISSR and RAPD', *CURRENT SCIENCE*, vol. 92, no. 4, pp. 524-6.

Garcia, A, Al-Yousif, M & Hirt, H 2012, 'Role of AGC kinases in plant growth and stress responses', *Cellular and Molecular Life Sciences*, vol. 69, no. 19, pp. 3259-67.

Garrett, A 1995, 'The pollination biology of papaw (*Carica papaya* L.) in central Queensland', PhD Thesis thesis, Central Queensland University.

Gilliland, FD, Islam, T, Berhane, K, Gauderman, WJ, McConnell, R, Avol, E & Peters, JM 2006, 'Regular smoking and asthma incidence in adolescents', *Am J Respir Crit Care Med*, vol. 174, no. 10, pp. 1094-100.

Goldbach, R, Bucher, E & Prins, M 2003, 'Resistance mechanisms to plant viruses: an overview', *Virus Research*, vol. 92, no. 2, pp. 207-12.

Gomez-Gomez, L & Boller, T 2000, 'FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis', *Mol Cell*, vol. 5, no. 6, pp. 1003-11.

Gonsalves, D 1998, 'Control of papaya ringspot virus in papaya: A case study', *Annual Review of Phytopathology*, vol. 36, no. 1, pp. 415-37.

Gonsalves, D & Ishii, M 1980, 'Purification and serology of papaya ringspot virus', *Phytopathology*, vol. 70, pp. 1028–32.

Gonzalez-Jara, P, Fraile, A, Canto, T & Garcia-Arenal, F 2009, 'The multiplicity of infection of a plant virus varies during colonization of its eukaryotic host', *J Virol*, vol. 83, no. 15, pp. 7487-94.

Grandillo, S, Ku, HM & Tanksley, SD 1999, 'Identifying the loci responsible for natural variation in fruit size and shape in tomato', *Theoretical and Applied Genetics*, vol. 99, no. 6, pp. 978-87.

Gu, C, Chen, S, Liu, Z, Shan, H, Luo, H, Guan, Z & Chen, F 2011, 'Reference gene selection for quantitative real-time PCR in Chrysanthemum subjected to biotic and abiotic stress', *Mol Biotechnol*, vol. 49, no. 2, pp. 192-7.

Gutha, L, Casassa, L, Harbertson, J & Naidu, R 2010, 'Modulation of flavonoid biosynthetic pathway genes and anthocyanins due to virus infection in grapevine (Vitis vinifera L.) leaves',

BMC Plant Biology, vol. 10, no. 1, p. 187.

Gutierrez, L, Mauriat, M, Guénin, S, Pelloux, J, Lefebvre, J-F, Louvet, R, Rusterucci, C, Moritz, T, Guerineau, F, Bellini, C & Van Wuytswinkel, O 2008, 'The lack of a systematic validation of reference genes: a serious pitfall undervalued in reverse transcription-polymerase chain reaction (RT-PCR) analysis in plants', *Plant Biotechnology Journal*, vol. 6, no. 6, pp. 609-18.

Hall, CB 1964, 'The effect of short periods of high temperature on the ripening of detached tomato fruits', *Proc. Amer. Soc. Hort. Sci*, vol. 84, pp. 501-6.

Hammond, RW & Zhao, Y 2000, 'Characterization of a tomato protein kinase gene induced by infection by Potato spindle tuber viroid', *Molecular Plant-Microbe Interactions*, vol. 13, pp. 903-10.

Hammond-Kosack, KE & Jones, JD 1996, 'Resistance gene-dependent plant defense responses', *The Plant Cell*, vol. 8, no. 10, pp. 1773-91.

Hammond-Kosack, KE & Kanyuka, K 2001, 'Resistance Genes (R Genes) in Plants', in JW Sons (ed.), *Encyclopedia of Life Sciences*, John Wiley & Sons, Ltd.

Hanks, SK & Hunter, T 1995, 'Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification', *FASEB J.*, vol. 9, no. 8, pp. 576-96.

Hansen, V 2005, *Papaya breeding and variety development, Report No. FR99018*, FR99018, Horticulture Australia Limited, Sydney, Australia.

Hellemans, J, Mortier, G, De Paepe, A, Speleman, F & Vandesompele, J 2007, 'qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data', Genome biology, vol. 8, no. 2, p. R19.

Hofmeyr, JDJ 1938, 'Genetical studies of *Carica papaya L.*', *S. Afr. J. Sci*, vol. 35, pp. 300-4.

—— 1939, 'Sex-linked inheritance in *Carica papaya* L.', *S Afr J Sci*, vol. 36, pp. 283–5.

Hong, SY, Seo, PJ, Yang, MS, Xiang, F & Park, CM 2008, 'Exploring valid reference genes for gene expression studies in Brachypodium distachyon by real-time PCR', *BMC Plant Biol*, vol. 8, p. 112.

Horovitz, S & Jimenez, H 1967, 'Cruzamientos interespecíficos e intergenéricos en Caricaceas y sus implicaciones fitotécnicas', *Agronomía Tropical*, vol. 17, no. 323-344.

Hu, L, Benson, ML, Smith, RD, Lerner, MG & Carlson, HA 2005, 'Binding MOAD (mother of all databases)', *Proteins: Structure, Function, and Bioinformatics*, vol. 60, no. 3, pp. 333-40.

Idstein, H, Keller, T & Schreier, P 1985, 'Volatile constituents of mountain papaya (*Carica candamarcensis*, syn. *C. pubescens* Lenne et Koch) fruit', *Journal of Agricultural and Food Chemistry*, vol. 33, no. 4, pp. 663-6.

Inohara, Chamaillard, McDonald, C & Nunez, G 2005, 'NOD-LRR proteins: role in host-microbial interactions and inflammatory disease', *Annu Rev Biochem*, vol. 74, pp. 355-83.

International Board for Plant Genetic Resources (IBPGR) 1988, *Descriptors for Papaya*, IBPGR, Rome.

Ishitani, M, Majumder, AI, Bornhouser, A, Michalowski, CB, Jensen, RG & Bohnert, HJ 1996, 'Coordinate transcriptional induction of myo-inositol metabolism during environmental stress', *Plant J*, vol. 9, no. 4, pp. 537-48.

Iwaki, M & Arakawa, Y 2006, 'Transformation of Acinetobacter sp. BD413 with DNA from commercially available genetically modified potato and papaya', *Letters in applied microbiology*, vol. 43, no. 2, pp. 215-21.

Jain, M, Tyagi, A & Khurana, J 2006, 'Molecular characterization and differential expression of cytokinin-responsive type-A response regulators in rice (Oryza sativa)', *BMC Plant Biology*, vol. 6, no. 1, p. 1.

Jarosova, J & Kundu, JK 2010, 'Validation of reference genes as internal control for studying viral infections in cereals by quantitative real-time RT-PCR', *BMC Plant Biol*, vol. 10, p. 146.
Jensen, DD 1949, 'Papaya virus diseases with special reference to papaya ringspot', *Phyt pathology*, vol. 39, pp. 191-211.

Jobin-Decor, MP, Graham, GC, Henry, RJ & Drew, RA 1997, 'RAPD and isozyme analysis of genetic relationships between *Carica papaya* and wild relatives', *Genet. Resour Crop Evol*, vol. 44, no. 5, pp. 471-7.

Jones, DA & Jones, JDG 1996, 'The role of leucine-rich repeat proteins in plant defences', *Adv. Bot. Res. incorporating Adv. Plant Pathol*, vol. 24, pp. 89–167.

Jones, JDG & Dangl, JL 2006, 'The plant immune system', *Nature*, vol.444, no.7117, pp.323-9.

Jost, W, Baur, A, Nick, P, Reski, R & Gorr, G 2004, 'A large plant beta-tubulin family with minimal C-terminal variation but differences in expression', *Gene*, vol. 340, no. 1, pp. 151-60.

Kajava, A 1998, 'Structural diversity of leucine-rich repeat proteins', *Journal of Molecular Biology*, vol. 277, no. 3, pp. 519-27.

Kanchana-udomkan, C, Ford, R & Drew, R 2014, 'Molecular Markers in Papayas', in R Ming & PH Moore (eds), *Genetics and Genomics of Papaya*, Springer New York, vol. 10, pp. 355-75.

Kang, BC, Yeam, I, Jahn, MM & Jahn, MM 2005, 'Genetics of plant virus resistance', *Annu Rev Phytopathol*, vol. 43, pp. 581-621.

Keen, NT 1990, 'Gene-for-gene complementarity in plant-pathogen interactions', *Annu Rev Genet*, vol. 24, pp. 447-63.

Kim, MS, Moore, PH, Zee, F, Fitch, MM, Steiger, DL, Manshardt, RM, Paull, RE, Drew, RA, Sekioka, T & Ming, R 2002, 'Genetic diversity of *Carica papaya* as revealed by AFLP markers', *Genome 503-12.*, vol. 45, no. 3, pp. 503-12.

Kinkade, MP & Foolad, MR 2013, 'Validation and fine mapping of lyc12.1, a QTL for increased tomato fruit lycopene content', *Theor Appl Genet*, vol. 126, no. 8, pp. 2163-75.

Klinge, S, Voigts-Hoffmann, F, Leibundgut, M, Arpagaus, S & Ban, N 2011, 'Crystal structure of the eukaryotic 60S ribosomal subunit in complex with initiation factor 6', *Science*, vol. 334, no. 6058, pp. 941-8.

Kohler, A, Rinaldi, C, Duplessis, S, Baucher, M, Geelen, D, Duchaussoy, F, Meyers, BC, Boerjan, W & Martin, F 2008, 'Genome-wide identification of NBS resistance genes in Populus trichocarpa', Plant molecular biology, vol. 66, no. 6, pp. 619-36.

Kørner, CF, Klauser, D, Niehl, A, Dominguez-Ferreras, A, Chinchilla, D, Boller, T, Heinlein, M & Hann, DR 2013, 'The immunity regulator BAK1 contributes to resistance against diverse RNA viruses', *Mol Plant Microbe Interact*, vol. 26, no. 11, pp. 1271-80.

Kozera, B & Rapacz, M 2013, 'Reference genes in real-time PCR', *J Appl Genet*, vol. 54, no. 4, pp. 391-406.

Kung, Y-J, Bau, H-J, Wu, Y-L, Huang, C-H, Chen, T-M & Yeh, S-D 2009, 'Generation of Transgenic Papaya with Double Resistance to Papaya ringspot virus and Papaya leaf-distortion mosaic virus', Phytopathology, vol. 99, no. 11, pp. 1312-20.

Kyndt, T, Romeijn-Peeters, E, Van Droogenbroeck, B, Romero-Motochi, JP, Gheysen, G & Goetghebeur, P 2005, 'Species relationships in the genus *Vasconcellea* (*Caricaceae*) based on molecular and morphological evidence.', *Am. J. Bot.*, vol. 92, no. 6, pp. 1033-44.

Lai, CWJ, Yu, Q, Hou, S, Skelton, RL, Jones, MR, Lewis, KLT, Murray, J, Eustice, M, Guan, P, Agbayani, R, Moore, PH, Ming, R & Presting, GG 2006, 'Analysis of papaya BAC end sequences reveals first insights into the organization of a fruit tree genome', *Molecular Genetics and Genomics*, vol. 276, no. 1, pp. 1-12.

Lawton, MA, Yamamoto, RT, Hanks, SK & Lamb, CJ 1989, 'Molecular cloning of plant transcripts encoding protein kinase homologs', *Proc Natl Acad Sci USA*, vol. 86, pp. 3140-4.

Leipe, DD, Koonin, EV & Aravind, L 2004, 'STAND, a Class of P-Loop NTPases Including Animal and Plant Regulators of Programmed Cell Death: Multiple, Complex Domain Architectures, Unusual Phyletic Patterns, and Evolution by Horizontal Gene Transfer', *Journal of Molecular Biology*, vol. 343, no. 1, pp. 1-28.

Lemos, EGM, Silva, CLSP & Zaidan, HA 2002, 'Identification of sex in *Carica papaya* L. using RAPD markers', *Euphytica*, vol. 127, no. 2, pp. 179-84.
Lilly, ST, Drummond, RS, Pearson, MN & MacDiarmid, RM 2011, 'Identification and validation of reference genes for normalization of transcripts from virus-infected Arabidopsis thaliana', *Mol Plant Microbe Interact*, vol. 24, no. 3, pp. 294-304.

Liu, D, Shi, L, Han, C, Yu, J, Li, D & Zhang, Y 2012a, 'Validation of reference genes for gene expression studies in virus-infected Nicotiana benthamiana using quantitative real-time PCR', *PLoS ONE*, vol. 7, no. 9, p. e46451.

Livak, KJ & Schmittgen, TD 2001, 'Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method', *Methods*, vol. 25, no. 4, pp. 402-8.

Loebenstein, G & Akad, F 2006, 'The local lesion response', in G Loebenstein & JP Carr (eds), *Natural Resistance Mechanisms of Plants to Viruses*, Springer, Dordrecht, The Netherlands, pp. 99-124.

Ma, H, Moore, PH, Liu, Z, Kim, MS, Yu, Q, Fitch, MMM, Sekioka, T, Paterson, AH & Ming, R 2004, 'High-density linkage mapping revealed suppression of recombination at the sex determination locus in papaya', *Genetics*, vol. 166, no. 1, pp. 419-36.

Mafra, V, Kubo, KS, Alves-Ferreira, M, Ribeiro-Alves, M, Stuart, RM, Boava, LP, Rodrigues, CM & Machado, MA 2012, 'Reference genes for accurate transcript normalization in citrus genotypes under different experimental conditions', *PLoS ONE*, vol. 7, no. 2, p. e31263.

Magdalita, PM, Drew, RA, Adkins, SW & Godwin, ID 1997, 'Morphological, molecular and cytological analyses of *Carica papaya* × *C. cauliflora* interspecific hybrids', *Theoretical and Applied Genetics*, vol. 95, no. 1-2, pp. 224-9.

Magdalita, PM, Drew, RA, Godwin, ID & Adkins, SW 1998, 'An efficient interspecific hybridisation protocol for *Carica papaya* L. x C. cauliflora Jacq', *Australian Journal of Experimental Agriculture*, vol. 38, pp. 523-30.

Mangrauthia, S, Singh Shakya, V, Jain, RK & Praveen, S 2009, 'Ambient temperature perception in papaya for papaya ringspot virus interaction', *Virus Genes*, vol. 38, no. 3, pp. 429-34.

Manshardt, RM 1992, 'Papaya', in FA Hammerschlag & RE Litz (eds), *Biotechnology in Agriculture No. 8 Biotechnology of Perennial Fruit Crops*, Alden Press, Oxford.

Manshardt, RM & Drew, RA 1998, 'Biotechnology of Papaya', *International Symposium on Biotechnology of Tropical and Subtropical Species - Part Ii*, no. 461, pp. 65-73.

Manshardt, RM & Wenslaff, TF 1989, 'Interspecific hybridization of papaya with other *Carica* species', *J. Amer. Soc. Hort. Sci.*, vol. 114, no. 4, pp. 689-94.

Marathe, R & Dinesh-Kumar, SP 2003, 'Plant defense: one post, multiple guards?', *Mol Cell*, vol. 11, no. 2, pp. 284-6.

Marcotte, EM 2000 'Computational genetics: finding protein function by nonhomology methods', *Curr Opin Struct Biol*, vol. 10, no. 3, pp. 359-65.

Maróstica, MR & Pastore, GM 2007, 'Tropical fruit flavor', in RG Berger (ed.), *Flavours and fragrances: Chemistry, bioprocessing and sustainability* Springer, Berlin, p. 189–201.

Martin, G, Brommonschenkel, S, Chunwongse, J, Frary, A, Ganal, M, Spivey, R, Wu, T, Earle, E & Tanksley, S 1993, 'Map-based cloning of a protein kinase gene conferring disease resistance in tomato', *Science*, vol. 262, no. 5138, pp. 1432-6.

Martin, GB 1999 'Functional analysis of plant disease resistance genes and their downstream effectors', *Curr Opin Plant Biol.*, vol. 2, no. 4, pp. 273-9.

Martin, GB, Bogdanove, AJ & Sessa, G 2003, 'Understanding the functions of plant disease resistance proteins', *Annu Rev Plant Biol*, vol. 54, pp. 23-61.

Mascia, T, Santovito, E, Gallitelli, D & Cillo, F 2010, 'Evaluation of reference genes for quantitative reverse-transcription polymerase chain reaction normalization in infected tomato plants', *Molecular plant pathology*, vol. 11, no. 6, pp. 805-16.

Matsui, H, Miyao, A, Takahashi, A & Hirochika, H 2010b, 'Pdk1 kinase regulates basal disease resistance through the OsOxi1-OsPti1a phosphorylation cascade in rice', *Plant Cell Physiol*, vol. 51, no. 12, pp. 2082-91.

Matsui, H, Yamazaki M Fau - Kishi-Kaboshi, M, Kishi-Kaboshi M Fau - Takahashi, A, Takahashi A Fau - Hirochika, H & Hirochika, H 2010a, 'AGC kinase OsOxi1 positively regulates basal resistance through suppression of OsPti1a-mediated negative regulation', *Plant Cell Physiol*, vol. 51, no. 10, pp. 1731-44.

Mauch-Mani, B & Mauch, F 2005, 'The role of abscisic acid in plant-pathogen interactions', *Curr Opin Plant Biol*, vol. 8, no. 4, pp. 409-14.

McDowell, JM & Woffenden, BJ 2003 'Plant disease resistance genes: recent insights and potential applications', *Trends Biotechnol*, vol. 21, no. 4, pp. 178-83.

Meyers, BC, Kozik, A, Griego, A, Kuang, H & Michelmore, RW 2003, 'Genome-Wide Analysis of NBS-LRR–Encoding Genes in Arabidopsis', *The Plant Cell*, vol. 15, no. 4, pp. 809-34.

Mezard, C 2006, 'Meiotic recombination hotspots in plants', *Biochem Soc Trans*, vol. 34, no. Pt 4, pp. 531-4.

Michelmore, RW & Meyers, BC 1998, 'Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process', *Genome Res*, vol. 8, no. 11, pp. 1113-30.

Ming, R, Hou, S, Feng, Y, Yu, Q, Dionne-Laporte, A, Saw, JH, Senin, P, Wang, W, Ly, BV, Lewis,

KLT, Salzberg, SL, Feng, L, Jones, MR, Skelton, RL, Murray, JE, Chen, C, Qian, W, Shen, J, Du, P, Eustice, M, Tong, E, Tang, H, Lyons, E, Paull, RE, Michael, TP, Wall, K, Rice, DW, Albert, H, Wang, M-L, Zhu, YJ, Schatz, M, Nagarajan, N, Acob, RA, Guan, P, Blas, A, Wai, CM, Ackerman, CM, Ren, Y, Liu, C, Wang, J, Wang, J, Na, J-K, Shakirov, EV, Haas, B, Thimmapuram, J, Nelson, D, Wang, X, Bowers, JE, Gschwend, AR, Delcher, AL, Singh, R, Suzuki, JY, Tripathi, S, Neupane, K, Wei, H, Irikura, B, Paidi, M, Jiang, N, Zhang, W, Presting, G, Windsor, A, Navajas-P√©rez, R, Torres, MJ, Feltus, FA, Porter, B, Li, Y, Burroughs, AM, Luo, M-C, Liu, L, Christopher, DA, Mount, SM, Moore, PH, Sugimura, T, Jiang, J, Schuler, MA, Friedman, V, Mitchell-Olds, T, Shippen, DE, dePamphilis, CW, Palmer, JD, Freeling, M, Paterson, AH, Gonsalves, D, Wang, L & Alam, M 2008, 'The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus)', *Nature*, vol. 452, no. 7190, pp. 991-6.

Ming, R, Moore, PH, Zee, F, Abbey, CA, Ma, H & Paterson, AH 2001, 'Construction and characterization of a papaya BAC library as a foundation for molecular dissection of a tree-fruit genome', *Theoretical and Applied Genetics*, vol. 102, no. 6, pp. 892-9.

Ming, R, Yu, Q & Moore, PH 2007, 'Sex determination in papaya', *Seminars in cell & developmental biology*, vol. 18, no. 3, pp. 401-8.

Moore, GA & Litz, RE 1984, *Biochemical markers for Carica papaya, C. cauliflora, and plants from somatic embryos of their hybrid*, vol. 109, American Society for Horticultural Science, Alexandria, VA.

Mora, A, Komander, D, van Aalten, DMF & Alessi, DR 2004, 'PDK1, the master regulator of AGC kinase signal transduction', *Semin Cell Dev Biol*, vol. 15, no. 2, pp. 161-70.

Morel, J-B & Dangl, JL 1997, 'The hypersensitive response and the induction of cell death in plants', *Cell death and differentiation*, vol. 4, no. 8, pp. 671-83.

Morillo, SA & Tax, FE 2006 'Functional analysis of receptor-like kinases in monocots and dicots', *Curr Opin Plant Biol*, vol. 9, no. 5, pp. 460-9.
Morton, JF 1987, 'Papaya *Carica papaya* L', in NC Winterville (ed.), *Fruits of Warm Climates*, Creative Resources Inc, pp. 336-46.

Nagarajan, N, Navajas-Pérez, R, Pop, M, Alam, M, Ming, R, Paterson, A & Salzberg, S 2008, 'Genome-Wide Analysis of Repetitive Elements in Papaya', *Tropical Plant Biology*, vol. 1, no. 3, pp. 191-201.

Nakajima, Y, Iwakabe, H, Akazawa, C, Nawa, H, Shigemoto, R, Mizuno, N & Nakanishi, S 1993, 'Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate', *J Biol Chem*, vol. 268, no. 16, pp. 11868-73.

Nakasone, HY & Paull, RE 1998, *Tropical fruits*, CAB International, Oxon, UK.

Nazeeb, M & Broughton, WJ 1978., 'Storage conditions and ripening of papaya 'Bentong' and 'Taiping.' ', *Sci. Hort*, vol. 9, pp. 265-77.
Nelson, RS, McCormic, SM, Delannay, X, Dube, P, Layton, J, Anderson, EJ, Kaniewska, M, Proksch, RK, Horsch, RB, Rogers, SG, Fraley, RT & Beachy, RN 1988, 'Virus tolerance, plant growth and field performance of transgenic tomato plants expressing coat protein from tobacco mosaicvirus', *Biotechnology*, vol. 6, pp. 403-9.

Newton, AC 2003, 'Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm', *Biochemical Journal*, vol. 370, no. Pt 2, pp. 361-71.

Nicot, N, Hausman, J, Hoffmann, L & Evers, D 2005, 'Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress', *J Exp Bot.*, vol. 56, no. 421, pp. 2907-14

Niroshini, E, Everard, J, Karunanayake, EH & Tirimanne, TLS 2008, 'Detection of sequence characterized amplified region (SCAR) markers linked to sex expression in *Carica papaya* L', *JOURNAL OF THE NATIONAL SCIENCE FOUNDATION OF SRI LANKA*, vol. 36, no. 2, pp. 145-50.

Noorda-Nguyen, K, Jia, R, Aoki, A, Yu, Q, Nishijima, W & Zhu, YJ 2010, 'Identification of disease tolerance loci to *Phytophthora palmivora* in *Carica papaya* using molecular marker approach', *Acta Hort*, no. 851, pp. 189-96.

O'Brien, CM & Drew, RA 2010, 'Marker-assisted hybridisation and backcrossing between *Vasconcellea* species and *Carica papaya* for PRSV-P resistance', *Acta Hort. (ISHS)*, vol. 859, pp. 361-8.

O'Brien, CM & Drew, RA 2009, 'Potential for using *Vasconcellea parviflora* as a bridging species in intergeneric hybridisation between *V. pubescens* and *Carica papaya*', *Australian Journal of Botany*, vol. 57, no. 7, pp. 592-601.

Ocampo Pérez, J, Coppens d'Eeckenbrugge, G, Risterucci, AM, Dambier, D & Ollitrault, P 2007, 'Papaya genetic diversity assessed with microsatellite markers in germplasm from the Caribbean region', *Acta Hort*, vol. 740, pp. 93-101.

OECD 2005, *Consensus document on the Biology of papaya (Carica papaya)* Organisation for Economic Co-operation and Development, Paris, France.

O'Hare, P 1993, *Growing Papayas in South Queensland*, Queensland Government Department of Primary Industries, Brisbane, Queensland.

Oliveira, EJ, Amorim, VO, Matos, EL, Costa, JL, Silva Castellen, M, Pádua, J & Dantas, J 2010, 'Polymorphism of microsatellite markers in papaya (*Carica papaya L.*)', *PLANT MOLECULAR BIOLOGY REPORTER*, vol. 28, no. 3, pp. 519-30.

Palukaitis, P & Carr, JP 2008, 'Plant resistance responses to viruses', *J Plant Pathol*, vol. 90, pp. 153–71.

Papaya-Seed-Australia 2007, *Information*, viewed 10 March 2014, http://www.papayaseed.com.au/information.htm.

Parasnis, AS, Gupta, VS, Tamhankar, SA & Ranjekar, PK 2000, 'A highly reliable sex diagnostic PCR assay for mass screening of papaya seedlings', *Molecular Breeding*, vol. 6, no. 3, pp. 337-44.

Parasnis, AS, Ramakrishna, W, Chowdari, KV, Gupta, VS & Ranjekar, PK 1999, 'Microsatellite (GATA)n reveals sex-specific differences in Papaya', *Theoretical and Applied Genetics*, vol. 99, no. 6, pp. 1047-52.

PâRez, JO, Dambier, D, Ollitrault, P, DÄo Eeckenbrugge, GC, Brottier, P, Froelicher, Y & Risterucci, AÄ 2006, 'Microsatellite markers in *Carica papaya* L.: isolation, characterization and transferability to *Vasconcellea* species', *Molecular Ecology Notes*, vol. 6, no. 1, pp. 212-7.

Parker, PJ & Parkinson, SJ 2001, 'AGC protein kinase phosphorylation and protein kinase C', *Biochem Soc Trans*, vol. 29, no. 6, pp. 860-3.

Pearce, LR, Komander, D & Alessi, D, R. 2010, 'The nuts and bolts of AGC protein kinases', *Nat Rev Mol Cell Biol*, vol. 11, no. 1, pp. 9-22.
Pegg, AE 2009, 'S-Adenosylmethionine decarboxylase', *Essays Biochem*, vol. 46, pp. 25–46

Peterson, TR, Laplante, M, Thoreen, CC, Sancak, Y, Kang, SA, Kuehl, WM, Gray, NS & Sabatini, DM 2009, 'DEPTOR Is an mTOR Inhibitor Frequently Overexpressed in Multiple Myeloma Cells

and Required for Their Survival', *Cell*, vol. 137, no. 5, pp. 873-86.

Pfaffl, M, Tichopad, A, Prgomet, C & Neuvians, T 2004, 'Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations', *Biotechnology Letters*, vol. 26, no. 6, pp. 509-15.

Pfaffl, MW 2001, 'A new mathematical model for relative quantification in real-time RT-PCR', *Nucleic Acids Res.*, vol. 29, no. 9, p. e45.

Pino, E, Campos, AM & Lissi, EA 2003, '8-Hydroxy- 1,3,6-pyrene trisulfonic acid (pyranine) bleaching by 2,2'-azobis(2-amidinopropane) derived peroxyl radicals', *Int J Chem Kinetics*, vol. 35, pp. 525-31.

Pino, JA, Almora, K & Marbot, R 2003, 'Volatile components of papaya (*Carica papaya L.*, Maradol variety) fruit.', *Flavour Fragrance J*, vol. 18, no. 6, pp. 492–6.

Pitzschke, A & Hirt, H 2009, 'Disentangling the Complexity of Mitogen-Activated Protein Kinases and Reactive Oxygen Species Signaling', *Plant Physiology*, vol. 149, no. 2, pp. 606-15.

Pollination Aware Report 2008, https://rirdc.infoservices.com.au/downloads/10-129

Porter, BW, Paidi, M, Ming, R, Alam, M, Nishijima, WT & Zhu, YJ 2009a, 'Genome-wide analysis of *Carica papaya* reveals a small NBS resistance gene family', *Molecular Genetics and Genomics*, vol. 281, no. 6, pp. 609-26.

Porter, BW, Zhu, YJ, Webb, DT & Christopher, DA 2009b, 'Novel thigmomorphogenetic responses in *Carica papaya*: touch decreases anthocyanin levels and stimulates petiole cork outgrowths', *Annals of botany*, vol. 103, no. 6, pp. 847-58.

Purcifull, DE, Edwardson, JR, Hiebert, E & Gonsalves, D 1984, *Papaya ringspot virus CMI/AAB despriptions of plant viruses No. 292*, CAB International, Wallingford, UK.

Quemada, HD, Gonsalves, D & Slightom, JL 1991, 'Expression of coat protein gene from cucumber mosaic virus strain C in tobacco: Protection against infections by CMV strains transmitted mechanically or by aphids', *Phytopathology*, vol. 81, pp. 794-802.

Rairdan, GJ & Moffett, P 2006, 'Distinct domains in the ARC region of the potato resistance protein Rx mediate LRR binding and inhibition of activation', *Plant Cell*, vol. 18, no. 8, pp. 2082-93.

Ratchadaporn, J, Sureeporn, K & Khumcha, U 2007, 'An analysis on DNA fingerprints of thirty papaya cultivars (*Carica papaya* L.), grown in Thailand with the use of amplified fragment length polymorphisms technique', *Pakistan journal of biological sciences: PJBS*, vol. 10, no. 18, p. 3072.

Razean Haireen, MR 2013, 'Identification, characterisation and expression of PRSV-P resistance genes in *Carica* and *Vasconcellea*', Ph.D. thesis, Griffith University, Brisbane.

Razean Haireen, MR & Drew, RA 2014, 'Isolation and characterisation of PRSV-P resistance genes in *Carica* and *Vasconcellea*', *International Journal of Genomics*, vol. 2014, p. 8.

Reis, FO, Campostrini, E, Sousa, EF & Silva, MG 2006, 'Sap flow in papaya plants: Laboratory calibrations and relationships with gas exchanges under field conditions', *Sci Hortic*, vol. 110, no. 3, pp. 254–9.

Remans, T, Smeets, K, Opdenakker, K, Mathijsen, D, Vangronsveld, J & Cuypers, A 2008, 'Normalisation of real-time RT-PCR gene expression measurements in Arabidopsis thaliana exposed to increased metal concentrations', *Planta*, vol. 227, no. 6, pp. 1343-9.

Rentel, MC, Lecourieux, D, Ouaked, F, Usher, SL, Petersen, L, Okamoto, H, Knight, H, Peck, SC, Grierson, CS, Hirt, H & Knight, MR 2004, 'OXI1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis', *Nature*, vol. 427, no. 6977, pp. 858-61.

Romeis, T, Ludwig, AA, Martin, R & Jones, JDG 2001, 'Calcium-dependent protein kinases play an essential role in a plant defence response', *The EMBO Journal*, vol. 20, no. 20, pp. 5556-67.

Rozen, S & Skaletsky, H 2000, 'Primer3 on the WWW for general users and for biologist programmers', *Methods Mol Biol.*, vol. 132, pp. 365-86.

Ruiz, D & Egea, J 2008, 'Phenotypic diversity and relationships of fruit quality traits in apricot (*Prunus armeniaca L.*) germplasm', *Euphytica*, vol. 163, pp. 143–58.

Sanders, PR, Sammons, B, Kaniewski, W, Haley, L, Layton, J, Lavallee, BJ, Delannay, X & Tumer, NE 1992, 'Field resistance of transgenic tomatoes expressing the tobacco mosaic virus or tomato mosaic virus coat protein genes', *Phytopathology*, vol. 82, pp. 683-90.

Santos, SC, Ruggiero, C, Silva, CLSP & Lemos, EGM 2003, 'A microsatellite library for *Carica papaya* L. cv. Sunrise solo', *REVISTA BRASILEIRA DE FRUTICULTURA*, vol. 25, no. 2, pp. 263-7.

Scheel, D 1998, 'Resistance response physiology and signal transduction', *Curr Opin Plant Biol*, vol. 1, no. 4, pp. 305-10.

Schenk, PM, Kazan, K, Manners, JM, Anderson, JP, Simpson, RS, Wilson, IW, Somerville, SC & Maclean, DJ 2003, 'Systemic Gene Expression in Arabidopsis during an Incompatible Interaction with Alternaria brassicicola', *Plant Physiology*, vol. 132, no. 2, pp. 999-1010.

Sessa, G & Martin, GB 2000, 'Protein kinases in the plant defense response', in JA Callow, M Kreis & JC Walker (eds), *Advances in Botanical Research*, Academic Press, London, UK, vol. 32, pp. 379–404.

Sharon, D, Hillel, J, Vainstein, A & Lavi, U 1992, 'Application of DNA fingerprints for identification and genetic analysis of *Carica papaya* and other *Carica* species', *Euphytica*, vol. 62, no. 2, pp. 119-26.

Shi, C, Ingvardsen, C, Thümmler, F, Melchinger, A, Wenzel, G & Lübberstedt, T 2005, 'Identification by suppression subtractive hybridization of genes that are differentially expressed between near-isogenic maize lines in association with sugarcane mosaic virus resistance', *Molecular Genetics and Genomics*, vol. 273, no. 6, pp. 450-61.

Shiu, SH & Bleecker, AB 2003, 'Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis', *Plant Physiol*, vol.132, no.2, pp. 530-43.

Siar, SV, Beligan, GA, Sajise, AJC, Villegas, VN & Drew, RA 2011, 'Papaya ringspot virus resistance in *Carica papaya* via introgression from *Vasconcellea quercifolia'*, *Euphytica*, vol. 181, no. 2, pp. 159-68.

Simmonds, NW 1979, 'Principles of crop improvement', in *Simmonds, NW: Principles of crop improvement.*

Singh, I 1990, *Papaya*, Oxford and IBH Publishing, New Delhi.

Singh, R & Green, MR 1993, 'Sequence-specific binding of transfer RNA by glyceraldehyde-3-phosphate dehydrogenase', *Science*, vol. 259, pp. 365–8.

Skelton, RL, Yu, Q, Srinivasan, R, Manshardt, R, Moore, PH & Ming, R 2006, 'Tissue differential expression of lycopene [beta]-cyclase gene in papaya', *Cell Res*, vol. 16, no. 8, pp. 731-9.

Somsri, S & Bussabakornkul, S 2008, ' Identification of certain papaya cultivars and sex identification in papaya by DNA Amplification Fingerprinting (DAF). Acta Hort 787:197-206', *Acta Hort. (ISHS)*, vol. 787, pp. 197-206.

Sondur, SN, Manshardt, RM & Stiles, JI 1996, 'A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers', *Theoretical and Applied Genetics*, vol. 93, no. 4, pp. 547-53.

Song, W-Y, Wang, G-L, Chen, L-L, Kim, H-S, Pi, L-Y, Holsten, T, Gardner, J, Wang, B, Zhai, W-X, Zhu, L-H, Fauquet, C & Ronald, P 1995, 'A Receptor Kinase-Like Protein Encoded by the Rice Disease Resistance Gene, Xa21', *Science*, vol. 270, no. 5243, pp. 1804-6.

Sooriyapathirana, SS, Khan, A, Sebolt, AM, Wang, D, Bushakra, JM, Lin-Wang, K, Allan, AC, Gardiner, SE, Chagné, D & Iezzoni, AF 2010, 'QTL analysis and candidate gene mapping for skin and flesh color in sweet cherry fruit (*Prunus avium* L.)', *Tree Genetics & Genomes*, vol. 6, no. 6, pp. 821-32.

Souza, BSd, Durigan, JF, Donadon, JR & Teixeira, GHdA 2005, 'Conservação de mamão 'Formosa' minimamente processado armazenado sob refrigeração', *REVISTA BRASILEIRA DE FRUTICULTURA*, vol. 27, pp. 273-6.

Stiles, JI, Lemme, C, Sondur, SN, Morshidi, MB & Manshardt, R 1993, 'Using randomly amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars', *Theoretical and Applied Genetics*, vol. 85, no. 6, pp. 697-701.

Storey, WB 1938, 'Segregation of sex types in solo papaya and their application to the selection of seed', *Proc. Amer. Soc. Hort. Sci*, vol. 35, pp. 83-5.

—— 1969, 'Papaya', in FP Ferwerda & F Wit (eds), *Outlines of Perennial Crop Breeding in the Tropics*, H. Vienman and Zonen N. V. Wageningen, pp. 389-407.

—— 1976, 'Papaya', in NW Simmonds (ed.), *Evolution of crop plants* Longman, England, UK., pp. 21-4.

Takken, FLW, Albrecht, M & Tameling, WIL 2006, 'Resistance proteins: molecular switches of plant defence', *Current Opinion in Plant Biology*, vol. 9, no. 4, pp. 383-90.

Tan, SC & Weinheimer, EA 1976, 'The isoenzyme patterns of developing fruit and mature leaf of papaya (*Carica papaya* L.)', *Sains Malays J Nat Sci*, vol. Jan 5, no. 1, pp. 7-14.

Tennant, P, Fermin, G, Fitch, MM, Manshardt, RM, Slightom, JL & Gonsalves, D 2001, 'Papaya ringspot virus resistance of transgenic Rainbow and SunUp is affected by gene dosage, plant development, and coat protein homology', *European Journal of Plant Pathology*, vol. 107, no. 6, pp. 645-53.

Tennant, PF, Gonsalves, C, Ling, KS, Fitch, MM, Manshardt, RM, Slightom, LJ & Gonsalves, D 1994, 'Differential protection against papaya ringspot virus isolates in coat protein gene transgenic papaya and classically cross-protected papaya', *Phytopathology*, vol. 84, no. 11, pp. 1359-66.

Thellin, O, Zorzi, W, Lakaye, B, De Borman, B, Coumans, B, Hennen, G, Grisar, T, Igout, A & Heinen, E 1999, ' Housekeeping genes as internal standards: use and limits', *J Biotechnol*, vol. 75, pp. 291-5.

Ton, J, Flors, V & Mauch-Mani, B 2009, 'The multifaceted role of ABA in disease resistance', Trends Plant Sci, vol. 14, no. 6, pp. 310-7.

Torii, KU 2004, 'Leucine-rich repeat receptor kinases in plants: structure, function, and signal

transduction pathways', *Int Rev Cytol*, vol. 234, pp. 1- 46.

Tripathi, S, Ferreira, SA & Gonsalves, D 2008, 'Papaya ringspot virus-P: characteristics, pathogenicity, sequence variability and control', *Molecular plant phatology*, vol. 9, no. 3, pp. 269-80.

Ulrich, D & Olbricht, K 2011, 'Fruit Organoleptic Properties and Potential for Their Genetic Improvement', in MA Jenks & P Bebeli (eds), *Breeding for Fruit Quality*, Wiley-Blackwell, pp. 41-56.

Urasaki, N, Tarora, K, Uehara, T, Chinen, I, Terauchi, R & Tokumoto, M 2002a, 'Rapid and highly reliable sex diagnostic PCR assay for papaya (*Carica papaya* L.)', *BREEDING SCIENCE*, vol. 52, no. 4, pp. 333-5.

Urasaki, N, Tokumoto, M, Tarora, K, Ban, Y, Kayano, T, Tanaka, H, Oku, H, Chinen, I & Terauchi, R 2002b, 'A male and hermaphrodite specific RAPD marker for papaya (*Carica papaya* L.)', *TAG Theoretical and Applied Genetics*, vol. 104, no. 2, pp. 281-5.

Van der Biezen, EA & Jones, JD 1998, 'Plant disease-resistance proteins and the gene-for-gene concept', *Trends Biochem Sci*, vol. 23, no. 12, pp. 454-6.

Van der Knaap, E & Tanksley, SD 2001, 'Identification and characterization of a novel locus controlling early fruit development in tomato', *Theoretical and Applied Genetics*, vol. 103, no. 2-3, pp. 353-8.

Van Droogenbroeck, B, Breyne, P, Goetghebeur, P, Romeijn-Peeters, E, Kyndt, T & Gheysen, G 2002, 'AFLP analysis of genetic relationships among papaya and its wild relatives (Caricaceae) from Ecuador', *Theoretical and Applied Genetics*, vol. 105, no. 2, pp. 289-97.

Van Ooijen, G, Mayr, G, Kasiem, MMA, Albrecht, M, Cornelissen, BJC & Takken, FLW 2008, 'Structure–function analysis of the NB-ARC domain of plant disease resistance proteins', *Journal of Experimental Botany*, vol. 59, no. 6, pp. 1383-97.

Vandesompele, J, De Preter, K, Pattyn, F, Poppe, B, Van Roy, N, De Paepe, A & Speleman, F 2002, 'Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes', *Genome biology*, vol. 3, no. 7, p. research0034.

Vandesompele, J, Kubista, M & Pfaffl, MW 2009, 'Reference gene validation software for improved normalization', *Real-time PCR: current technology and applications*, pp. 47-64.

Wai, CM, Ming, R, Moore, PH, Paull, RE & Yu, Q 2010, 'Development of Chromosome-specific Cytogenetic Markers and Merging of Linkage Fragments in Papaya', *Tropical Plant Biology*, vol. 3, no. 3, pp. 171-81.

Waigmann, E, Ueki, S, Trutnyeva, K & Citovsky, V 2004 'The ins and outs of non-destructive cell-to-cell and systemic movement of plant viruses', *Crit Rev Plant Sci*, vol. 23, pp. 195–250.

Walton, D & Yi, L 1995, 'Abscisic Acid Biosynthesis and Metabolism', in P Davies (ed.), *Plant Hormones*, Springer Netherlands, pp. 140-57.

Wan, H, Zhao, Z, Qian, C, Sui, Y, Malik, AA & Chen, J 2009, 'Selection of appropriate reference genes for gene expression studies by quantitative real-time polymerase chain reaction in cucumber', *Anal Biochem*, vol. 399, no. 2, pp. 257-61.

Wan, H, Zhao, Z, Qian, C, Sui, Y, Malik, AA & Chen, J 2010, 'Selection of appropriate reference genes for gene expression studies by quantitative real-time polymerase chain reaction in cucumber', *Analytical Biochemistry*, vol. 399, no. 2, pp. 257-61.

Wang, J, Chen, C, Na, J-K, Yu, Q, Hou, S, Paull, RE, Moore, PH, Alam, M & Ming, R 2008, 'Genome-Wide Comparative Analyses of Microsatellites in Papaya', *Tropical Plant Biology*, vol. 1, no. 3, pp. 278-92.

Warmke, H, Cabanillas, E & Cruzado, H 1954, 'A new interspecific hybrid in the genus Carica', in *Proceedings of the American Society for Horticultural Science*, vol. 64, pp. 284-8.

Watson, B 1997, *Agronomy/agroclimatology notes for the production of papaya*, MAFFA, Australia.

World Health Organization 2007, *Micronutrient Deficiencies-Vitamin A Deficiency*, World Health Organization (WHO), viewed 21 December 2014, <http://www.who.int/nutrition/topics/vad/en/>.

Yamamoto, HY 1964, 'Comparison of the carotenoid in yellow-and red-fleshed *Carica papaya*', *Nature*, vol. 201, pp. 1049–50.

Yang, Y, Shah, J & Klessig, DF 1997, 'Signal perception and transduction in plant defense responses', *Genes Dev.*, vol. 11, no. 13, pp. 1621-39.

Ying, KC 2008, 'Breeding Papaya (*Carica papaya L.*)', in *Breeding Plantation Tree Crops: Tropical species*, Springer, pp. 128-31.

Yu, Q, Tong, E, Skelton, RL, Bowers, JE, Jones, MR, Murray, JE, Hou, S, Guan, P, Acob, RA, Luo, M-C, Moore, PH, Alam, M, Paterson, AH & Ming, R 2009, 'A physical map of the papaya genome with integrated genetic map and genome sequence', *BMC genomics*, vol. 10, no. 1, pp. 371-.

Zaitlin, M & Hull, R 1987, 'Plant Virus-Host Interactions', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 38, pp. 291–315.

Zeevaart, JAD & Creelman, RA 1988, 'Metabolism and physiology of abscisic acid', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 39, no. 1, pp. 439-73.

Zeng, S, Liu, Y, Wu, M, Liu, X, Shen, X, Liu, C & Wang, Y 2014, 'Identification and Validation of Reference Genes for Quantitative Real-Time PCR Normalization and Its Applications in Lycium', *PLoS ONE*, vol. 9, no. 5, p. e97039.

Zhang, Y, He, J & McCormick, S 2009, 'Two Arabidopsis AGC kinases are critical for the polarized growth of pollen tubes', *Plant J*, vol. 58, no. 3, pp. 474-84.

Zhu, X, Li, X, Chen, W, Chen, J, Lu, W, Chen, L & Fu, D 2012, 'Evaluation of new reference genes in papaya for accurate transcript normalization under different experimental conditions', *PLoS ONE*, vol. 7, no. 8, p. e44405.

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Appendices

Appendix 1

Chapter 1 of PhD thesis of Dr. Chat Kanchana-udomkan

This chapter provides a complete literature review of publications relevant to the research in this report and additional research undertaken in the PhD program.

Chapter 1:

Literature Reviews

1.1 Published reviews by Kanchana-udomkan et al. (2014)

1.1.1 Background

Molecular markers are effective tools and have been used to facilitate genetic improvement in many crop species including *Carica papaya* L. (Eustice et al. 2008). The main purpose of using molecular markers within a breeding program is to either determine the relatedness among genotypes for germplasm resource management and parental choice, for true-to-type and hybrid identification; or, to identify and select for particular sequences that are associated with traits of interest. DNA markers are generally stable, unaffected by environment, present at all stages of plant growth and in all tissue types. They have been adopted within papaya breeding programs as accurate selection tools for traits of interest (Eustice et al. 2008; Ma et al. 2004; Porter et al. 2009a; de Oliveira et al. 2010b; Deputy et al. 2002; Dillon et al. 2006b).

Recent research has led to some important developments in this field. In the search for DNA markers linked to the genes that condition the traits of interest, a genetic and physical map of the papaya genome was developed (Yu et al. 2009). The papaya genome was sequenced and has been used to identify a library of SSR (Simple Sequences Repeat; microsatellite) loci (Eustice et al. 2008; Santos et al. 2003; Wang et al. 2008). In addition, several gene sequences with associated functions have become available through the papaya genome project database (Ming et al. 2008).

This chapter will focus on the development and application of molecular markers that have been used to assess genetic diversity and to improve papaya breeding objectives.

Table 1.1 DNA markers and their primer sequences linked to sex type identification in *Carica papaya*

Marker name	Primer sequence (5' ---> 3')	Product size	Type of marker	Sex detection	Note	Reference
OPT12	OPT12:GGGTGTGTAG	Na	RAPD		*Sex1*: 7cM	Sondur et al. (1996)
OPT1C	OPT1: GGGCCACTCA	na	RAPD		*Sex1*: 7cM	Sondur et al. (1996)
T1	T1F: TGCTCTTGATATGCTCTCTG T1R: TACCTTCGCTCACCTCTGCA	1.3 kb	SCAR	All sex	*Sex1*: n/a	Deputy et al. (2002)
T12	T12F: GGGTGTGTAGGCACTCTCCTT T12R: GGGTGTGTAGCATGCATGATA	800 bp	SCAR	Hermaphrodite and Male	*Sex1*: 0.3 cM	Deputy et al. (2002)
W11	W11F: CTGATGCGTGTGTGGCTCTA W11R: CTGATGCGTGATCATCTACT	800 bp	SCAR	Hermaphrodite and Male	*Sex1*: 0.3 cM	(Deputy et al. 2002)
PSDM	IBRC-RP07: TTGGCACGGG	450 bp	RAPD	Hermaphrodite and Male	*Sex1*: n/a	(Urasaki et al. 2002b)
SCARps	SDP-1: GCACGATTTAGATTAGATGT SDP-2: GGATAGCTTGCCCAGGTCAC	225 bp	SCAR	Hermaphrodite and Male	*Sex1-H* and *Sex1-M*: n/a	(Urasaki et al. 2002b)
Papain	P5': GGGCATTCTCAGCTGTTGTA P3': CTCCCTTGAGCGACAATAAC	221 bp	EST		Papain	(Urasaki et al. 2002a)
SCARpm	SDP-2: GGATAGCTTGCCCAGGTCAC SDP-3: GGTAAGAGTTTTTCCCAAGC	347 bp	SCAR	Hermaphrodite and Male		(Urasaki et al. 2002a)
$BC210_{438}$	BC210: GCACCGAGAG	438 bp	RAPD	Hermaphrodite		(Lemos et al. 2002)
$OP\text{-}Y7_{900}$	OP-Y7: AGAGCCGTCA	900 bp	RAPD	Male		(Chaves-Bedoya and Nuñez 2007)
SCAR SDSP	CFw: AAACTACCGTGCCATTATCA CRv: AGAGATGGGTTGTGT CACTG	369 bp	SCAR	Hermaphrodite and Male		(Chaves-Bedoya and Nuñez 2007)

$(GATA)_4$	Genomic DNA digested with *Hin*fI	5 kb	RFLP	Hermaphrodite and Male		(Parasnis et al. 1999)
$(GATA)_4$	Genomic DNA digested with *Hae*III	4 kb	RFLP	Hermaphrodite and Male		(Parasnis et al. 1999)
OPC09-1.7	OPC09: CTCACCGTCC	1.7 kb	RAPD			(Niroshini et al. 2008)
OPE03-0.4	OPE03: CCAGATGCAC	0.4 kb	RAPD			(Niroshini et al. 2008)
C09/20	C09/20FP: CTCACCGTCCATTTTAATTA	1.7 kb	SCAR	Hermaphrodite and Male		(Niroshini et al. 2008)
	C09/20RP: CTCACCGTCCGCGGCATCAATGTA	978 bp				
$(GACA)_4$	(GACA)4: GACAGACAGACAGACA		ISSR			(Gangopadhyay et al. 2007)
OPF2-0.8	OPF2: GAGGATCCCT	831 bp	RAPD	Male	Acc#AF148540	(Parasnis et al. 2000)
	F-Napf-76: GAGGATCCCTATTAGTGTAAG	831 bp	SCAR	Male		(Parasnis et al. 2000)
	R-Napf-77: GAGGATCCCTTTTGCACTCTG					
	F-Napf-70: GGATCCCT ATTAG	0.83 kb	SCAR	Male	Mass screening	(Parasnis et al. 2000)
	R-Napf-71: GAGGATCCCTTTTGC					
GC	F-GN-C: CGAAATCGGTAGACGATACG	0.6 kb		Sex neutral		(Parasnis et al. 2000)
	R-GN- D: GGGGATAGAGGGACTTGAAC					
OPA06	OPA 06: 5' GGT CCC TGAC 3'	365 bp	DAF	Hermaphrodite and Male		(Somsri and Bussabakornkul 2008)
		360 bp				

1.1.2 Development of molecular marker in papaya

The first markers were morphological, which Hofmeyr (1938) mapped onto the initial papaya genetic map in 1938. Subsequently, from the 1970s to the 1990s, biochemical markers (isozymes) were used to study the development of papaya fruits and mature leaves (Tan and Weinheimer 1976); hybridity of *C. papaya* and *C. cauliflora* (Jacq.) A.DC. (Moore and Litz 1984); and genetic relationships between *C. papaya* and wild *Vasconcellea* relatives (Jobin-Decor et al. 1997).

The first report of the use of DNA markers in a papaya genomic study was in 1992. Southern blot detection of restriction fragment length polymorphisms (RFLPs), produced by digesting total genomic DNA with restriction enzymes and detection by micro- and mini-satellite probes, were used to detect polymorphisms between *C. papaya* genotypes and between *C. papaya* and its related species (Sharon et al. 1992). The report suggested that *C. papaya* genotypes and their related species could be identified and distinguished by their unique DNA fingerprints.

The invention of the polymerase chain reaction (PCR) in the early 1990s then led to rapid advances in the development and application of molecular markers. The first PCR-based markers for papaya were random amplified polymorphic DNA (RAPD) markers, which are dominant markers that are amplified by arbitrary short primer sequences. These were used to evaluate genetic relationships among papaya cultivars (Stiles et al. 1993) and between papaya and wild related *Vasconcellea* species. RAPD sourced markers were also applied to determine sex of papaya prior to flowering. However, RAPD markers can be difficult to reproduce on different equipment and by different researchers. In addition, although assumptions have been made in the past, RAPD markers cannot be used to determine allele differences at a particular locus without fragment sequencing. A variation of this technique, that employs increased annealing temperature and polyacrylamide gel detection, randomly amplified DNA fingerprinting (RAF), identified markers linked to Papaya Ringspot Virus type P (PRSV-P) resistance in the related *Vasconcellea cundinamarcensis* Badillo, also known as *V. pubescens* (Dillon et al. 2005).

Subsequently, amplified fragment length polymorphism (AFLP) markers, integrating RFLP with PCR, were applied to assess genetic relationships within the Caricaceae (Kim et al. 2002; Van Droogenbroeck et al. 2002; Ocampo Pérez et al. 2007; Ratchadaporn et al. 2007). AFLP markers have also been placed on papaya genome maps (Ma et al. 2004; Blas et al. 2009). Approximately 1500 AFLP markers were mapped onto 12 linkage groups (LGs) (Ma et al. 2004). However, using the same population, only 20% of these markers were mapped along with other types of markers into 9 major and 5 minor linkage groups (Blas et al. 2009). These markers, RAPD, RAF and AFLP, which are non-specific and dominant markers, could be useful for papaya crop improvements as they do not require much genetic information i.e. DNA sequence analysis. However, the amplification patterns of these markers are complicated and they can not identify single loci and alleles. Hence, more reliable, stable and useful markers were needed.

Simple sequence repeats (SSR), otherwise known as microsatellite markers have many advantages over anonymous dominant markers. They are targeted to flanking sequences to amplify the tandem short repeat units dispersed throughout the genome. Therefore, they are generally locus specific and may be co-dominant. Also, due to the phenomenon of conserved slippage they may be highly polymorphic among individuals within a species although they are generally not well transferred between species. The *C. papaya* SSR

markers have been used for sex identification (Parasnis et al. 1999), in genetic diversity studies (Pérez et al. 2006; Ocampo Pérez et al. 2007; de Oliveira et al. 2008; Eustice et al. 2008; de Oliveira et al. 2010a; de Oliveira et al. 2010b), for the construction of genetic maps (Blas et al. 2009; Chen et al. 2007) and integration to a physical map, and for comparing cytogenetic markers to merge linkage fragments (Yu et al. 2009; Wai et al. 2010).

1.1.3 Application

1.1.3.1 Molecular markers and papaya genomic studies

Papaya belongs to the order Brassicales which comprises 17 families including Caricaceae that contains papaya, and Arabidopsis, the model plant in Brassicaceae. The family Caricaceae contains six genera and 35 species including *Carica papaya*. Papaya is the only member in the genus *Carica*, and has a relatively small genome size of 372 Mb (Arumuganathan and Earle 1991); diploid 2n = 18. Papaya has been identified as a model for biotechnology applications in tropical fruit species because it is an economically important fruit crop in tropical and subtropical regions, it has a small genome size; and it has a short generation time (9-15 months). The genome was successfully sequenced in 2008 for 'SunUp', which is a commercial virus-resistant transgenic genotype of papaya (Ming et al. 2008). The papaya genome was the fifth flowering plant to be sequenced. Compared to the other four plant genomes that were sequenced, papaya contains 24746 genes, which is 20%, 34%, 46% and 19% less than *Arabidopsis*, rice, poplar and grape, respectively.

The first genetic map of papaya comprised three morphological markers; sex type, flower color and stem color, covering 41 cM (Hofmeyr 1939) of the genome. The second papaya genetic map (F_2: Hawaiian cultivar 'Sunrise' x UH breeding line 356) was established 60 years later comprising 61 RAPD markers and one morphological locus (*SEX1*) within 11 linkage groups and comprising a total map distance of 999.3 cM (Sondur et al. 1996). In 2004, another map was produced using 54 F_2 plants derived from cultivars Kapoho x Sunup and containing 1498 AFLP markers, a PRSV-P coat protein marker and two morphological markers; that determined sex type and fruit flesh colour (Ma et al. 2004). These markers were mapped into 12 linkage groups with a total length of 3294.2 cM and an average distance of 2.2 cM. This map was then integrated into a recent genetic map (Blas et al. 2009).

SSR markers have been used widely in papaya research and SSR libraries for papaya have been developed (Eustice et al. 2008; Santos et al. 2003; Wang et al. 2008). The patterns of SSR distribution are similar within genomic or genic regions and the most abundant motif repeats are dinucleotides. The AT/TA motif repeats are predominant across several studies (Eustice et al. 2008; Santos et al. 2003; Wang et al. 2008; Nagarajan et al. 2008). The most abundantly detected trinucleotides motif differs among studies (AAG/TTC (44.1%; Eustice et al. 2008); AAT/TTA (55.8%; Wang et al. 2008); and TAC/ATG, AGA/TCT and ATT/TAA were reported to be very common in the papaya genome sequence (Nagarajan et al. 2008). This is likely due to the different methods used to isolate the SSR sequences. SSRs were identified from the papaya genome sequence using Microsatellite Analysis Server (Eustice et al. 2008), Perl program, Simple Sequence Repeat Identification Tool (SSRIT; Wang et al. 2008) and the Tandem Repeats Finder software (Nagarajan et al. 2008).

SSR markers were included within a subsequent map constructed with 54 F_2 plants that were derived from AU9 x SunUp. This comprised 706 SSR markers and one morphological marker for fruit flesh colour within 12 linkage groups over 1068.9 cM and an average distance of 1.51 cM (Chen et al. 2007). The nine major linkage groups represent nine chromosomes in the papaya genome, covered 993.5 cM with 683 map loci with an average marker density of 1.45 cM. The three short linkage groups covered 75.4 cM with 24 mapped loci and an average distance of 3.1 cM between adjacent markers.

Codominant markers have greatly increased the resolution and accuracy of papaya genetic maps. The map containing SSR markers was far more compact (1068.9 cM) than that produced by AFLP markers (3294.2 cM). The threefold reduction in genetic distance was due to the ability of codominant markers to separate the three classes of genotypes within the F_2 population; homozygous dominant, homozygous recessive and heterozygous.

A recent genetic map of papaya was saturated with 712 SSR, 277 AFLP and one morphological markers spanning 945.2 cM (Blas et al. 2009). This was constructed with the same 54 F_2 AU9 x Sunup population and comprised 14 linkage groups; nine major and five minor. The nine major linkage groups incorporated 939 marker loci over a total 849.4 cM at 1.6 cM or less intervals. The five minor linkage groups comprised 51 loci and spanned 95.8 cM with 3.0 cM or less intervals. Comparing the previous map (Chen et al. 2007) to this recent map, the addition of AFLP markers allowed six unmapped SSR markers from previous map to be linked, but did not join the gap between three previous minor linkage groups and the nine major linkage groups. However, the number of gaps that were greater than 5 cM between adjacent loci was reduced from 48 to 27 and the total map length was reduced by approximately 11.5%. The addition of AFLP markers resulted in an order of locus rearrangement.

Prior to the availability of the whole genome sequence, bacterial artificial chromosome clones containing papaya genomic DNA were produced and assembled. The first papaya BAC library consisted of 39168 clones from two separate ligation reactions (Ming et al. 2001). The average insert size of 18700 clones from the first ligation was 86 kb, while 20468 clones from the second ligation contained inserts twice as large, averaging 174 kb. The entire BAC library was estimated to provide a 13.7 x papaya genome coverage. In 2006, a total of 50661 BAC end sequence (BES) chromatograms were generated from 26017 BAC clones (Lai et al. 2006) from the BAC clone library by Ming et al (2001). After eliminating all unused sequences, 35472 high-quality sequences from 20842 BAC clones were generated. The total number of high-quality bases was 17483563 or 4.7% of the papaya genome. Two years later, a 3x draft genome of cultivar 'SunUp' was reported within which 1.6 million high-quality reads were assembled into contigs containing 271 Mb and scaffolds spanning 370 Mb (Ming et al. 2008). Subsequently, 652 BAC and whole genome sequence derived SSRs were used to anchor 167 Mb of contigs and 235 Mb of scaffolds to the 12 linkage groups of papaya on the current genetic map (Ming et al. 2008).

A physical map of the papaya genome, that integrated with the genetic map and genome sequence, was published in 2009 (Yu et al. 2009). The BAC-based physical map of papaya covered 95.8% of the genome, while 72.4% was aligned to a sequence-tagged SSR genetic map (Chen et al. 2007). This BAC library initially included 39168 BAC clones;

however, after evaluation and reviews, 26466 BAC clones were assembled into 963 contigs having an average number of fragments for each clone of 69.4. The average physical distance per centimorgan was approximately 348 kb. The integrated genetic and physical map when aligned with the genome sequence revealed recombination hot spots as well as regions suppressed for recombination across the genome, particularly on the sex chromosome, namely LG1 (Yu et al. 2009). A total of 1181 overgos representing conserved sequences of *Arabidopsis* and genetically mapped *Brassica* loci were anchored on the integrated genetic and physical map and the draft genome sequence of papaya. These overgos are direct links among papaya, *Arabidopsis* and *Brassica* genomes for comparative genomic research among species within the order Brassicales. The combined information of physical and genetic maps will enhance the capacity for map-based cloning and identification of underlying genes controlling traits of interest in papaya. It will also expedite the mapping and cloning of target genes and promote marker-assisted selection for papaya breeding.

Recently, chromosome-specific cytogenetic markers were developed and merged with linkage groups of papaya using the integrated technique of fluorescence in situ hybridisation (FISH) and BAC clones harboring mapped SSR markers as probes (Wai et al. 2010). Minor linkage groups 10, 11 and 12 from the previous map were assigned to major LGs 8, 9 and 7, respectively. Thus, the nine linkage groups in the genetic map corresponded to the haploid number of papaya chromosomes. This integrated map will facilitate genome assembly, quantitative trait locus mapping, and the study of cytological, physical and genetic distance relationships between papaya chromosomes. It is an even more powerful and accurate tool for trait selection.

1.1.3.2 Molecular markers and genetic diversity in Caricaceae

Variations in *Carica papaya* in terms of phenotypic, morphological and horticultural characteristics such as fruit size, fruit shape, flesh colour, texture, flavour and sweetness, stamen carpellody and carpel abortion, sex type, length of juvenile period, plant stature, plant canopy size, can be detected at high levels between different genotypes of papaya in the field. However, most of this morphological diversity in papayas has not been correlated to genetic diversity, specific genes or molecular markers despite many studies on genetic diversity. Different techniques have been used to study genetic diversity in papaya and relationships between plants within *Caricaceae* including AFLPs, isozymes, RAPDs, microsatellites and SSRs.

Kim et al. (2002) studied genetic relationships between papayas and related *Vasconcellea* species using samples which had a wide range of morphology and climate adaptation variation, from tropical, subtropical and temperate regions. But only 12% of genetic variation was detected among this diverse group of material using AFLP markers, and it was not representative of the wide range of morphological characteristics that were observed in the field. The accessions that Kim et al. (2002) used in their study consisted of breeding lines, unimproved germplasm and related species. Five cultivars of papayas were initially screened for polymorphisms by 64 sets of *Eco*RI-*Mse*I primers with three nucleotide extensions. The number of polymorphic markers ranged from 0 to 9 with an average of 3.2. Nine primers were selected to assess all samples and generated 186 polymorphic markers (42%) from 445 readable fragments. The estimation of genetic similarity using pairwise comparison among 63 papaya accessions ranged between 0.74

and 0.98 (mean 0.88). Cluster analysis of 71 papaya accessions and related species showed the genetic relationship among individual genotypes which developed in different geographic regions. The first cluster included all 15 Solo-type cultivars and breeding lines. The second cluster included dioecious Australian cultivars and Indian cultivars that grow in subtropical or temperate regions. The third cluster was a group of cultivars originating from different countries. AFLP markers were used to study genetic relationships between papaya and wild relatives by Van Droogenbroeck et al. (2002) who analysed 95 accessions of papayas and wild relatives from Ecuador. Five primer combinations were used and revealed 951 bands ranging in length from 50 to 500 base pairs. Only 512 bands were scored of which 16 were monomorphic. All papaya genotypes were clustered separate to two other clusters comprising individuals from *Jacaratia* or *Vasconcellea*. Genetic similarity ranged from 0.39 to 0.81 among the *Vasconcellea* species that were assessed.

Genetic relationships between *C. papaya* and wild relatives were studied by Jobin-Décor et al. (1997) by comparing isozyme and RAPD techniques. A total of 47 bands were generated by nine enzymes and 188 bands were generated from 14 RAPD primers. Both techniques gave similar measures of genetic distance of 70% dissimilarity between *C. papaya* and the other *Carica* species (later renamed *Vasconcellea* species) and approximately 50% dissimilarities among *Vasconcellea* species. RFLP markers were used to study phylogenetic relationships using the chloroplast DNA (cpDNA) of 12 wild and cultivated species of *Cairca* (Aradhya et al. 1999). Twenty-three accessions, representing 14 taxa, were analysed in the cpDNA intergenic spacer region by amplification of the region via PCR technique and then the PCR product was digested by 14 restriction enzymes. A total of 138 fragments accounting for 137 restriction sites were examined and the results confirmed the close association among South American wild *Carica* (*Vasconcellea*) species.

From a microsatellite-enriched library developed using $(GA)_n$ and $(GT)_n$ probes (Pérez et al. 2006), 45 primer pairs giving the best resolution and allelic differentiation were used for evaluation of 29 accessions of *C. papaya* and 11 accession of *Vasconcellea*. Of these, 24 revealed polymorphisms between these two genera. A total of 99 alleles were observed in papaya with an average of 3.8 alleles per locus. In the *Vasconcellea* samples, 22 alleles were identified from four loci. These two genera had a clear allelic divergence for the loci that they shared. This strong differentiation gave further support to the hypothesis of the early divergence of *Vasconcellea* from *Carica* (Pérez et al. 2006). Many more SSR markers were identified from the genome sequence project (Eustice et al. 2008). These were tested for polymorphism in seven genotypes of papaya. Of the 938 SSR markers that were defined, 812 were from genomic sequences and 126 from genic sequences (Eustice et al. 2008). Overall, 52.9% were polymorphic. SSR primers developed in 2008 (Oliveira et al. 2008) were screened on 30 papaya accessions and 18 landraces (de Oliveira et al. 2010b). Of the 100 SSR primers, 81 successfully amplified PCR products of high quality and were selected for further studies. Of these, 59 produced easily scorable markers and detected a total of 237 alleles with 2 to 11 per locus. In a separate study using 27 of the same SSR loci developed in 2008 (Oliveira et al. 2008), the relationships among 83 papaya lines were assessed (de Oliveira et al. 2010b). Of the 27 primers, 20 were polymorphic and identified a total of 86 alleles, with an average of 3.18 alleles per primer. Since cultivated *C. papaya* is proposed to have a low or narrow genetic base (Ratchadaporn et al. 2007; Stiles et al. 1993), many important genes conditioning traits of interest (such as disease resistances and abiotic stress tolerances) may have been lost or excluded in the pursuit of other traits (such as fruit colour and

sweetness). Indeed, many disease resistances are found in wild relative *Vasconcellea* species and future breeding may require interspecific recombination to reintrogress these back into the elite cultivated genomes (d'Eeckenbrugge et al. 2014).

A number of taxonomy studies have confirmed the diversity between *C. papaya* and *Vasconcellea* species and have supported the separation into two genera by Badillo (2000). Most studies supported the early divergence of *C. papaya* from the wild relatives and this has been verified by different molecular marker techniques; isozyme and RAPD (Jobin-Decor et al. 1997), RFLP (Aradhya et al. 1999), AFLP (Kim et al. 2002; Van Droogenbroeck et al. 2002). The most closely related species were reported as *V. stipulata* (Badillo) and *V. pubescens* (Jobin-Decor et al. 1997; Sharon et al. 1992); and *V. goudotiana* Tr. et Pl. and *V. pubescens* (Kim et al. 2002). The species most distant from *C. papaya* were reported to be *V. cauliflora* (Jobin-Decor et al. 1997) and *V. goudotiana* (Sharon et al. 1992; Kim et al. 2002). In particular, Kim et al. (2002) reported that the average genetic similarity between papaya and other *Vasconcellea* species was 0.43 and the average similarity among six different species of *Vasconcellea* was 0.73, much closer to each other than to *C. papaya*. *C. goudotiana* was the most distantly related species to papaya (0.36 similarity), while it was closely related to *C. pubescens* (*V. pubescens*) with a similarity of 0.87.

Previously, Jobin-Décor et al. (1997) had reported *C. papaya* to be distinct from the other *Carica* species, *C. cauliflora*, *C. parviflora*, *C. pubescens*, *C. goudotiana*, *C. stipulata* and *C. quercifolia* with a mean dissimilarity of 0.73 and 0.69 using isozyme and RAPD analysis, respectively. As this work was done before 2000 all these species were considered to be in *Carica* genus at that time. The other *Carica* (*Vasconcellea*) species were more closely related to each other with a mean dissimilarity of 0.46. The closest two species were *V. stipulata* and *V. pubescens* with the dissimilarity of 0.13 and 0.18 0.87 and 0.82 using isozyme and RAPD analysis respectively. However, from RAPD analysis, the *Vasconcellea* species most distant from *C. papaya* was *cauliflora.* It was reported that *C. papaya* and other species had band sharing between 25% and 48% (Sharon et al. 1992). *C. goudotiana* is more distant from *C. papaya* with band sharing of 25%. *C. stipulata* and *C. pubescens* were very closely related species with band sharing of 71%.

In summarising their genetic diversity, it should be noted that the *Vasconcellea* species have recently been divided into three clades (D'Eeckenbrugge et al. 2014). They are (1) *V. weberbaueri* (Harms)*, V. stipulata, V. x heilbornii* (Badillo) Badillo and *V. parviflora* A.DC.*;* (2) *V. chilensis* (Planch. ex A.DC.) A.DC.*, V. candicans* (Gray) A.DC.*, V. quercifolia* St.-Hil. and *V. glandulosa* A.DC. and (3) a clade holding all other species of the genus (D'Eeckenbrugge et al. 2014).

1.1.3.3 Molecular marker and sex determination in *Carica papaya*

Carica papaya is a polygamous species with three basic sex types; female, male and hermaphrodite. Although the male plants occasionally produce hermaphrodite flowers on the abaxial end of inflorescences, they do not produce commercial fruit. However, in dioecious plantings, which are common in subtropical regions, male plants are still needed for pollen. Usually the ratio of male: female plants is 1:10, thus multiple seedlings are planted at each site and then the plants are thin to achieve the required ratio of female and male plants. In tropical regions hermaphrodite trees are preferred

because every tree is capable of producing marketable fruits. In plantations comprising hermaphrodite plants, female plants are unwanted and removed after flowering. Commercial papaya growers have to plant 3-5 plants per site, then evaluate sex type after flowering and cull the undesired plants. The process is time consuming, laborious, and cost ineffective. In addition, competition between multiple plants at each planting site causes poor root systems, elongated plants and increased height to the first flower and fruit. Therefore, the use of DNA markers to discriminate sex of papaya plants at the earliest plant growth stage has the potential to greatly increase efficiency within the papaya production system. Thus much research has been applied to this subject in recent years.

Genetic control of sex of papaya has been studied since 1938. Hofmeyr (1938) and Storey (1938) independently proposed the hypothesis that sex determination in papaya is controlled by a single dominant gene with three alleles, named M^1, M^2 and m by Hofmeyr, and M, M^h and m by Storey. They proposed the genotype of male, hermaphrodite and female plants are Mm (M^1m), M^hm (M^2m) and mm respectively, and explained that homozygous dominant alleles are lethal. Therefore segregation of sex type from selfed hermaphrodite trees is 2 hermaphrodites: 1 female. Whereas seeds from female trees segregate at the ratio of 1:1 female: hermaphrodite if the plant is crossed with a hermaphrodite tree, but that of 1:1 female: male when it is crossed with a male tree. Subsequently, other researchers have proposed other hypotheses for genetic control of sex in papaya and this was reviewed by Ming et al. (2007).

Most research on DNA marker-assisted sex selection in papaya has been done on Hawaiian papaya genotypes. The first report of a sex linked marker in papaya was reported by Sondur et al. in 1996. They created a genetic linkage map using RAPD markers and investigated the genetics of sex determination in papaya using an F_2 population of Hawaiian cultivar Sunrise x IH breeding line 356. Of 596 10-base primers screened, two; OPT1 and OPT12, produced two marker bands; OPT1C and OPT12, flanking the *SEX1* locus at 7 cM for both markers. RAPD and DNA amplification fingerprinting (DAF) were compared and showed that DAF reactions produced at least five times more fragments than equivalent RAPD reactions in terms of ability to detect variation. They also revealed that DAF reactions were more reliable (Somsri et al. 1998). Bulk segregant analysis was used to define a large number of DAF markers present in only male or hermaphrodite pooled DNAs. Preliminary analyses for linkage associations indicated these markers were closely linked to the sex- determining alleles. Ten years later, Somsri and Bussabakornkul (2008) employed DAF to study the relationships between fourteen cultivars of papaya in Thailand. Using 11 primers, a total of 129 distinct fragments were amplified. Primer OPA06 could be used to identify the sex type of papaya plants. This primer produced two polymorphic bands: at 365 bp from the hermaphrodite bulk DNA and 360 bp from the male bulk DNA. Neither band was detected for females. Evaluation of the accuracy of OPA 06 analysis was verified using 254 plants of different generations and their original parents, and the analysis correctly identified sex type for 88.18% of the plants. In the final experiment, 47 hermaphrodite plants of the Khaeg Dum cultivar, that were grown in tissue culture, were examined using OPA 06, and the sex type was identified correctly for 100% of the plants.

From 2000 onwards, the sequence characterized amplified region (SCAR) technique has been used to increase specificity of priming sites from RAPD primers to the target DNA for sex determination of papaya. Eighty RAPD primers from Operon kits were screened on 12 different papaya varieties and the marker OPF2-0.8 kb was identified as male-

specific (Parasnis et al. 2000). The marker was converted into a SCAR marker, by designing a 20-bp primer pair as a sex specific primer. They also developed an internal control for the PCR reaction using primer GC, which is a neutral sex, meaning the marker presents in all sex types of papaya. For mass screening, they developed a single step DNA extraction and used a 15 bp-SCAR primer at a lower annealing temperature for sex detection.

The information from Sondur et al. (1996) was applied by cloning three RAPD products and SCAR primers were designed on these sequences. Two RAPD markers, OPT1C and OPT12, flanked the *SEX1* gene (Sondur et al. 1996), however Deputy et al. (2002) identified another marker, W11, that did not show recombination in the population that they used. So, W11 was included in this study along with T1 and T12. A SCAR T1 primer was designed on the interval sequences of the T1 marker, however SCAR T1 can amplify all sex types; female, male and hermaphrodite at 1300 bp. This could be because one or more point mutations exist in the original 10-base primer site that can distinguish females from hermaphrodites. Therefore, T1 was used as positive (or internal) control for PCR amplification. T12 and W11 were designed on the original 10-base plus a further 10-11 bases to make the primer more specific to papaya sequences. Both primers successfully differentiated sex type male/hermaphrodite from female plants generating the marker at 800 bp for both primers. The linkage analysis of SCAR markers W11 and T12 in 182 F_2 plants from the cross of Sunup x Kapaho indicated that these markers were within 0.3 cM of *SEX1*.

RAPD techniques were used to determine the sex of 11 Hawaiian cultivars of *C. papaya* for three sex types, male female and hermaphrodite (Urasaki et al. 2002b). Twenty-five arbitrary 10-mer primers were tested with papaya DNA. The IBRC-RP07 primer produced a fragment named PSDM at 450 bp in all male and hermaphrodite but not female plants. The fragment was analysed and a SCAR marker named SCARps was designed and produced a PCR product of 225 bp in the male and hermaphrodite plants only. A multiplex-PCR assay was developed for a sex-specific SCARpm marker and a marker for a papain gene as an internal control to minimize false negatives (Urasaki et al. 2002a). The marker was tested for amplification on a hermaphrodite plant of *C. papaya* 'Sunrise Solo'. This marker differentiated hermaphrodite and male plants from female plants.

Lemos et al. (2002) screened 152 RAPD primers on female and hermaphrodite plants of *C. papaya* cv. Baixinho de Santa Amália, cv. Sunrise Solo and cv. Improved Sunrise Solo 72/12. Primer BC210 produced a marker band at 438 bp ($BC210_{438}$) that was present in hermaphrodite but not in female plants. The marker was tested with 195 different samples from three cultivars and was present only in hermaphrodite plants. Published sex linked markers were validated on a selection of Brazilian commercial genotypes; two varieties of a Solo group and 2 hybrids of the Formosa group (de Oliveira et al. 2007). Four SCAR markers (Deputy et al. 2002; Parasnis et al. 2000; Urasaki et al. 2002b; Chaves-Bedoya and Nuñez 2007) revealed the presence of both false positives and negatives in some varieties, while the RAPD marker $BC210_{438}$ (Lemos et al. 2002) could predict papaya sex type correctly.

In another study, the sex linked markers that were described previously were tested on three Columbian papayas, but could not distinguish between male/hermaphrodite and female plants (Chaves-Bedoya and Nuñez 2007). Therefore, 32 arbitrary 10-mer Operon primers were screened and the OP-Y7 primer that generated a PCR product that had 900 bp and was present only in male plants and absent in female and hermaphrodite plants.

The marker was analysed and converted into a SCAR marker that could differentiate female plants from male and hermaphrodite plants. The SCAR SDSP marker at 369 bp, was present in male and hermaphrodite but not female plants.

In Sri Lanka, Niroshini et al. (2008) screened 100 arbitary decamer primers in ten plants of each papaya sex type. The plants were selected from home gardens near Kadawatha, Sri Lanka. Of the 100 primers that were tested, two primers; OPC09 and OPE03 produced two DNA maker bands specific to male/hermaphrodite plants at 1.7 and 0.4 kb, respectively. The markers were then sequenced and SCAR primers were designed by use of extension oligonucleotides at both ends of the sequence. SCAR primer C09/20 amplified two fragments of length 1.7 kb and 978 bp in both male and hermaphrodite plants. However, SCAR primer E03/20FP and E03/20RP that were designed from OPE03-0.4 did not detect polymorphisms among plants of different sex types.

Other markers based on microsatellites have been used for sex detection in papaya by Santos et al. (2003). It was possible to design primers from the library from sequences enriched with the probe $(TCA)_{10}$. Thirty-two pairs of SSR primers were designed, however, none of them could identify sex type in this study. Commercial cultivars of papaya and a wild species *V. cauliflora* were screened by digesting genomic DNA with various restriction enzymes and microsatellite sequences were used as probes by Parasnis et al. (1999). The microsatellite repeats $(GATA)_4$ and $(GAA)_6$ detected sex-specific differences in *Hin*fI or *Hae*III digested samples. However, only the repeat $(GATA)_4$ showed male/hermaphrodite specific bands at 5 and 4 kb after digesting the genomic DNA with *Hin*fI and *Hae*II, respectively, while the repeat $(GAA)_6$ could detect polymorphisms for sex in some cultivars only.

Inter simple sequence repeat (ISSR) and RAPD techniques were used to determine sex of 200 seedlings of a local variety of papaya (Gangopadhyay et al. 2007) in Kolkata, India. Ten RAPD primers (OPA01-OPA05 and OPB01-OPB05) failed to show polymorphisms among the three sex types. Of three ISSR primers used in this study, one ISSR primer, $(GACA)_4$, could distinguish female or hermaphrodite from male plants.

In conclusion, much marker research has been applied to the identification of DNA markers for the differentiation and selection of male, female and hermaphrodite plants. Although many of these markers can be used for marker assisted selection in breeding programs, there is a need to adapt some of these markers into low-cost techniques that can identify sex of seedlings and thus facilitate commercial production of papayas. Over planting, then thinning to achieve the desired sex ratios after plants reach flowering stage is still a major and potentially unnecessary cost for papaya producers in both tropical and subtropical regions.

1.1.3.4 Molecular marker assisted selection for papaya breeding

1.1.3.4.1 Disease resistance

Lack of disease resistance genotypes in *Carica* is the major problem for crop improvement, while resistance to all diseases that attack papaya can be found in wild relative *Vasconcellea* species (D'Eeckenbrugge et al. 2014). Therefore the relationship between these two genera has been studied with the aim of transferring resistant genes from *Vasconcellea* spp. to papaya.

Papaya ringspot disease, caused by *Papaya ringspot virus* type P; PRSV-P, is widely reported as the most devastating disease of papaya production worldwide. The sustainable method to control this disease in the papaya industry is to produce improved papaya varieties that are resistant to the pathogen. A transgenic papaya variety that was resistant to PRSV-P was successfully developed in 1990 (Fitch et al. 1990), however GMOs plants are not accepted in many countries, and transgenic resistant varieties can be virus-strain specific in their resistance (Tennant et al. 1994). Therefore, conventional breeding for PRSV-P resistance in papaya is a viable option for long-term control of the disease, and until GMO food crops are more universally accepted worldwide.

Resistance to PRSV-P has been reported in *V. cauliflora, V. stipulata, V. pubescens and V. quercifolia* (Manshardt and Drew 1998). The gene for PRSV-P resistance was successfully backcrossed from *V. pubescens* into *V. parviflora* from F_3 interspecific hybrids containing the homozygous dominant allele of the gene (O' Brien and Drew 2009). This suggested that a single dominant gene controlled PRSV-P resistance in *V. pubescens* and was consistent with earlier reports on the PRSV-P resistance in a generic hybrid between *V. pubescens* and *C. papaya* (Drew et al. 1998b). This is consistent with reports of single gene dominance regulating the PRSV-P resistance in F_1 intergeneric hybrids of *C. papaya* and *V. cauliflora* (Magdalita et al. 1997). Similar results were reported in interspecific hybrids between *V. pubescens* x *V. parviflora* (Dillon et al. 2006a; b). Genetic mapping studies of these hybrids further supported the concept that PRSV-P resistance in *V. pubescens* is controlled by a single dominant gene (Dillon et al. 2006c). This was confirmed with molecular markers which were linked to a single locus resistance gene (*prsv-1*) that was identified in *V. pubescens* (Dillon et al. 2005; Dillon et al. 2006b).

A genetic map of PRSV-P resistance gene(s) based on RAF markers was constructed from100 F_2 plants of *V. cundinamarcensis* (*V. pubescens*) and *V. parviflora* (Dillon et al. 2005). Mapping of dominant markers in repulsion phase in F_2 populations can cause an incorrect estimation of genetic distance, therefore, RAF markers were mapped to separate parental maps. For *V. pubescens*; markers were mapped to ten linkage groups that covered 745.4 cM with an average distance of 9.68 cM between adjacent markers. The PRSV-P resistance locus (*prsv-1*) was mapped within 4 and 2.8 cM of adjacent markers Pbw15_40 and OPA15_8 on LG7. For *V. parviflora*, markers were mapped to ten linkage groups that covered 630.2 cM separated by an average distance of 7.95 cM between adjacent markers. The markers Pbw15_40 and OPA15_8, flanking *prsv-1* locus, were near but not on the resistance gene-coding region, as they did not co-locate with the resistant phenotype.

Markers linked to *prsv-1* have been used in the marker-assisted breeding programs described above because of their dominant inheritance, and because resistance to the Australian strain of PRSV-P imparted by *prsv-1* has been shown to be robust (Drew et al. 1998a; Magdalita et al. 1997). Five DNA markers, which were developed by use of RAF on bulked segregants of virus resistant and susceptible populations, were identified in the cross of *V. parviflora* x *V. pubescens* (Dillon et al. 2006b). The markers were mapped to the same linkage group, LG7, flanking prsv-1 at the distance of 2.1, 5.4, 9.7 and 12.0 cM for the marker Opa_16r, Opk4_1r, Opk4_2r and Opb8_1r, respectively, while the another marker, Opa11_5R co-located with prsv-1. The two

candidate markers, Opa11_5R and Opk4_1r, were sequenced and converted to SCAR markers. A SCAR marker, Opk4_1r, was converted into a CAPS marker, *Psilk4*, by digesting the amplicon with *Psi*1 and was shown to be diagnostic for the 3 alleles of *prsv-1*. The SCAR marker Opk4_1r detected similar band sizes for *V. pubescens, V. cauliflora* and *V. goudotiana* with the size of 360 bp, 360 bp and 361 bp, respectively. However, the amplicons of *V. parviflora*, *V. quercifolia* and *V. stipulata* were slightly larger with the size of 372 bp. The application of this SCAR and CAPS marker for marker assisted breeding was confirmed in research on interspecific populations of *V. pubescens* x *V. parviflora*; F_2 and F_3 populations produced from the *V. pubescens* and *V. parviflora* F_1; and, BC_1 and BC_2 generations when *V. pubescens* x *V. parviflora* F_3 RR plants were backcrossed to *V. parviflora* (O'Brien and Drew 2010). The Opk4_1r SCAR marker amplified in other *Vasconcellea* spp*. quercifolia, goudotiana,* and *cauliflora* however, the CAPS marker was not consistent in determining the allele of *prsv-1* in crosses involving *Vasconcellea* spp. other than *pubescens* and *parviflora* (O'Brien and Drew 2010).

Transgenic papayas, resistant to PRSV-P have been developed in many countries; and, DNA markers have been used to detect the transgenic plants. A detection protocol for characterisation of PRSV coat protein transgenic papaya lines was demonstrated by use of PCR (Fan et al. 2009). PCR patterns using primers designed from the left or right flanking DNA sequence of the transgene insert in transgenic papaya lines were specific and reproducible.

In addition to PRSV-P, papayas are susceptible to many other pathogens. However, there are few reports on the use of DNA markers to identify other disease genes. However, tolerance to *Phytophthora palmivora* in papaya was identified and molecular markers linked to this resistance were developed by use of AFLPs (Noorda-Nguyen et al. 2010). Several polymorphic bands linked with the tolerance trait in a F_2 population, derived from an F_1 of the most tolerant Hawaiian cultivar Kamiya, crossed with a highly susceptible cultivar SunUp, have been identified. These markers were further characterized to form sequence characterized amplified region markers. Nineteen genes were selected for gene expression analysis for resistance to *P. palmivora* (Porter et al. 2009b). Of these genes, a predicted peroxidase, β-1,3-glucanase, ferulate 5-hydroxylase and hypersensitive-induced response protein were pathogen upregulated, while a second peroxidase (Cp9) and aquaporin (Cp15) were downregulated.

1.1.3.4.2 Hybrid identification

To be able to identify hybrids at an early stage of plant growth is preferable in crop improvement studies, especially confirmation of F_1 hybrids in populations to facilitate further crossing. Seventeen RAPD primers were selected and screened to confirm hybridity of 120 putative interspecific cross of *C. papaya* x *C. cauliflora* (whereas, nowadays, they are intrageneric cross of *C. papaya* x *V. cauliflora*; Magdalita et al. 1997). A range of 1-5 primers consistently confirmed that all 120 plants were genetic hybrids. A single primer can not guarantee accurate results thus more than one marker is necessary to analyse for hybridity. This is because chromosome elimination can occur during meiosis thus absence of a single marker may represent elimination of part of a chromosome.

1.1.3.4.3 Fruit quality traits

Even though papaya genomic research is exceptionally advanced in many aspects, there are many other characteristics of papaya that need to be studied. Very little information on other characteristics of papaya has been studied in the past few years. One of the characteristics that need to be identified for papaya marketing is fruit flesh colour. The Australian papaya industry is clearly split between growers who grow either the yellow-fleshed (commonly described as Paw Paw) or the red-fleshed varieties (known as Papaya) or both types (Australian Papaya Industry Strategic Plan 2008-2012). A single major gene for yellow flesh that is dominant over red flesh color, has been found in a simple Mendelian segregation in flesh fruit colour of papaya by recognized papaya breeders (Blas et al. 2010). The carotenoid composition profiles of red- and yellow-fleshed Hawaiian Solo papayas showed a strong accumulation of lycopene in red-fleshed fruit, while none was detected in yellow flesh (Yamamoto 1964). The flesh colour locus was mapped near the end of LG7 and the two flanking markers were located at 3.4 and 3.7 cM, respectively (Ma et al. 2004). Recently, a high-density genetic map of papaya using SSR markers was established, fruit flesh colour was mapped at the end of LG5 and the closet marker was located at 13 cM (Chen et al. 2007). Blas et al. (2010) reported the cloning and characterisation of the papaya chromoplast-specific lycopene β-cyclase, *cpCYC-b*, and geneomic analysis of the surrounding region included a recombination hot spot in papaya. They found tomato chromoplast-specific lycopene β-cyclase (*CYC-b*) and SunUp *CpCYC-b*, shared 75% sequence identity over a 682-bp genomic sequence length. Quantitative RT-PCR analysis and subsequent functional analysis in bacteria confirmed the role of *CpCYC-b* in controlling fruit flesh color in papaya. The elevated expression of *CpCYC-b* and papaya β-carotene hydroxylase (*CpCHY-b*) between yellow-fleshed Kapoho and red-fleshed SunUp validated activation of the carotenoid biosynthesis pathway in yellow-fleshed papaya. The disruption of this pathway in red flesh varieties is caused by a frame-shift mutation induced by a 2-bp insertion (Blas et al. 2010). A PCR-based marker was developed the CPFC1 marker (Table 1.2), which is 530 bp away from *CpCYC-b*. The marker showed approximately 98% recombination frequency to flesh colour in 219 F_2 (KD3 x 2H94). It should be noted that this tightly linked marker, only 580 bp away from the target gene, is still not 100% accurate due to the extremely high recombination rate in this region of the genome.

Table 1.2: DNA markers for PRSV-P and other genetic traits in *Carica papaya*

Characteristics	Markers name	Primer sequence	Reference
PRSV-P coat protein region detection	Cp	F: GAGAAGTGGTATGAGGGAGTG R: CCATACCTGCCGTCACAATCA	Dillon et al. (2005)
PRSV-P resistance; *prsv-1* locus	Opa5_11R	PBA115R F: CAATCGCCGTAGGAAAATTC PBA115R R: CAATCGCCGTAGAGGAGGAGG	Dillon et al. (2006a)
PRSV-P resistance; *prsv-1* locus	Opk4_1r	PBK41R F: CCGCCCAAACTGCGGAACAC PBK41R R: CCGCCCAAACCCCCAACTAG	Dillon et al. (2006a)
Flesh colour; CpCYC-b	CPFC1	F: GACGTGTTAGTGTCCGACAA R: GACCAGGAAGCAAATTTTGTAA	Blas et al. (2012)

1.1.4 Quantitative trait loci in papaya

Many agronomically important traits, i.e. fruit size, fruit shape, flesh flavour and skin quality are quantitative traits that are influenced by multiple genes. Various quantitative trait loci (QTLs) have been identified in many crops; however, surprisingly not many QTLs have been identified in papaya breeding research. Recently, in 2012, a QTL analysis for papaya fruit size and shape has been reported (Blas et al. 2012). Fifty-four SSR markers, the morphological flesh colour locus and CPF1 and CPF2 SCAR markers were mapped to 11 LGs using a population of 219 F_2 plants (KD X 2H94). Fourteen QTLs having phenotypic effects ranging from 5 to 23% were identified across six linkage groups. These loci contain homologs to the tomato fruit QTL *ovate*, *sun* and *fw2.2* regulating fruit size and shape.

1.1.5 Potential and future

Papaya genomic research is exceptionally advanced in many aspects. There is a recent genetic map which correlates with the number of chromosomes. A physical map was analysed, a draft genome of the plant was sequenced and anchored on the genetic map, the genome was thoroughly analysed for simple sequence repeats and *NBS* gene families, and SSR libraries have been developed. Thus much basic information has been revealed in the past few years. However, there are many other characteristics of papaya that need to be studied and the molecular markers that have been identified, particularly the SSR markers, need to be linked to the many important traits of interest for MAS in papaya breeding.

Future papaya genomics approaches to develop more precise tools for trait selection are likely to involve the identification and mapping of candidate genes from the full genome sequence. These must then be tested for functional validation to the trait of interest, potentially through expression analysis. Meanwhile, with affordability becoming a reality, it is likely that expression of representative RNA sequences of the geneome will be analysed and functional genes isolated directly from the transcriptome in response to a particular target trait, such as disease resistance. We predict that in the very near future, that suits of expressed papaya genes and their predicted pathways will be commonly available for e-mapping and trait-associated marker-assisted selection.

1.2 Additional information from the previous reviews

1.2.1 *Vasconcellea quercifoia*

V. quercifolia was reported to be resistant to PRSV-P virus in Florida (Conover 1964), Hawaii (Manshardt and Wenslaff 1989) and Australia (Drew et al. 2006a; b). However, another report classified *V. quercifolia* as one of the susceptible species in Venezuela (Horovitz and Jimenez 1967). This reflects variation in resistance within the species and/or varying response to different PRSV-P strains. *V. quercifolia* was classified in clade 2 of *Vasconcellea* along with *V. chilensis*, *V. candicans* and *V. glandulosa* (d'Eeckenbrugge et al. 2014). This clade is the basal clade of genus *Vasconcellea* which means that they diverged early in evolution from other species of the genus. *V. quercifolia* was successfully crossed to papaya (Drew et al. 1998a; 2005; 2006a; 2006b; Manshardt and Wenslaff 1989; Siar 2011). It is proposed that this species is genetically less distant from *C. papaya* and hence the best option for intergeneric hybridisation with *C. papaya* (Siar et al. 2011).

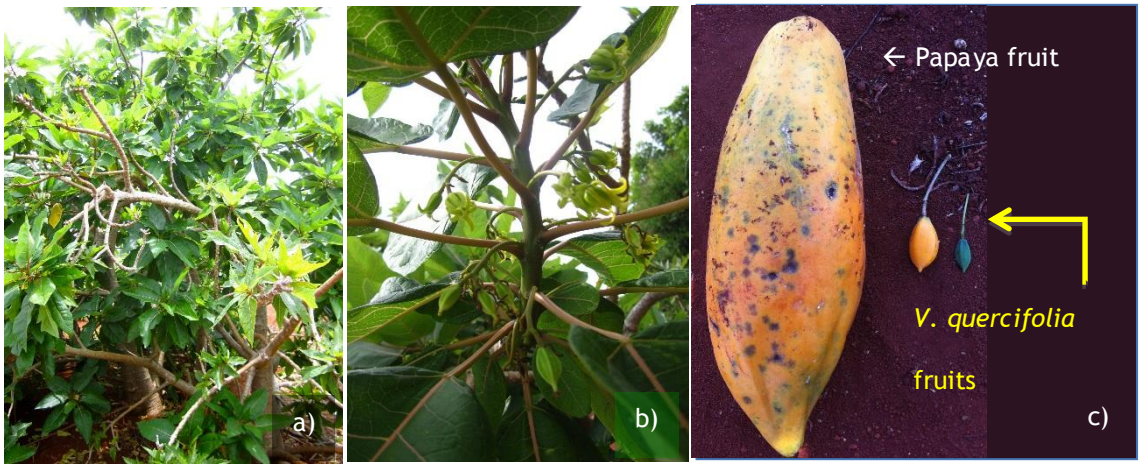

Figure 1.1: *V. quercifolia* a) tree, b) fruits and flowers and c) a comparison to a papaya fruit in Thornlands, Queensland

1.2.2 Papaya ringspot virus

Papaya ringspot virus (PRSV), a positive sense RNA, is a member of *Potyvirus*, the aphid-transmitted genus in the family *Potyviridae*. This virus can also be transmitted mechanically, and is typically not seed-transmitted. It is transmitted by many species of aphids (mainly *Myzus persicae* and *Aphis gossypii*) by sap sucking in a non-persistent manner.

The virus has and continues to be a destructive disease and a major biotic problem for papaya and cucurbit production worldwide (Purcifull et al. 1984). There are two types of PRSV based on host range infection; papaya-infecting type-P (PRSV-P) and non-papaya – infecting type-W (PRSV-W). PRSV-P isolates can infect plants in the family's Caricaceae, Cucurbitaceae and Chenopodiaceae, while the isolate of PRSV-W can infect plants in the families Cucurbitaceae and Chenopodiaceae. There was no significant difference between sequence of the coat protein gene for the Australian isolates, PRSV type P or W which suggested that PRSV-P could have arose from PRSV-W in Australia (Bateson et al. 1994).

The name of the disease, papaya ringspot, was taken from the ringed spots that develop on fruits and infected trees (Jensen 1949). In addition to the typical ringspots, PRSV infection produces a wide range of symptoms including leaf mosaic and chlorosis, water soaked oily streaks on the petiole and upper part of the trunk, distortion of young leaves, stunting of infected plants and flower abortion. Consequently, fruit production can be severely decreased and fruit quality can be reduced by decreased sugar concentration (Gonsalves 1998).

PRSV-P is considered an important threat to the Australian papaya industry although it has not yet occurred in the major growing region of North Queensland (Drew et al. 2006b). In 1991, the disease was detected in Australia for the first time in Southeast Queensland (Thomas and Dodman 1993), and could spread to North Queensland.

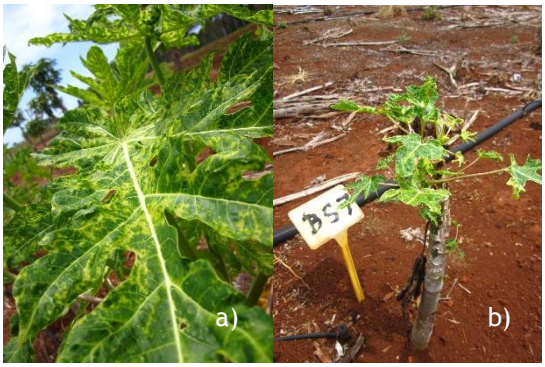

Figure 1.2: Papaya a) leaves and b) tree infected by PRSV-P in Thornlands, Queensland

1.2.3 Crop improvement for PRSV-P resistance in papaya

The sustainable method to control this disease in the papaya industry is to produce improved papaya varieties that are resistant to the pathogen. A transgenic papaya variety that was resistant to PRSV-P was successfully developed in 1990 (Fitch et al. 1990). The strategy is termed coat protein-mediated resistance and the first transgenic line was called 55-1. Transgenic papayas in other countries have been reported in Brazil for 'Sunrise' and 'Sunset' cultivars (Souza et al. 2005), Venezuela (Fermin et al. 2004), Jamaica (Cai et al. 1999), Taiwan (Bau et al. 2003; 2004; Kung et al. 2009) and Thailand (reviewed by Tripathi et al. 2008). These lines showed excellent PRSV-P resistance and horticultural characteristics. They had increased yields when compared to the non-transgenic controls that were infected with the virus.

Although GM is generally considered to be the best strategy for long-term virus control (Gonsalves 1998), they are not accepted in many countries (Tennant et al. 1994; 2001). Furthermore, transgenic resistant varieties can be virus-strain specific in their resistance (Nelson et al. 1988; Quemada et al. 1991; Sanders et al. 1992; Nakajima et al. 1993) as the virus and transgene must have more than 98% sequence homology for the technique to be effective (Gonsalves 1998). Therefore, conventional breeding for PRSV-P resistance in papaya is a viable option for long-term control of the disease. Reviews of breeding for PRSV-P resistance was covered in 1.1.

1.2.4 Molecular study of PRSV-P resistance genes in *V. pubescens*

Recent work was undertaken by Razean Haireen (2013) to further characterise PRSV-P resistance genes in *V. pubescens.* The marker Opa11_5r, which was shown to be linked to PRSV-P resistance in *V. pubescens* Dillon *et al.* (2006a), was sequenced, aligned to the NCBI database and shown to have similarity to a Kinase gene. Novel genes of VP_STK1, VP_STK2 and VP_LRR1 were characterized from *V. pubescens* (Razean Haireen, 2013). The full sequence of STK genes revealed one bp insertion/deletion in the coding region of *V. pubescens* and was predicted to be derived from an alternative splicing process. Gene expression supported the function of VP_STK2 in relation to resistance to PRSV-P in *V. pubescens* as it was up-regulated at 15 days after inoculation.

1.2.5 Resistance to plant virus

Resistance to disease of plants can be caused by the ability of the pathogen to infect a host (host and non-host resistance) or by physical/chemical response to pathogen (passive and active resistance). In plant viruses, host resistance has been investigated in detail because it is genetically accessible (Kang et al. 2005). The term host or specific resistance means a virus may or may not able to multiply to some extent, but the spread of viral particles is restricted relative to susceptible hosts. Active defense mechanisms are more related to resistance to a virus in a plant because typically the virus initiates infection by penetrating the plant cell wall through wounds either by mechanical abrasion or vectors such as insects (Goldbach et al. 2003; Kang et al. 2005)

Plant viruses have to undergo multiple steps to complete their life cycles. The processes include entry into plant cells, uncoating of nucleic acid, translation of viral proteins, replication of viral nucleic acid, assembly of progenies virions, cell-to-cell movement, systemic movement (long distance movement) and plant-to-plant movement (Carrington et al. 1996). Resistance can operate at four levels: inhibition of replication, cell-to-cell movement, long distance movement and defense responses restricting infection to a limited number of cells (Zaitlin and Hull 1987).

1.2.5.1 Type of resistance

Complete resistance to virus infection in plants is referred to as ***immunity*** (Bruening 2006). The immunity is usually a result of prevention of virus replication. If immunity occurs against all biotypes of a pathogen and in all cultivars or accessions of plant species, it is referred to as non-host resistance. The terms of ***extreme resistance*** (ER) or cellular resistance convey immunity (Fraser 1986; 1990). The most common mechanism associated with active defence is ***hypersensitive response*** (HR), which is a rapid development of cell death at and immediately surrounding infection sites (Morel and Dangl 1997). As a result, HR disrupts cell-to-cell movement of plant viruses and results in prevention of further spread of a virus (Kang et al. 2005). The HR and ER are referred to as a type of ***innate resistance*** where both are associated with a dominant resistance gene (reviewed by Gilliland et al. 2006; Loebenstein and Akad 2006). ***Tolerance*** is another level of resistance where plants may show mild or no symptoms. It is usually associated with a reduction of viral titre in the infected plants (Palukaitis and Carr 2008).

Because viruses are intercellular obligate parasites, they need the host cell to replicate themselves. Cell death or HR appears to be the best system to block multiplication of the viral particles. Fraser (1990) reported more than 65% of viral resistance genes were not associated with HR, but rather with the reduction of viral multiplication (Loebenstein and Akad 2006). Thus, HR is not the major resistance mechanism of a plant against viral infection (Morel and Dangl 1997). Local lesion infection was observed to be one of the most remarkable resistance responses of plant to virus and is used by breeders to obtain resistant cultivars. The resistance to PRSV-P in *V. pubescens* showed a sign of HR (Figure 1.3) in F_3 (RR), BC_2 (Rr) and BC_4 (Rr) plants in interspecific cross of *V. parviflora* (susceptible to PRSV-P) and *V. pubescens* (Razean Haireen 2013). However, self-limiting necrotic, chlorotic and ring-like patterns at local lesions has been described as localised acquired resistance rather HR (Loebenstein and Akad 2006).

Figure 1.3: A hypersensitive response showed rapid cell death and yellow spots in a BC_2F_3 plant of interspecific cross of *V. parviflora* and *V. pubescens* after inoculation with PRSV-P after 45 days (Picture from Razean Haireen, 2013)

1.2.5.2 Gene for gene interaction

The classic concept of "gene-for-gene" interaction has been used to explain resistance mechanisms in plants for the correspondence between plant a resistance gene (*R*) and a pathogen's avirulence gene (*Avr*) (Flor 1971). The model proposes the resistance reaction occurs when the complementary compatible of dominant *R* gene and *Avr* gene are present. Conversely, a loss or alteration to either the *R* gene or *Avr* gene leads to susceptibility to a disease (compatibility). This simple model has been used to explain many biotroph pathogens, including fungi, viruses, bacteria and nematodes (Crute and Pink 1996; Keen 1990).

1.2.5.3 Class of R genes

Despite the wide range of pathogens and their pathogenicity, *R* genes encode only five classes of protein (Dangl and Jones 2001). Figure 1.4 represents the five classes of proteins related to *R* genes. The largest class of R genes encodes a nucleotide-binding site plus leucine-rich repeat (NB-LRR) type of protein and all R genes conferring viral resistance have been identified in this class (Goldbach et al. 2003). In papaya, 54 NBS class resistance genes were identified (Porter et al. 2009a) and 61% of these had NBS domains homolog to *Vitis vinifera*. Of the 54 genes identified, 18 genes have only NBS domains, 23 genes have NBS-LRRs, seven genes show N-terminal TIR domains and six genes are predicted to encode CC motifs (Porter et al. 2009a).

LRR domains are also presented in the most class of R proteins. It shows function of protein-protein binding, peptide-ligand binding or protein-carbohydrate interaction (Jones and Jones 1996; Kajava 1998). *Cf* gene in tomato reported to be an extracellular receptor-like protein that involved in pathogen detection (reviewed by Dickinson 2003). Dixon et al. (1996) suggested LRR might involve in facilitating the interaction of *R* gene products with other proteins involved in defence signal transduction. The ARC domain in between the NBS and LRR domains has been identified to play a role in the recruitment of the LRR domain to the N-terminal region (Rairdan and Moffett 2006). LRR location is known to influence the timing of detection of the invading pathogen and affects the resistance response. This resulted in a variation in degrees of hypersensitive reactions and pathogen colonisation in different *R* gene/*Avr* gene-dependent interactions (Dickinson 2003).

Protein kinases play a central role for signalling transduction during pathogen recognition and the subsequent activation of plant defence mechanisms (Romeis et al. 2001). A

kinase gene may work together with an NBS-LRR gene by forming a molecular complex to detect more than one pathogenic organism. This resulted in initiation of defence response to multiple pathogens, which is known as a guarding mechanism (Jones and Dangl 2006). In papaya, fourteen of the flanking genes have significant similarity to the gene encoding kinases, including receptor kinases and kinases associated with LRRs (Porter et al., 2009a). Furthemore, Dillon et al. (2006c) described a marker, Opa11_5r collocated with the *prsv-1* resistance locus and is homologous to a serine/threonine protein kinase gene. Recently, two STK genes, VP_STK1 and VP_STK2 which were characterized from SC28.106 and SC28.105, showed relation to PRSV-P resistance in *V. pubescens* (Razean Haireen, 2013).

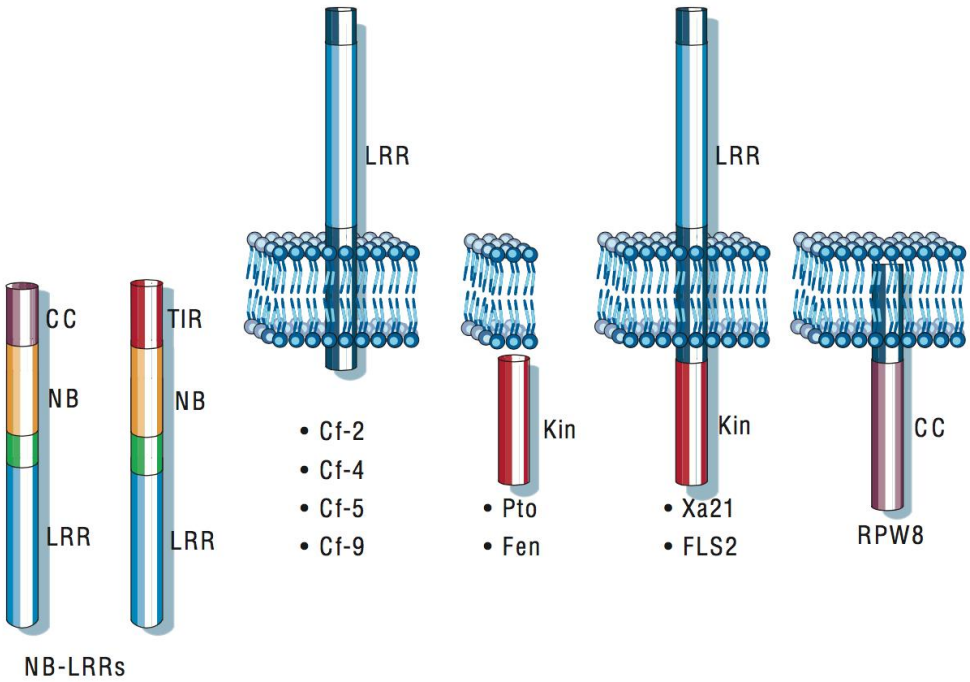

Figure 1.4: Representative functional domains of five main classes of disease resistance proteins (Dangl and Jones 2001)

1) NB-LRRs: are cytoplasmic and carry distinct N-terminal domain,
2) Cf-X proteins: extracellular LRR,
3) *Pto* gene: intracellular or cytoplasmic serine threonine kinase (STK)
4) Xa21: extracellular LRR and cytoplasmic protein kinase,
5) RPW8: putative signal anchor at the N terminus.

1.2.6 Productivity and fruit quality traits of papaya

In addition to disease resistance, papaya faces another problem of consumer acceptance, especially in the Australian market. This is mainly due to the unpleasant taste of papaya fruit that was sold on the Australian market. Consumer choice is generally influenced by appearance and sensory response to fruits. Therefore, improvement in eating and skin quality are the keys to increased consumption of papaya. Furthermore, production efficiency and high marketable yield are also important from growers' perspectives. Thus, breeding for these traits is essential.

Papaya is a polygamous species. A cross can populate either dioecious (either male or female) plants or gynodioecious (either female or hermaphrodite) plants. These forms exist due to human interference and deliberate selection against non-productive male trees (Storey 1969). The advantage of dioecious populations is the uniformity of fruit size, shape and appearance because female flowers do not display instability in sex expression of flowers compared to hermaphrodites (Storey 1976; Ying 2008). Papaya sex expression was complicated and influenced by environment variables including temperature, humidity, and soil nutrients which may modify functional gender of a plant when it flowers (OECD 2005). In hot and dry conditions (temperature greater than 35°C), hermaphrodite flowers became functional male with a poorly developed female reproductive system (Watson 1997; Nakasone and Paull 1998). Conversely, at low temperatures (less than 20°C) the flowers may become female only. The distortion in fruit shape suggested to cause by stamens resembled to carpels (OEDC 2005).

A papaya plant has a single stem which provides structural support under its leaf canopy. The stem is responsible for most of the rigidity, body mass, storage capacity, defence substances, height and competitive ability. It carries a bidirectional flow of water, nutrients, various organic compounds, and chemical and physical signals that regulate root and shoot relations (Reis et al. 2006). Diameters of mature plants could be related to yield (Francisco et al. 2007). Morton (1987) demonstrated that a wide and thick base of the tree mechanically supported the entire weight of the plant. Height to first flower indicated the potential ability of bearing fruit early and tolerance to fruit dropping (Anh 2011). O'Hare (1993) noted that ideally, plants should start fruit set at as low as possible, however, this can be achieved by maximising leaf growth in young trees, cultural practices and propagation techniques.

Several researchers reported the effect of environments toward phenotypic expressions that eventually affected in yield. Dioecious outcrossed varieties generally produced higher yields than gynodioecious varieties (Drew et al. 1998b; Chay-prove et al. 2000). Change of temperature caused hermaphrodite trees to reverse sex and reduced yields (OECD 2005). Papaya Seed Australia (2007) observed that carpelloid fruit were produced abnormally by the fusion of ovary and stamens during unfavourable weather conditions. It resulted in production of deformed and unmarketable fruit. Some varieties produce higher numbers of carpellodic fruit. 'Solo" varieties produced 100% carpellody fruits when minimum temperature was less than 17°C (Nakasone and Paull 1998). Yield and fruit quality varied by location, variety and season as well as agronomic practice (Elder et al. 2000a; 2000b). Age of tree also affected yield as trees yielded well for the first two years, but after that production declined (Benson and Poffley 1998).

Yield gap represents inconsistency of fruit production on a tree and a section of stem where

no fruit sets between two harvesting times. It varied considerably and was affected by cross-pollination and other factors such as environmental conditions, floral characteristics, and flower receptivity (OEDC 2005). Papaya fruit production in Australia varied in seasons due to several factors including low pollen viability and absence of suitable pollinators (Pollination Aware Report, 2008). Pollen of papaya can be produced all year round, but varied by seasons and varieties (Magdalita et al. 1998). In general, there was a trend of decrease in quantity of pollen during winter and early spring, while the receptivity of papaya stigmas remained high throughout the year even in winter (Garrett 1995). Ninety percent of freshly dispersed pollen grains were viable in winter, but some lines presented only 45% of pollen availability and it was as low as 4.5% in other lines. Allan (1963) reported that high humidity reduced the storage life of papaya pollen.

Fruit shape is a sex-linked characteristic. A female flower usually develops a round, spherical or ovoid fruit shape, whereas, a hermaphrodite flower develops a pyriform or elongate one. The standard of fruit size, shape and quality are dependent on market preferences which is different from country to country. Small fruits are more preferred in European, USA and Chinese markets, while the medium fruits are preferred in Malaysia. In Australia, yellow-fleshed papaya is preferred to be round female fruit from dioecious varieties and red-fleshed papaya is preferred from elongated hermaphrodite fruit (Ying 2008).

There are a number of papaya varieties that have been developed worldwide to meet a range of expectations including disease tolerance, improved fruit quality and yield, such as 'Solo' from Hawaii, 'Tainung' from Taiwan, 'Eksotika I, II and III' from Malaysia, Maradol from Cuba and Hortus Gold from South Africa (Fitch 2005; OECD 2005). In the Australian market, the majority of papaya fruit for commercial production were from varieties hybrid 1B (approximately 60%) and 'Sunrise Solo' (approximately 30%) (Hansen 2005). Even through these two varieties accounted for some desirable characteristics compared to other varieties available in the market, they both have some poor characteristics. Hybrid 1B fruit has good appearance, but lacks flavour, while 'Sunrise Solo' has good flavour, but low yields and the fruit has poor shape, thin flesh and blemished skin (Drew 2005). The current commercial varieties in North Queensland are Hybrid 1B and Hybrid 13 for yellow-flesh papaw; and Hybrid RB1, Hybrid RB2 and Hybrid RB4 for red-flesh papaya (Kath, personal communication).

Breeding programs to improve productivity and fruit quality traits have been attempted in many fruit crops during the last century. However, genetic improvement for productivity and fruit quality traits has some limitations from low diversity and unknown inheritability of traits. That led to insufficient genetic variability in gene pools for use in conventional breeding (Grandillo et al. 1999; Ulrich and Olbricht 2011). Moreover, fruit quality traits such as yield, size, shape, colour, texture, sweetness, flavour, appearance, and shelf life are complex because there are many factors involved including genetic and environmental factors. Some traits are controlled by multiple genes and express in relation to environmental factors (Fernandez-Trujillo 2011). Consequently, breeding for productivity and fruit quality requires improvement of breeding and the establishment of efficient methods for selection.

Several breeding methods and techniques have been employed to develop superior varieties for commercial production. There was a report of using cycles of random pollination to break linkage between high sugar content and susceptibility to winter spot (Hansen 2005). *In vitro* propagation to propagate promising emerging lines is ideal to speed up breeding programs. An efficient protocol for papaya micropropagation was published by Drew (1992). As the

result of advances in genome studies in papaya, molecular breeding is an alternative and effective way to improve papaya genotypes. Details of the development of molecular markers in papayas was reviewed by Kanchana-udomkan et al. (2014) and is presented in Section 1.1.

Appendix 2

Chapter 4 of Ph.D. thesis of Dr. Chat Kanchana-udomkan

This chapter provides complete experimental details of research associated with the breeding trial as described in this report

Chapter 4:

Development of Papaya Breeding Populations and Analysis of Productivity and Fruit Quality Traits and Phenotypes

INTRODUCTION:

Even though PRSV-P devastates the papaya industry worldwide and causes economic losses for producers, the most important character trait that affects demand by consumers is fruit quality. In Australia, the papaya industry is relatively small in comparison to other countries, and has an estimated yield of 10,000 -13,000 tons per year worth AUD18-25 million (Diczbalis et al. 2012). The industry has potential to expand. One of the biggest challenges for the industry is to produce a uniform cultivar with consistently good eating quality all year-round. The traditional flavour of the yellow fleshed papaya is an acquired taste that is less popular among the younger generation and red-fleshed varieties are gaining in popularity. Consumers in Australia have come to associate yellow flesh with round fruit and red flesh with elongate fruit. Therefore, a breeding program for papaya is needed to improve the quality of cultivated varieties within Australia.

Papaya is the only member of the *Carica* genus in the family Caricaceae (Badillo 2000). Its ploidy level is 2x, it is generally dioecious ensuring a high degree of heterogeneity, and can be inbred and cross-pollinated with relative ease making it subject to a range of plant improvement methods (Simmonds 1979). Papaya germplasm shows much phenotypic variation for many important traits (Kim et al. 2002). These include fruit quality and production traits such as yield, pattern of fruit production, peduncle length for ease of harvesting, fruit size, shape, flesh colour, flavour and sweetness.

There are few cultivars grown commercially in Australia, therefore, it is a necessity to explore papaya germplasm to evaluate and select potential parental lines for breeding programs. Most Australian papaya breeding efforts in the past have moved gene frequency for various traits with relative ease by selecting in segregating plant populations. The implication is that some traits of commercial importance are quantitatively inherited and controlled mainly by additive gene action with quite high heritability. Nevertheless, within a narrow base of cultivars grown in Queensland, traits do vary significantly between environments including different crop management practices (Elder et al. 2000a; 2000b; 2002), implying heritability may be lower than thought. The main purpose of research in this chapter was to evaluate different lines of papaya for fruit quality and productivity traits, and then to select good trees to be parents in a breeding program with commercial varieties to improve fruit quality and tree productivity in a major producing region. In addition, other parent trees were selected and crossed so that DNA markers could later be identified to facilitate marker assisted breeding.

After consulting with reference groups of papaya growers in north Queensland, important traits that must be considered in applied plant breeding were identified. They include those relating to a tree's productivity: total yield, consistent set of fruit over time (i.e. no yield gap), consistent fruit size, ease of harvest and disease resistance and also to fruit quality traits: appearance and appeal, shape, flesh colour, sweetness and flavour. There is wide range in these traits observed in germplasm. In fact, most of these traits are quantitatively assessed (IBPGR 1988). Therefore

it is important to applied papaya breeding that proper assessment be made of the variability in commercially acceptable papaya in an Australian production area (north Queensland), and to produce segregating populations of plants by controlled plant breeding. Some of the many traits may be correlated. If so, fewer time-consuming assessments of trees might be possible for a population of segregating plants. This is vitally important to applied plant breeding with inherent high costs of land and labour.

RESEARCH AIMS:

1. Identify important productivity and fruit quality traits to be improved by applied payaya breeding.

2. Describe the phenotypes 27 papaya lines and choose lines and plants within lines for use as parents in a breeding program in north Queensland.

3. Establish breeding and segregating populations of plants for selecting the required traits for both commercial acceptability and DNA-marker analysis.

MATERIALS AND METHODS:

4.0 Overviews

Lines of papaya were grown for evaluation leading to selection of parental lines for two main purposes; firstly to improve eating-quality in commercial varieties and secondly to establish segregating populations to use for identifying DNA markers for other traits. The steps of work for this chapter are outlined graphically in Figure 4.0.

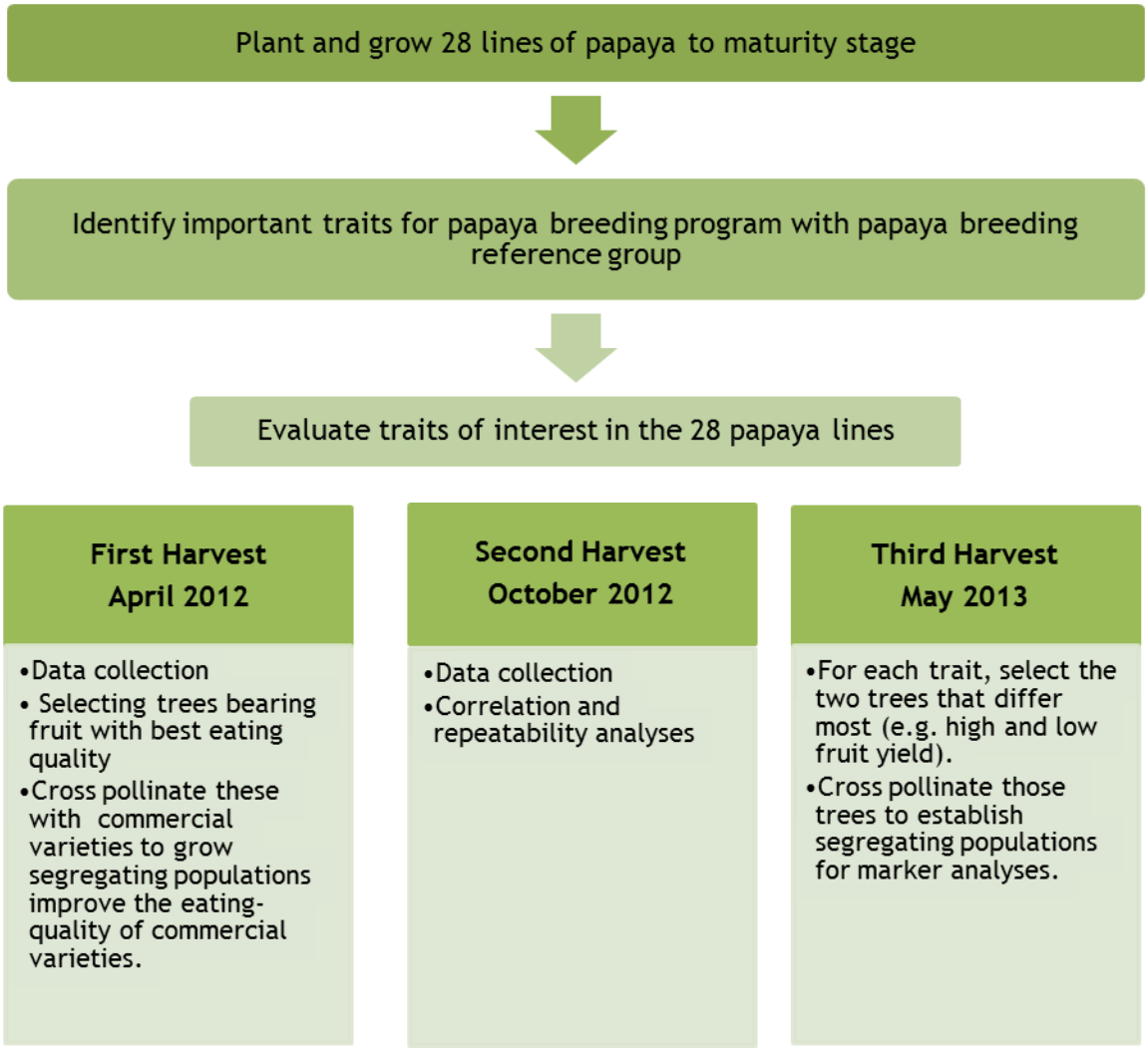

Figure 4.0: Steps of work for developing papaya breeding population and analysis of productivity and fruit quality traits and phenotypes

4.1 Plant materials

Twenty-eight different lines of papaya were obtained from three sources: Australian commercial varieties, crosses between Australian commercial varieties and selected lines from Department of Agriculture, Fisheries and Forestry, Queensland (QDAFF), and from collections held in Queensland but originally from Hawaii, Malaysia, Vietnam and Thailand. The latter collections are in the possession of Professor Rod Drew, Griffith University. Crosses between commercial varieties and QDAFF lines were made by Narenda Singh (QDAFF) in the industry-funded breeding project in 2010. The 28 lines include yellow- and red-fleshed papaya types and are detailed in Table 4.1. The trees were planted on a property owned by Lecker Farming, Mareeba, Queensland (latitude -16.96, longitude 145.34, average rainfall 918 mm/year).

4.2 Papaya seed preparation

Seeds of crosses between Australian commercial varieties and selected lines from DAFFQ were extracted from ripe fruits following cross-pollination. The fruits were cut in half and the seeds were collected then washed under tap water. The sarcotesta, which is the clear membrane covering the seed, was removed by gently rubbing the seeds between hessian fabrics. The seeds were then washed under running tap water eight times and placed on a sieve tray to dry in a cabinet that controlled temperature at 15ºC and relative humidity at 15%. Seeds of collections from Professor Rod Drew, which had been kept for more than five years, were first soaked in 2 mM gibberellic acid solution (Fermoz) for 15 minutes and then rinsed with tap water before sowing.

One hundred seeds of each line were sown in February 2011 in seed-raising mix (Searly, Australia) in 48-cell seedling trays. Two or three seeds per cell were placed on the mix before lightly covering with additional mix. Trays were held in a shade-house and were watered and treated according to the farm management practice at Lecker Farming in order to produce healthy seedlings ready for field-planting. Seedlings were field-planted three months after sowing. The number of seedlings field-planted per line of all but one line varied from 10 to 100 and reflected germination percentages.

Table 4.1: Identity, ancestry and source of 28 papaya lines planted at Lecker Farming, Mareeba, Queensland

Line number[1/]	Ancestry or variety name[2/]	Source of seed
R01	RB1 x 18-45	Cross of commercial red papaya RB1 and DAFFQ line #18-45
R02	RB1 x 24-29	Cross of commercial red papaya RB1 and DAFFQ line #24-29
R04	RB2 x 18-45	Cross of commercial red papaya RB2 and DAFFQ line #18-45
R06	RB2 x 25-5	Cross of commercial red papaya RB2 and DAFFQ line #25-5
R09	25-5 x RB1	Cross of DAFFQ line #25-5 and commercial red papaya RB1
Y11	24-29 x RB2	Cross of DAFFQ line #24-29 and commercial red papaya RB2
Y15	1B x 33-66	Cross of commercial yellow papaya 1B and DAFFQ line #33-66
Y16	7-82 x 1B	Cross of DAFFQ line #7-82 and commercial yellow papaya 1B
Y17	24-87 x 1B	Cross of DAFFQ line #24-87 and a commercial yellow papaya 1B
R19	24-29 Self	Self pollination of DAFFQ line #24-29
Y20	JC2	[3/]
R21	25-5 Self	Self pollination of a DAFFQ line #25-5
R22	TS2	[3/]
R23	Malaysian Red 1	[3/]
R24	Malaysian Red 2	[3/]
R25	Malaysian Red 3	[3/]
Y26	1B	A commercial yellow papaya
R27	RB2	A commercial red papaya
R28	RB4	A commercial red papaya
R29	Sunrise Solo	[3/]; it is a commercial red papaya in Hawaii
R30	Solo Linda	[3/]
R31	RD6 Self	[3/]
R33	Brazilian Solo	[3/]
Y34	2.54-14 self	[3/]
Y35	2.54-12 self	[3/]
R41	JC2 x Vietnam Red	[3/]
R42	TS2 Self	[3/]
R48	Red Lady, Taiwan	[3/]

[1/] The prefix R refers to red fleshed fruit; Y to yellow

[2/] Female parent is noted first in each cross.

[3/] From a collection of seed held by Professor Rod Drew, Griffith University, Brisbane, Queensland

Only one seed of R48 (Red Lady) germinated, therefore, this line has only one plant in the field. Trees were planted at 3.20 m intervals in rows 1.75 m apart and were treated according to the maintenance program of Lecker Farming. Fertiliser was applied by addition of nutrients to drip irrigation and trees were regularly and routinely treated with fungicides. Weeds were controlled by herbicide application according to standard farm management practice. Replicating plots of trees of the lines was not used.

4.3 Evaluation of traits of interest

Important commercial traits were identified during discussions with local commercial papaya producers. Traits were grouped into those relating to tree productivity and those relating to fruit quality.

Ten fruit-bearing trees of each line were selected at random to evaluate traits. They were evaluated at three different harvesting times, April 2012, October 2012 and May 2013, to confirm that the data represented the genetics of the trees rather than environmental effects.

4.3.1 Productivity traits

Nine productivity traits that related to performance of the trees were identified and recorded. They were:

i. Sex type: dioecious (male and female flowers on separate trees) or gynodioecious (hermaphrodite flowers on the same tree).

ii. Height to the first flower was measured in centimetres from the ground.

iii. Height to the first mature fruit was measured at harvest in centimetres from the ground.

iv. Height to the first marketable fruit was measured in centimetres from the ground. Number of side shoots was measured by counting on each tree before the first fruit was harvested.

v. Peduncle length was recorded using a 1,3 and 5 rating scale; where

1 = short (estimation of the length less than 3 cm),

3 = medium (estimation of the length between 3 and 5 cm) and

5 = long (estimation of the length greater than 5 cm).

Figure 4.1: Measuring length of fruit peduncle

vi. Yield of fruit of saleable quality was estimated by an experienced grower at each harvest time. The total number of marketable fruits per tree were counted. This number of fruits covered the duration of six months harvest time (April to October 2012, approximately). Therefore, this saleable yield was the estimation of tree production over the entire time. Number of fruit per cartons and weight of marketable fruit per carton was estimated. Saleable yield for each tree was calculated using Formula 4.1.

$$\text{Saleable yield (kg)} = \frac{\text{Number of fruits per tree}}{\text{Number of fruit per carton}} \times \text{weight per carton (kg)}$$

Formula 4.1: Equation used to calculate yield of saleable fruit

vii. Yield gap, which was a space on a tree where fruit were not produced between two harvesting times, was rated between 1 and 9; where

1 = no gap was observed between two harvesting times
3 = less than 20% of space of fruit set was observed between two harvesting times
5 = less than 40% of space of fruit set was observed between two harvesting times
7 = less than 60% of space of fruit set was observed between two harvesting times
9 = greater than 60% of space of fruit set was observed between two harvesting times

viii. Number of carpelliod fruits was counted for each tree.

4.3.2 Fruit quality traits

Thirteen traits that related to fruit quality were identified and recorded. One fruit at similar fruit harvesting stage of each tree was evaluated. Most traits were evaluated by using the standards of the International Board for Plant Genetic Resources (IBPGR 1988) as detailed below.

i. **Fruit shape:** Fruits from each tree were scored in numeric system as detailed in Figure 4.2.

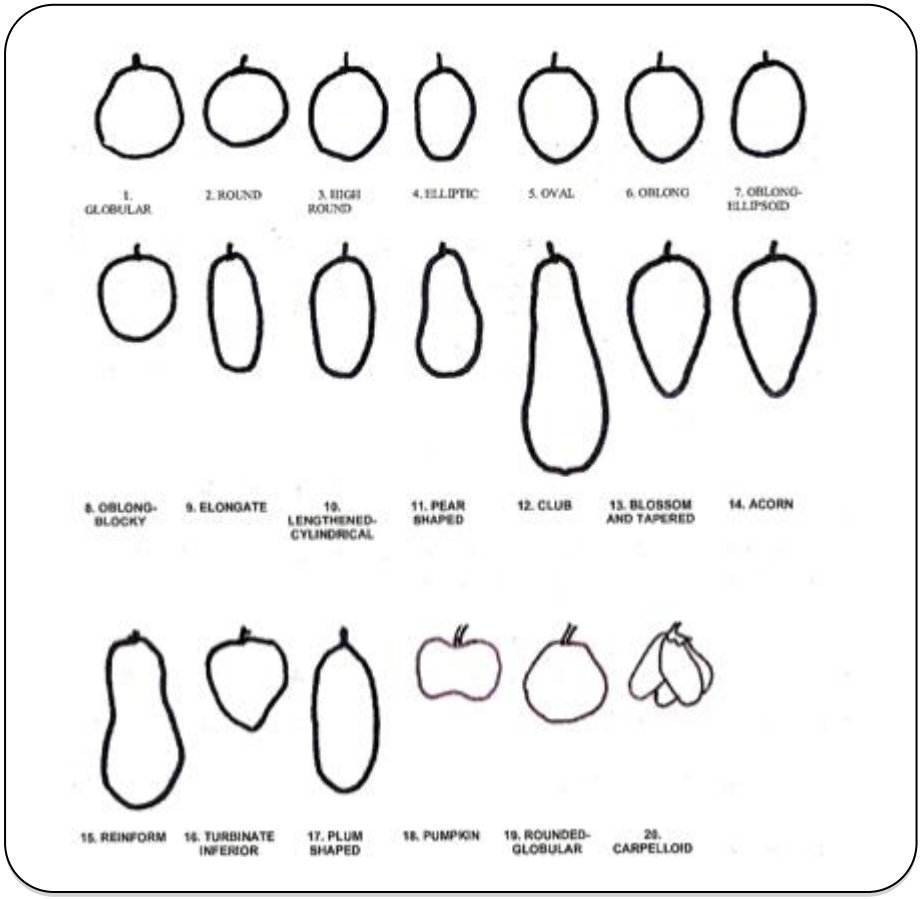

Figure 4.2: Fruit shape in papaya (IBPGR 1988)

ii. **Teat shape:** Teat of each fruit was scored in numeric system as detailed in Figure 4.3.

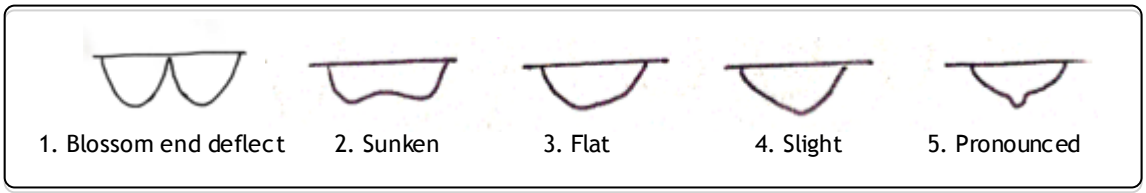

Figure 4.3: Teat shape in papaya (modified from IBPGR 1988)

iii. **Stalk insertion:** The insertion of stalk of each fruit was scored in a numeric system as detailed in Figure 4.4.

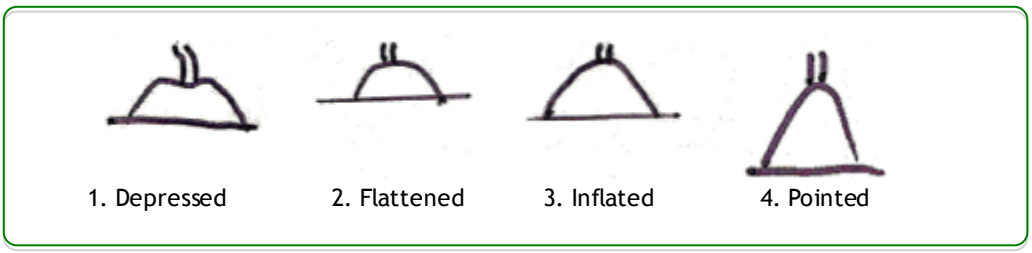

Figure 4.4: Stalk insertion in papaya (IBPGR 1988)

iv. **Skin quality:** Skin quality was scored using a rating system of 1 to 4 where

1 = poor, 2 = average, 3 = good, and 4 = excellent skin quality

v. **Skin freckle:** Skin freckle, which is skin blemish occurring on ripe fruit but not related to disease (Eloisa et al. 1994), were observed on mature fruits at the ripe full colour stage. The severity of skin freckle is recorded using a rating system of 0 to 4 rating as detailed in Figure 4.5.

Figure 4.5: Rating scale 0 to 4 for skin freckle in papaya; where 0 = skin freckle cover less than 1% of the surface, 1 = skin freckle cover 1% to 15%, 2 = skin freckle cover 16% to 30%, 3 = skin freckle cover 31% to 50%, and 4 = skin freckle cover more than 50%

vi. **Skin colour:** Skin colour was visually observed and recorded in a numeric rating system: 1 = Yellow, 3 = Yellow/Orange, 5 = Orange, 7 = Orange/Red and 9 = Red. All fruit rated 3 or less were phenotypically classed yellow, fruit rated >3 were classed red.

vii. **Cavity shape:** Each fruit was cross-sectioned laterally. The central cavity of each fruit was scored as detailed in Figure 4.6.

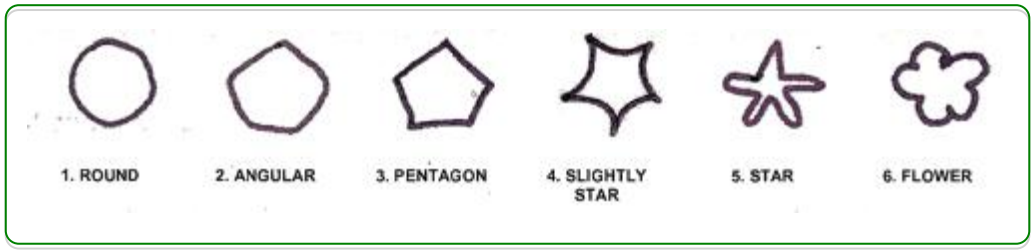

Figure 4.6: Shape of the central cavity of papaya fruit (IBPGR 1988)

viii. **Consistency in flesh colour**: Each fruit was cross-sectioned laterally in half and scored for consistency of flesh colour as detailed in Figure 4.7.

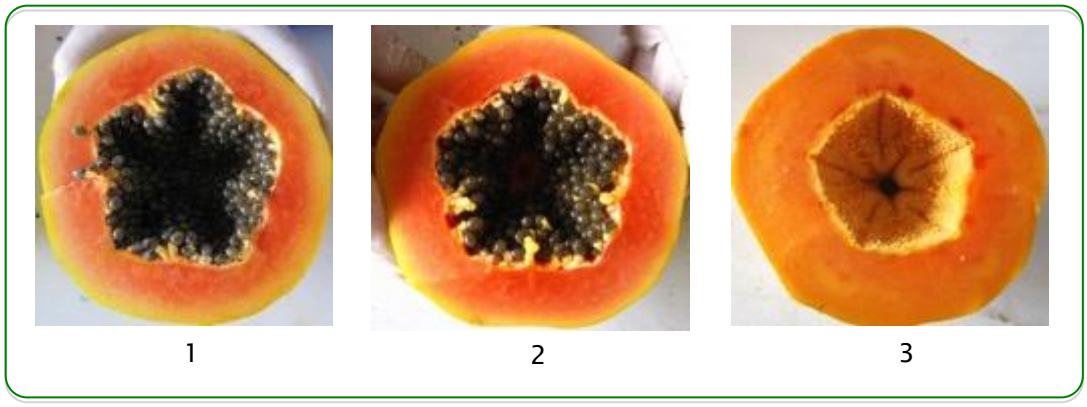

Figure 4.7: Numeric rating scale for consistency of flesh colour; where

1 = more than 50% colour inconsistency,
2 = colour inconsistency is between 50-75%, and
3 = flesh colour is 100% consistent

ix. **Flesh colour:** Each fruit was cross-sectioned in half laterally and scored for flesh colour as 1 = Yellow, 3 = Yellow/Orange, 5 = Orange, 7 = Orange/Red and 9 = Red.

Two flesh colours, yellow and red, were distinguished by using a cut-off value with scores of three and less representing for yellow flesh and above three for red flesh. Even though flesh colour is controlled by only a single dominant gene, quantitative expression can still be detected.

x. **Flesh firmness:** Flesh firmness was rated: 1, 3 or 5; where

1 = Soft, 3 = Intermediate and 5 = Firm.

xi. **Useable flesh thickness:** Flesh thickness was measured in millimetres from the skin to the seed cavity; one measurement per fruit.

xii. **Flesh sweetness:** Total soluble solids (TSS) were measured on ripe fruits by using a hand held refractometer. The measurement was recorded in ºT scale.

xiii. **Fruit flavour:** Each fruit was tasted at the ripe fruit stage. The details are described in Table 4.2.

Table 4.2: Papaya rating system for flesh flavour

Flavour rating system	Descriptions				
Type of flavour	Flavourless	Nasturtium	Solo	Khagdum	Musk
Overall taste	1 = Poor	2 = Average	3 = Good	4 = Excellent	
Strength of flavour	1 = Weak	2 = Intermediate	3 = Strong		

4.4 Data analysis

Analysis of Variance (ANOVA) was applied to data of the three harvests by use of XLSTAT software (Addinsoft, 2015). The harvest times were used as a fixed variable in ANOVA analysis. Duncan's multiple range test was used to calculate significant differences ($P<0.05$) between means.

Repeatability estimates (Falconer 1960) were calculated for each trait using the data from the first two harvests (Formula 4.2). If the data is consistent between assessments, it may not be a necessity to do more than one evaluation.

$$r = \frac{S_A^2}{S^2 + S_A^2} \qquad \textbf{Formula 4.2}$$

Where: r is repeatability, S^2_A is the between-groups variance component and S^2 is the within-group variance component.

These variance components are calculated from the mean squares in the analysis of variance using Formula 4.3 and Formula 4.4. Mean squares were calculated by the one-way analysis of variance in Microsoft Excel software.

$$S^2 = MS_{\text{within group}} \qquad \textbf{Formula 4.3}$$

$$S^2_A = \frac{MS_{\text{between group}} - MS_{\text{within group}}}{n_0} \qquad \textbf{Formula 4.4}$$

Where n_0 is a coefficient related to the sample size per group in the analysis of variance and in this case is equal to the group size = 2.

Correlation analysis was computed by XLSTAT software (Addinsoft, 2015) using data from first and second harvest. Clones were used as a fixed variable in ANOVA. Duncan's multiple

range test was used to calculate significant differences ($P<0.05$) between means.

4.5 Development of breeding populations to improve flesh flavour for commercial papayas

Fruits from each tree were tasted and scored according to Table 4.2 in April 2012. Trees that represented outstanding flavour and other eating quality traits were selected based on the result in the first harvest due to time limitation and the commitment to the project fund to produce crosses as soon as possible. Crosses were made from these selected trees to red and yellow fruited commercial papayas. Nevertheless, flavour characteristics of fruit from the selected trees were measured at all three harvesting times.

4.5.1 Controlled self- and cross-pollination

To self-pollinate hermaphrodite flowers, mature but tightly closed flowers were bagged with a paper bag to prevent out-crossing from other plants. The self-pollinated flower was labelled to record the variety and date of bagging.

Cross-pollination of selected trees was done by using either female or hermaphrodite flowers for the female parent, and either male or hermaphrodite flowers for the male parent. The tips of female flowers were carefully opened and the pollen from a male or a hermaphrodite parent was placed on stigmas. Hermaphrodite flowers were emasculated before pollen maturity. After three days, pollen from a selected male parent was placed on the stigma of the emasculated, hermaphrodite flower. Pollinated flowers were covered with cotton wool and bagged to prevent out-crossing from other plants. They were labelled as described above. The procedures of cross-pollination are detailed in Figure 4.8. The paper bag was removed when the fruit was set approximately 2 to 3 weeks after pollination. The fruit was tagged and covered with an orange mesh bag. It was then harvested at the maturing stage of 75% colour change, which was approximately 4 to 5 months after pollination, the seeds extracted and germinated to produce the second population of plants for selection.

4.6 Development of gene-mapping populations for papaya tree and fruit quality traits

Data from three harvesting times were used to calculate means of each trait for each line and to identify trees to be used as parents in cross-pollination. Only trees consistent in their traits across harvest times and which represented the widest variation in particular traits were selected for crossing. Trees were selected first on flesh colour (yellow and red). Crosses were made (detailed 4.5.1) between trees with poor and good traits in both yellow and red-fleshed types (Table 4.3). Reciprocal crosses were made where possible.

Figure 4.8: Cross-pollination process of papaya

i: A closed female flower at mature stage was selected. ii: The tip of the female flower was cut. iii: Petals of a selected male flower were peeled and anthesis was checked. iv: Pollen was gently tapped and brushed on the stigma. v: The flower of female parent was covered to protect it from out crossing by using cotton wool. vi: The female flower was covered again by using a paper bag and it was tagged using an aluminium tag which was recorded female and male parents, and date of pollination

Table 4.3: Traits selected for cross-pollination to produce hybrids for segregating populations for DNA marker analysis

Trait[1/]	Criteria to select parental lines for crossing for marker DNA analysis	
	Poor trait	**Good trait**
Fruit flavour	No Flavour	Solo
	No Flavour	Nasturtium
	No Flavour	Musk
	No Flavour	Khag Dum
Skin quality	Poor	Excellent
Skin colour	Light	Deep
Number of side shoots	High	Low
Height to first fruit	High	Low
Number of carpelloid fruit	High	Low
Peduncle length	Short	Long
Yield gap	High	Low
Flesh colour	Light	Deep
Flesh firmness	Soft	Firm
Flesh Thickness	Thin	Thick
TSS	Low	High
Flesh colour consistency	Inconsistent	Consistent

[1/] **Note:** Trees that had the required tree criteria of skin quality, skin colour, number of side shoots, height to first fruit, number of carpelloid fruit, peduncle length and yield gap were selected for crossing regardless of whether fruit were red- or yellow-fleshed.

However, trees with extremes of fruit quality traits (flesh colour, firmness, thickness, TSS and consistent flesh colour) were selected for each of red-and yellow-coloured fruit. Crosses were then made within each of the two fruit flesh colours.

RESULTS:

4.7 Difference of traits between harvest seasons

Means of maximum and minimum of temperature in Mareeba between 2011 and 2013 were obtained from Australian Bureau of Meteorology and show in Table 4.4. The means for each trait in each harvest time averaged across lines are presented in Table 4.5. They differed significantly between harvesting times for most traits, except numbers of carpelloid fruit that did not differ significantly in all harvest times. Yield of marketable fruit, peduncle length and flesh thickness were significantly less in the third harvest (May 2013) compared with the first two harvests, which did not differ significantly.

Table 4.4: Mean minimum and maximum temperature (°C) in Mareeba from 2011 to 2013 (Data from Australian Bureau of Meteorology)

Statistics	2011		2012		2013	
	Min[1/]	Max[2/]	Min[1/]	Max[2/]	Min[1/]	Max[2/]
January	21.2	30.2	21	31.4	21.2	32.2
February	21	29.5	21.4	30.7	21.3	31.9
March	21.2	29.4	21	29	20.8	29.8
April	19	28	18.2	28.5	18.8	28.6
May	14.9	26.4	17.1	25.9	17.8	26.6
June	12.6	25	13.6	25.2	15.6	25.9
July	12.9	24.5	14.5	24.4	15.1	24.7
August	13	25.9	13.3	25.8	12.8	27.1
September	14.6	28.2	15.2	28.3	15.1	29.4
October	17.5	31	16.8	30	17.5	31.1
November	18.8	30.4	18.5	31.4	19.9	31.2
December	21.3	32	20	33.1	19.3	31.5
Annual	17.3	28.4	17.6	28.6	17.9	29.2

[1/] Mean minimum temperature (°C)

[2/] Mean maximum temperature (°C)

Traits related to skin quality and flesh firmness trended lower over time. Skin quality and flesh firmness were rated highest at the first harvest (April 2012), while TSS and consistency of flesh colour were highest in the last harvest (May 2013). Fruits from winter (October 2012 harvest) had the highest score of skin freckle, while flesh colour rating was lowest in winter fruit compared with the other two harvests.

Table 4.5: Means of fruit productivity and quality traits at each harvest

Trait	Harvest time		
	April 2012	October 2012	May 2013
Peduncle length rating	2.69 a	2.80 a	2.21 b
Number of carpelloid fruit	2.55 a	2.33 a	2.01 a
Yield of marketable fruit (kg; estimated)	35.48 a	36.62 a	27.41 b
Skin quality	2.49 a	2.28 b	2.07 c
Skin freckle	1.59 b	2.36 a	1.04 c
Flesh colour	5.36 a	4.45 c	4.73 b
Flesh colour consistency	2.08 c	2.41 b	2.53 a
Flesh firmness	3.82 a	3.03 b	2.29 c
Flesh thickness (mm)	18.32 a	18.93 a	15.70 b
Total soluble solid (% Brix)	11.02 c	11.42 b	12.13 a

* Means followed by the same letter in each row are not significantly different (P>0.05)

In general, yield means over the first two harvest times were not significantly different, but a 25% reduction in yield was observed in the third harvest (P<0.0001).

After the second harvest in October 2012, some lines were culled as a result of poor performance of their trees in the field and they were not used in further breeding program. These lines were Y34 and Y35 in the yellow lines and R19, R21, R22, R23 and R30 for the red lines.

4.8 Repeatability of traits related to fruit

Flesh firmness showed the most consistent among all the traits in the two harvest times (repeatability of 0.69; Table 4.6). In general, traits related to productivity (yield, number of carpelloid fruits, peduncle length and yield gap) were relatively consistent with the repeatability between 0.23 and 0.34. High repeatability was also relatively high for fruit TSS (0.29). Other fruit quality traits (fruit shape, teat shape, consistency in flesh colour and flesh thickness had repeatability lower than 0.2. There were high P-values (> 0.05) associated with repeatability of stalk insertion, skin quality, skin freckle, skin colour, cavity shape and flesh firmness ratings. Repeatabilities of those traits were low because the variance among lines was small relative to within-lines.

Table 4.6: Repeatability of all traits between the first and the second harvest time in April and October 2012

Traits	Repeatability	P-Value
Yield	0.37	9.33E-13
Number of carpelloid fruits	0.26	6.41E-07
Peduncle length	0.23	8.54E-06
Yield gap	0.34	1.14E-07
Fruit shape	0.17	1.44E-03
Teat shape	0.14	7.60E-03
Stalk insertion	-0.11	0.9788
Skin quality	-0.03	0.7257
Skin freckle	-0.03	0.6751
Skin colour	-0.15	0.9965
Cavity shape	0.08	0.0697
Consistency in flesh colour	0.18	8.53E-04
Flesh colour	0.62	4.77E-33
Flesh firmness	-0.07	0.8884
Flesh thickness	0.16	2.09E-03
TSS	0.29	9.89E-08

4.9 Correlation analysis among traits related to fruit

Most traits showed moderate to low repeatability, so correlations between traits were calculated for each of two harvest times (Tables 4.7 and 4.8). Correlations between traits in most scenarios were significantly ($P < 0.05$) but at a low level (r^2 less than 0.5). The highest correlations were between skin freckle and skin quality (r^2 -0.543 and -0.522 in the first and second harvest, respectively); that is, the higher the rating for skin quality the lower the rating for skin freckle. Flesh colour in both harvests was comparatively highly related to skin colour and consistency in flesh colour ($r^2 > 0.3$); that is, the redder the flesh, the lower the rating for flesh colour consistency but the higher the rating for skin colour. Data from the second harvest revealed yield was then more highly correlated with number of carpelloid fruits, peduncle length and yield gap than at the first harvest.

Table 4.7: Correlation matrix (r^2 values) of Pearson's correlation coefficient of the first harvest in April 2012

Variables	Number of Carpelloid fruits	Peduncle length	Yield gap	Skin quality	Skin freckle	Skin colour	Consistent in flesh colour	Flesh colour	Flesh firmness	Flesh thickness	TSS
Yield	**-0.227**	**0.176**	**-0.136**	-0.012	-0.005	-0.008	-0.053	0.099	0.032	**0.211**	**-0.204**
Number of carpelloid fruit	1	**0.121**	0.001	-0.063	0.104	**0.267**	-0.064	0.105	**-0.110**	**-0.300**	**0.214**
Peduncle Length		1	-0.062	0.033	-0.023	0.059	0.044	**-0.158**	0.055	0.106	-0.022
Yield gap			1	0.060	**-0.119**	**-0.119**	0.040	-0.107	0.053	**0.144**	-0.037
Skin quality				1	**-0.543**	0.040	-0.039	**-0.140**	0.091	0.054	0.049
Skin freckle					1	0.098	0.048	**0.117**	-0.060	0.019	0.090
Skin colour						1	-0.051	**0.353**	-0.081	**-0.123**	**0.267**
Consistent in flesh colour							1	**-0.339**	**-0.108**	**0.159**	0.009
Flesh colour								1	-0.023	**-0.210**	**0.227**
Flesh firmness									1	**0.203**	**-0.130**
Flesh thickness										1	**-0.287**

Note: Values in bold are different from 0 with a significance level 0.05 (P > 0.05)

Table 4.8: Correlation matrix (r^2 values) of Pearson's correlation coefficient of the second harvest in October 2012

Variables	Number of Carpelloid fruits	Peduncle length	Yield gap	Skin quality	Skin freckle	Skin colour	Consistent in flesh colour	Flesh colour	Flesh firmness	Flesh thickness	TSS
Yield	**-0.315**	**0.344**	**-0.381**	**0.217**	**-0.185**	-0.004	-0.048	-0.110	-0.038	-0.038	**-0.230**
Number of carpelloid fruit	1	0.022	-0.008	-0.062	-0.021	0.057	0.015	0.042	**0.163**	**-0.162**	0.097
Peduncle Length		1	-0.050	**0.258**	**-0.225**	-0.107	0.065	**-0.317**	**0.256**	0.096	**-0.260**
Yield gap			1	-0.092	0.099	0.046	-0.120	0.051	0.031	**0.204**	**-0.191**
Skin quality				1	**-0.522**	0.035	-0.085	-0.025	**0.131**	**0.256**	**-0.147**
Skin freckle					1	0.011	0.023	**0.190**	-0.100	**0.175**	0.015
Skin colour						1	**0.119**	**0.321**	0.054	-0.023	**0.269**
Consistent in flesh colour							1	**-0.318**	0.056	**-0.150**	**0.200**
Flesh colour								1	-0.043	-0.030	**0.201**
Flesh firmness									1	**0.182**	**-0.182**
Flesh thickness										1	**-0.241**

Note: Values in bold are different from 0 with a significance level 0.05 (P > 0.05)

4.10 Analysis of tree productivity traits

The information on sex type, which can be found in Appendix 2, was recorded for future reference when selecting parental lines. Heights to the first flower, first fruit and first marketable fruit were highly, positively correlated (r^2 >0.65, p<0.001; Figure 4.9). The scatter plots of individual values and coefficients of determination (R^2) are detailed in Figure 4.9.

Mean values of each tree productivity trait of each line are presented in Table 4.9. Number of side shoots varied from zero to more than 20 (Figure 4.10). In general, yellow papaya had fewer side shoots than the red lines. The line with fewest side shoots was 1B, a commercial yellow papaya (Y26, Table 4.9), while the highest number was recorded in Solo type papayas (all red-fleshed): Solo Linda (R30), Sunrise Solo (R29), and Brazilian Solo (R33). Peduncle length varied from short to long (Figure 4.11); the result suggested the older the tree, the shorter the peduncle.

Total saleable yield of yellow lines was, in general, higher than that of red line (Figure 4.12). Yield of marketable fruits of yellow lines differed insignificantly over the three harvesting times, while that of most red papayas decreased significantly over time.

The yield gap ratings were between 10% and 60% of the space available for fruit set on the tree (Figure 4.13). The result was regardless of flesh colour.

The highest numbers of carpelloid fruits was observed in R22 (24.5 fruits per tree) and R41 (9.3 fruits per tree). The difference between number in R22 and all other lines was highly significant ($p < 0.0001$). All other lines had none to three carpelloid fruit per tree and their means did not differ significantly.

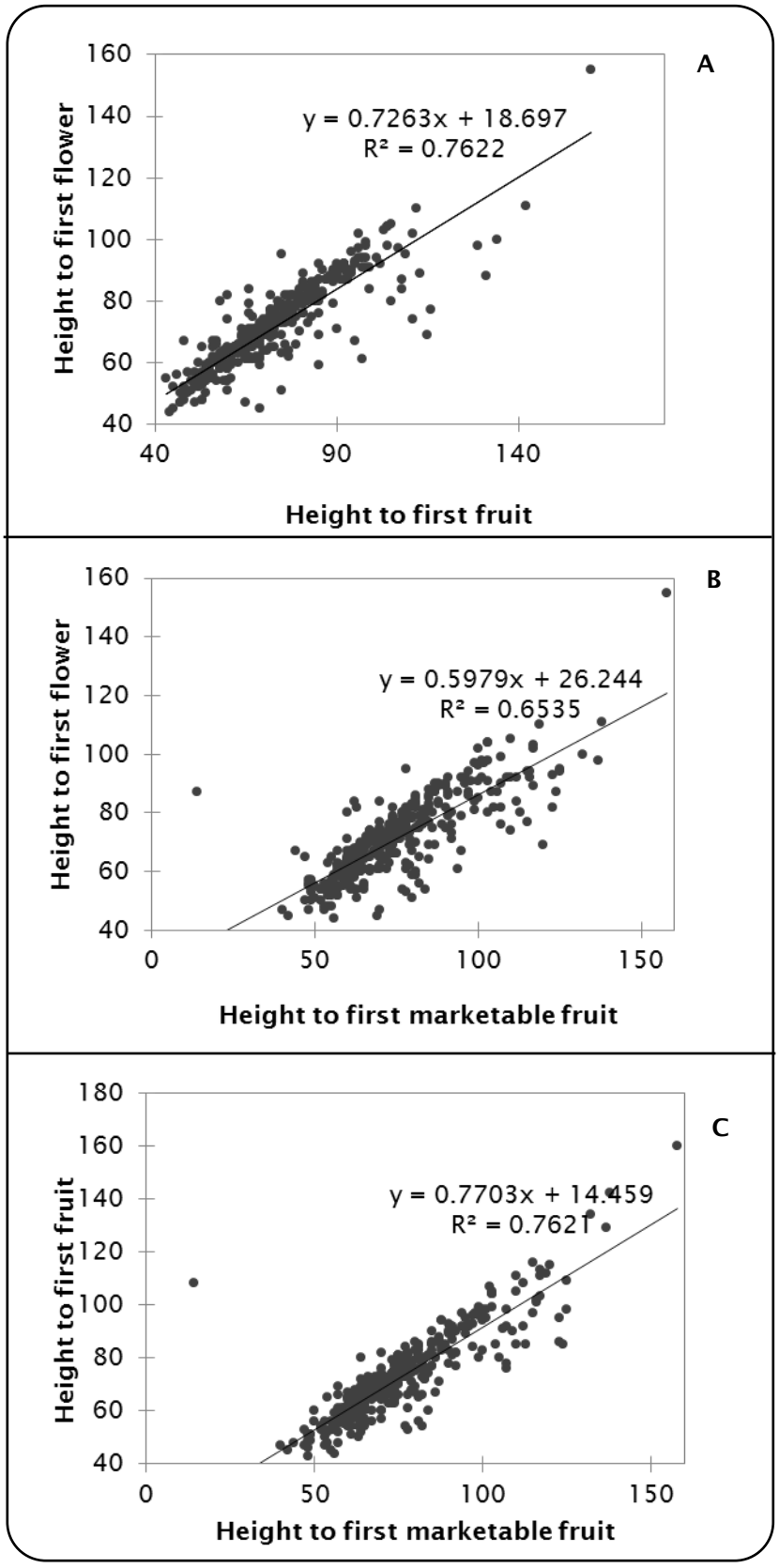

Figure 4.9 Scatter plots of A: height to first flower and height to first fruit, B: height to first flower and height to first marketable fruit, and C: height to first fruit and height to first marketable fruit

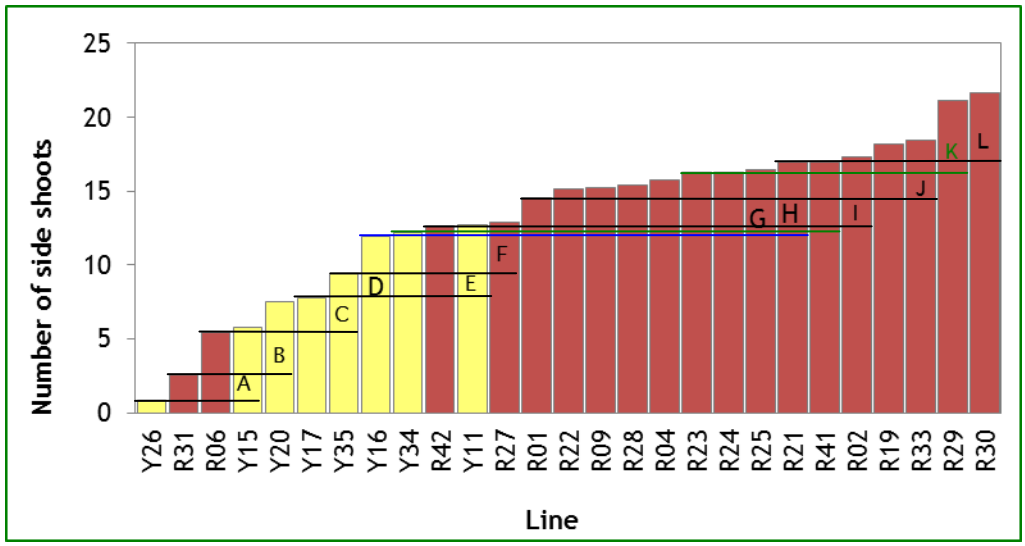

Figure 4.10: Number of side shoots of 27 lines papaya with yellow (yellow bars) and red (red bars) fruit. Means under the same line and letter are not significantly different from each other (P > 0.05)

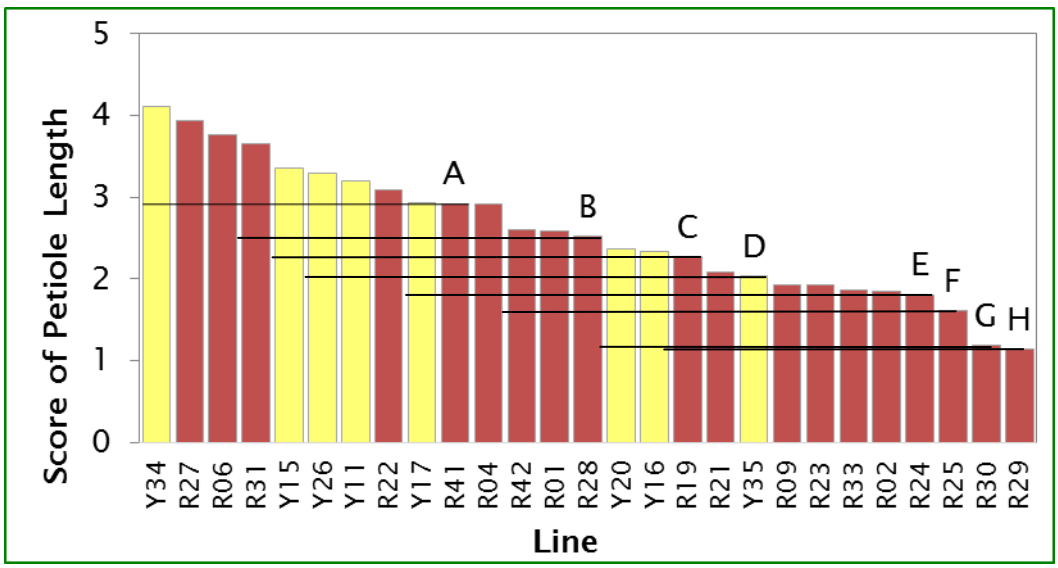

Figure 4.11: Rating of peduncle length of 27 lines of yellow (yellow bars) and red (red bars) papayas. Means under the same line and letter are not significantly different from each other (P > 0.05)

Table 4.9: Means of productivity traits for each line averaged across assessment times

Line	Height to 1st flower (cm)	#Side Shoot	Peduncle length	Saleable yield (kg/tree)	Yield gap	#Carpelloid fruits
R01	77.800	14.533	2.583	38.423	2.721	1.630
R02	67.200	17.333	1.848	34.621	4.149	1.164
R04	63.600	15.733	2.914	37.559	2.796	2.842
R06	79.267	5.467	3.764	**57.926**	2.865	0.131
R09	68.600	15.267	1.933	35.356	4.133	1.222
R19	56.267	18.133	2.267	25.552	2.921	1.644
R21	69.600	15.133	3.088	**6.907**	1.054	**24.857**
R22	70.867	16.267	1.926	23.947	4.206	0.336
R23	79.467	16.267	1.808	23.694	3.601	0.692
R24	70.400	16.400	1.615	35.783	4.220	1.071
R25	59.200	12.867	3.933	47.029	2.200	1.089
R27	68.786	15.429	2.518	35.939	2.538	1.244
R28	90.214	21.143	**1.143**	22.835	2.114	2.381
R29	**91.800**	**21.600**	1.182	20.734	**0.921**	2.028
R30	**54.867**	2.600	3.654	43.224	1.587	**0.000**
R31	64.533	18.467	1.869	23.816	1.968	2.296
R33	70.667	17.000	2.919	37.566	1.087	9.313
R41	64.600	12.600	2.596	43.058	3.692	2.387
R42	73.267	5.800	3.350	32.598	3.434	1.653
Y11	68.467	12.733	3.205	46.331	2.385	1.385
Y15	71.933	11.933	2.336	33.923	3.963	1.302
Y16	65.867	7.733	2.937	36.919	4.364	1.110
Y17	70.533	7.467	2.361	35.824	1.993	0.123
Y20	71.571	16.933	2.082	24.177	3.998	0.446
Y26	85.933	**0.800**	3.298	37.259	1.528	**0.000**
Y34	77.733	12.200	**4.107**	19.610	**5.830**	0.004
Y35	61.643	9.400	2.036	35.023	3.103	**0.000**
STD*	14.218	6.362	1.520	18.394	2.545	6.382

Note: Highlighted and bold figures are the highest and the lowest figures in each trait

* STD standard error of means for each data set

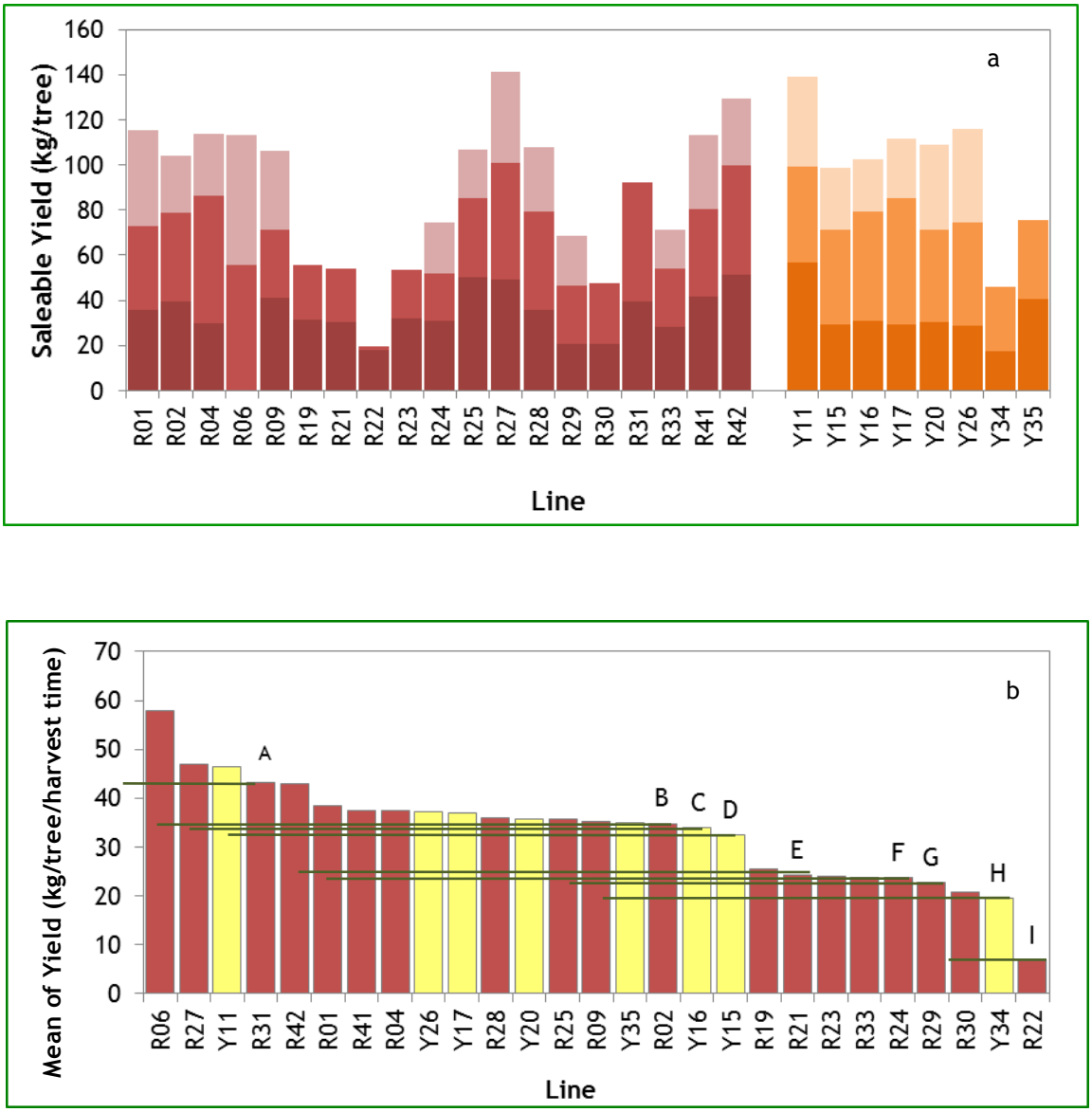

Figure 4.12: Saleable yield of 27 lines of papaya. a) Saleable yield over three harvest dates (1st harvest of red and yellow fruit, respectively, 2nd harvest and 3rd harvest). b) Mean of saleable yield of 27 lines of yellow (yellow orange bars) and red papaya (red bars). Means under the same line and letter are not significantly different from each other ($P > 0.05$)

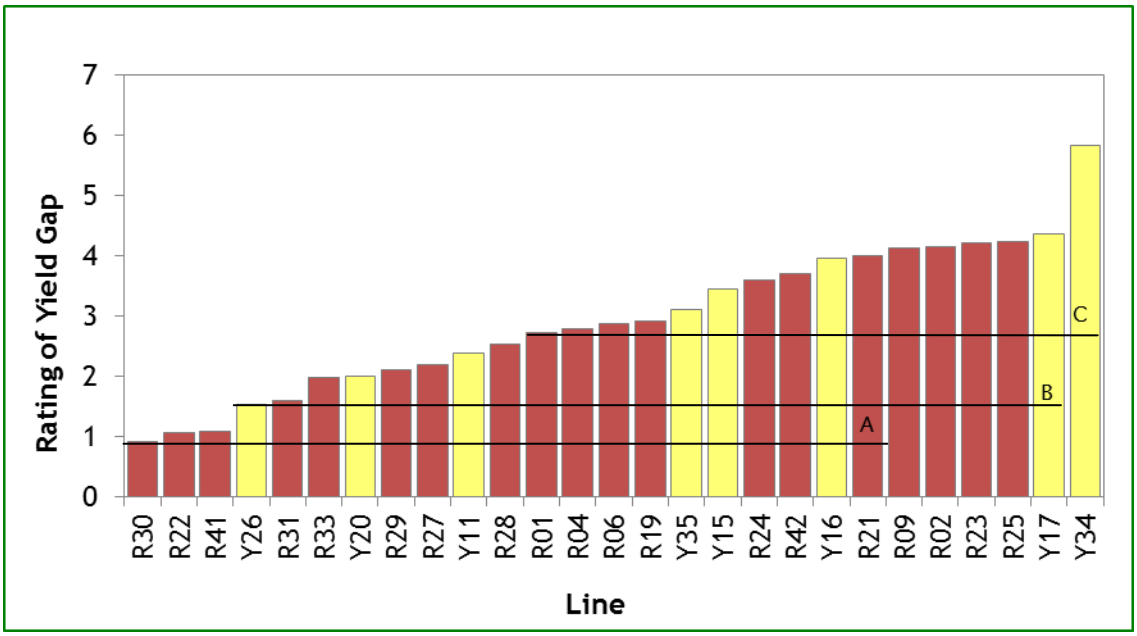

Figure 4.13: Rating of yield gap of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

4.11 Analysis of fruit quality traits

The data for shape of fruit, teat, stalk insertion and cavity is detailed in Appendix 2. This information was used as a reference when selecting parental trees. Histograms of the traits mention above are presented in Figure 4.14. The frequencies of each trait in each harvest season were different as shown in different patterns of distribution. Most traits showed continuous segregation except stalk insertion that the majority of fruits scored 2 (flattened).

Mean values of all the traits related to fruit quality for each line are shown in Table 4.10. Skin quality rating varied among lines in this study (Figure 4.15) and ranged between 1.5 and 3. The score of skin freckle varied among lines in this study (Figure 4.16) with red-fleshed fruits showing greater susceptibility to skin freckle than yellow.

The variation of skin colour in this population is reported in Figure 4.17. The chart showed yellow papaya tended to have lighter skin than the red flesh fruits. Line R06 and Y15 were the best two lines for skin traits, while R29 and R30 were the two lines with the lowest scores for both skin quality and skin freckle.

More than twice as many red fleshed lines than yellow fleshed lines were grown in this study (Figure 4.19). Lines with yellow flesh showed more consistency in flesh colour than those with red flesh (Figure 4.18). Flesh firmness and thickness varied among all the lines without any pattern (Figure 4.20 and 4.21). The measurement of total soluble solids in the 27 lines varied from eight to 15 (Figure 4.22). The highest TSS was detected in Solo type fruit (Linda and Sunrise Solo). However, the lowest TSS was found in an Australian commercial yellow papaya (1B). Fruit flavour was recorded to assist selection for parent lines to use in the next cycle of breeding program.

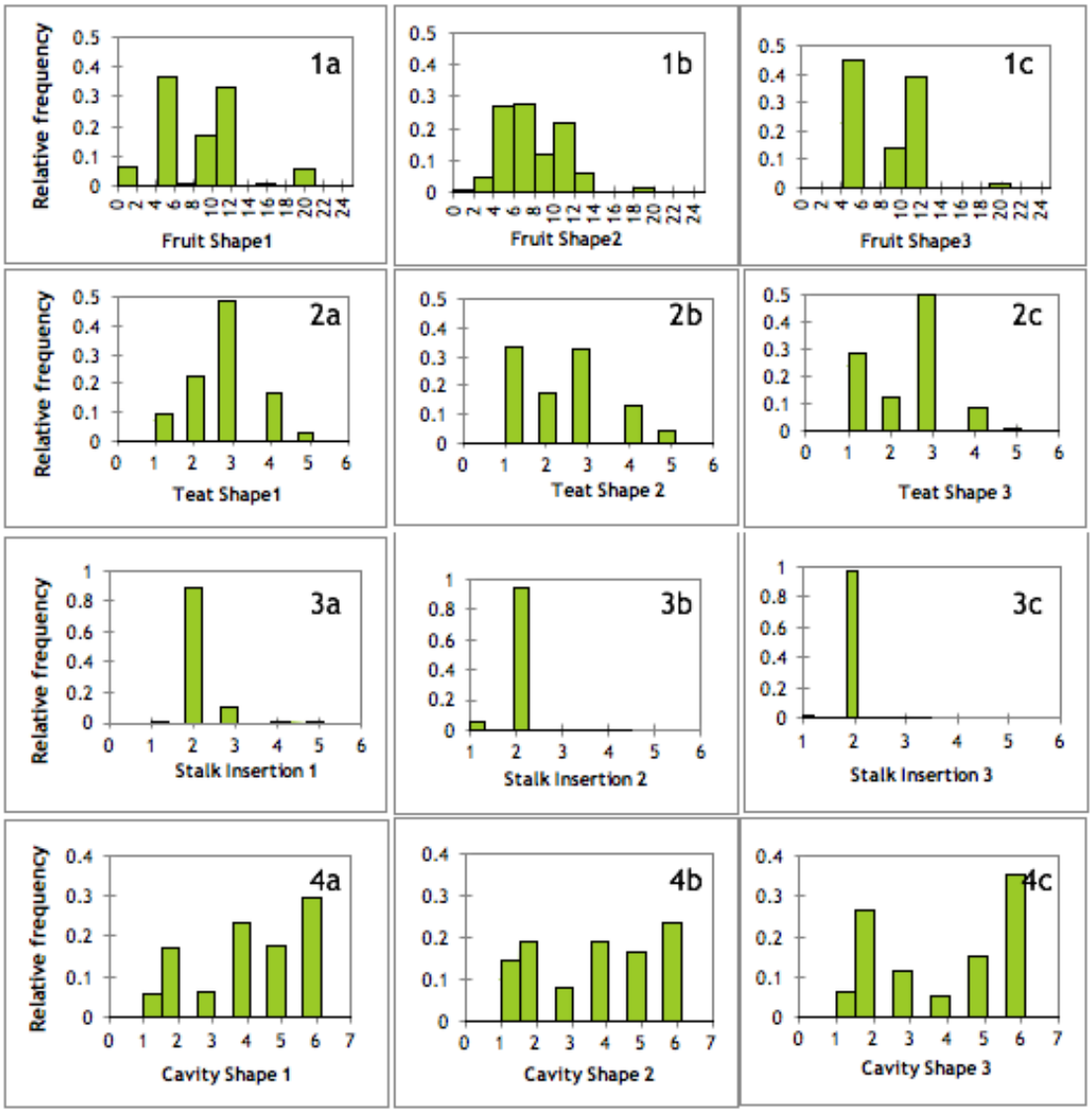

Figure 4.14: Histogram of papaya plants for 1) fruit shape, 2) teat shape, 3) stalk insertion and 4) cavity shape in three harvest times (a: first harvest in April 2012, b: second harvest in October 2012, and c: third harvest in May 2013)

Table 4.10: Means of fruit quality traits of each line

Line	Skin quality	Skin freckle	Skin colour	CFC[1/]	Flesh colour	Flesh firmness	UFT[2/] (mm)	TSS [3/] (ºBrix)
R01	2.215	1.731	3.148	1.833	6.733	3.370	18.630	10.948
R02	1.924	2.526	3.538	2.107	6.357	3.154	19.920	11.184
R04	2.694	1.048	3.368	2.000	6.378	3.368	18.432	11.274
R06	2.968	0.489	3.654	1.800	5.600	3.240	19.040	10.544
R09	2.119	2.024	4.143	1.800	5.689	3.571	18.762	11.571
R19	1.924	1.949	3.385	2.393	7.214	4.040	17.423	12.165
R21	2.508	1.148	3.650	2.036	5.310	2.850	19.375	11.946
R22	2.145	1.584	4.351	2.344	4.489	4.081	13.486	12.603
R23	2.161	1.774	4.040	1.643	6.286	4.040	19.760	12.228
R24	2.445	1.404	3.850	2.133	5.200	2.500	19.500	12.118
R25	2.339	1.644	4.250	2.356	6.200	2.950	18.000	11.168
R27	2.683	1.600	3.634	2.044	6.467	3.341	19.317	10.956
R28	2.366	2.070	3.857	2.262	6.000	2.943	16.514	11.780
R29	1.580	2.641	4.100	2.655	6.452	2.500	13.875	13.082
R30	1.490	2.821	3.476	2.511	5.333	2.100	12.897	13.295
R31	2.268	1.563	3.500	2.077	6.625	4.500	19.458	9.699
R33	1.886	2.198	3.864	2.422	6.000	2.318	16.455	12.675
R41	2.175	2.055	5.000	2.233	5.489	3.529	13.529	12.824
R42	2.100	2.001	3.857	2.100	6.067	2.464	20.143	11.514
Y11	2.338	1.433	3.080	2.786	2.000	4.680	19.960	10.295
Y15	2.728	0.950	2.571	2.833	1.286	3.286	18.286	11.744
Y16	2.622	1.479	2.588	2.905	1.738	3.000	17.971	10.913
Y17	2.093	1.688	3.300	2.458	1.250	3.800	19.700	9.853
Y20	2.348	1.143	3.000	3.000	1.308	2.545	17.409	11.349
Y26	2.105	1.583	2.905	3.000	1.182	2.333	22.619	9.238
Y34	2.647	1.333	2.941	2.795	1.154	3.059	19.500	12.865
Y35	2.673	0.996	2.667	2.125	3.444	3.333	20.708	11.251
STD*	0.764	1.137	1.300	0.726	2.380	1.516	4.329	1.710

Note: Highlighted and bold figures are the highest and the lowest figures in each trait

* STD standard error of means for the data set

1/ CFC means consistency in flesh colour

2/ UFT means useable flesh thickness

3/ TSS means total soluble solid for flesh sweetness

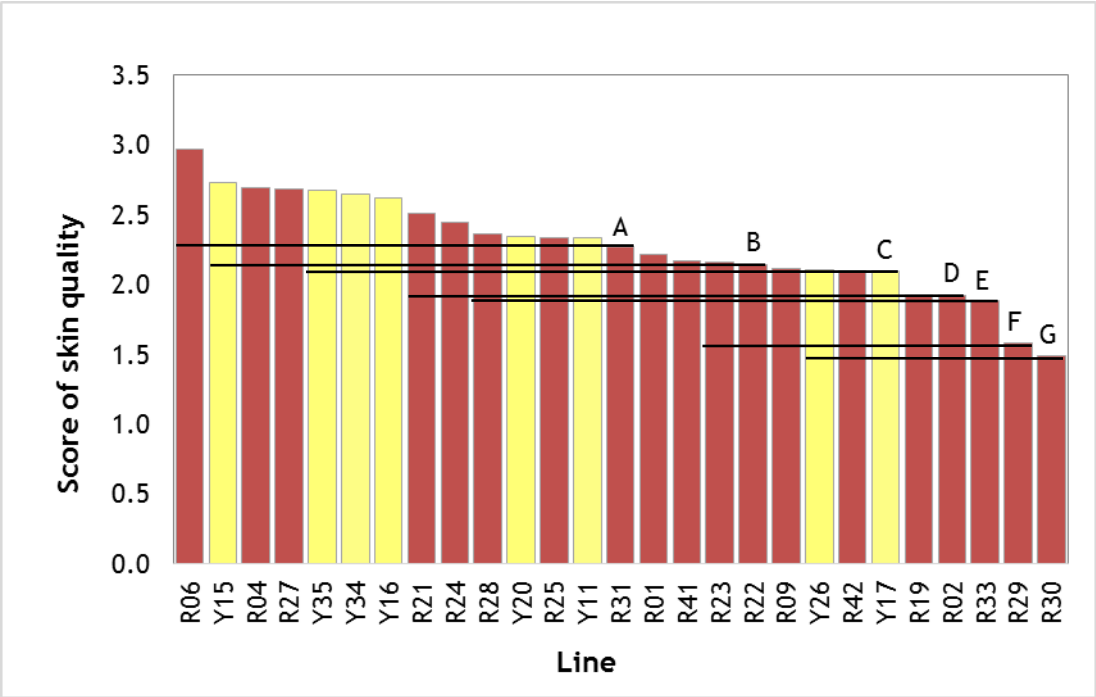

Figure 4.15: Score of skin quality of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

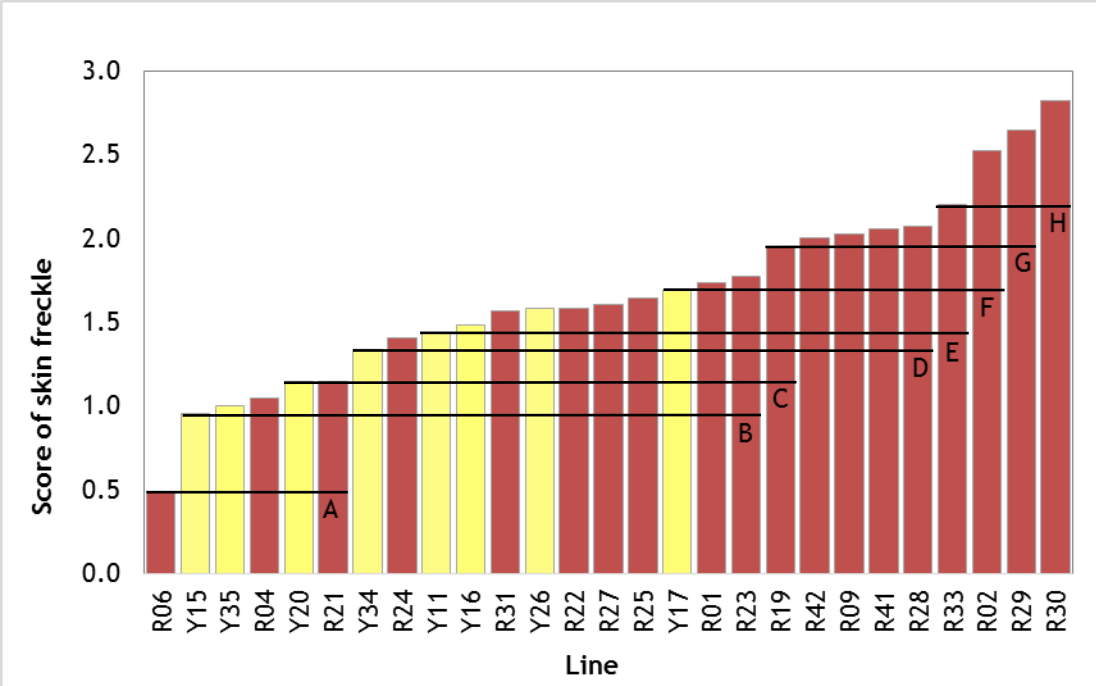

Figure 4.16: Score of skin freckle of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

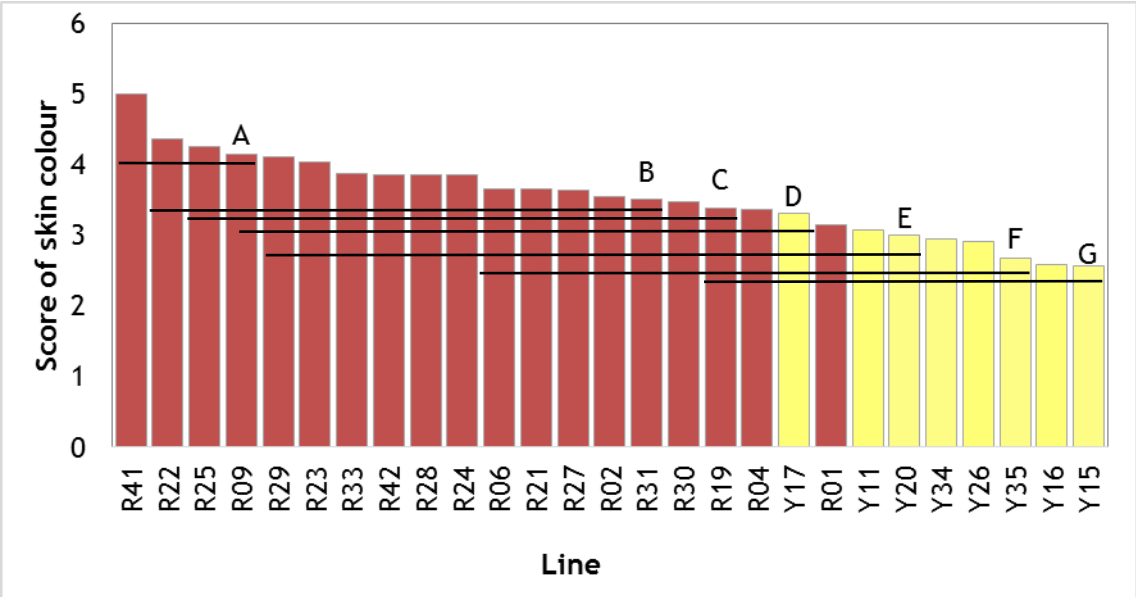

Figure 4.17: Score of skin colour of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

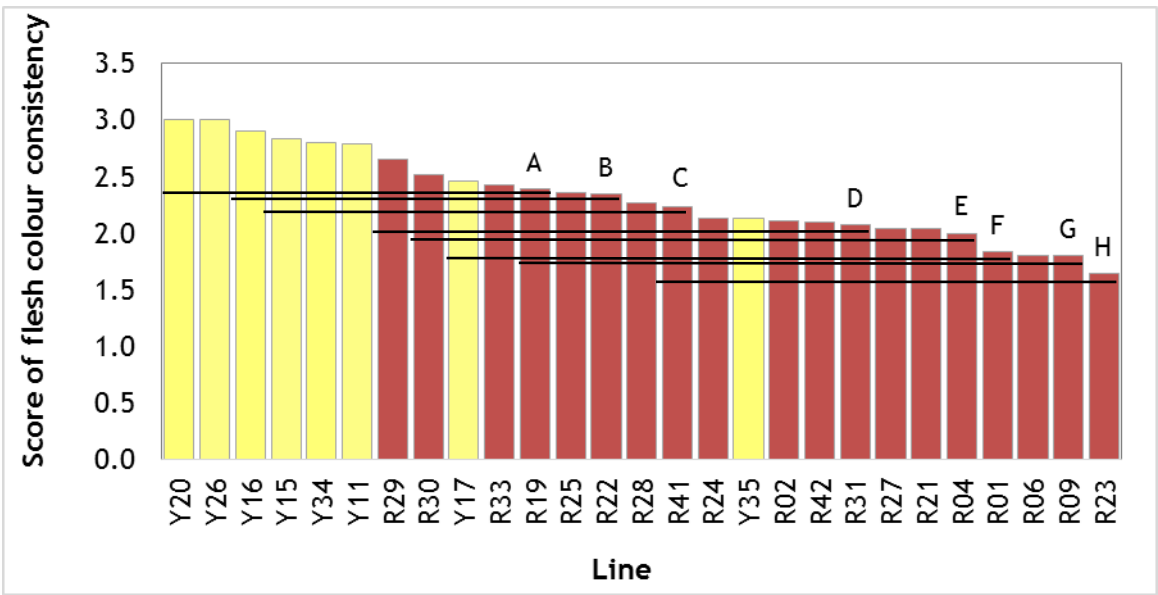

Figure 4.18: Score of consistency in flesh colour of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

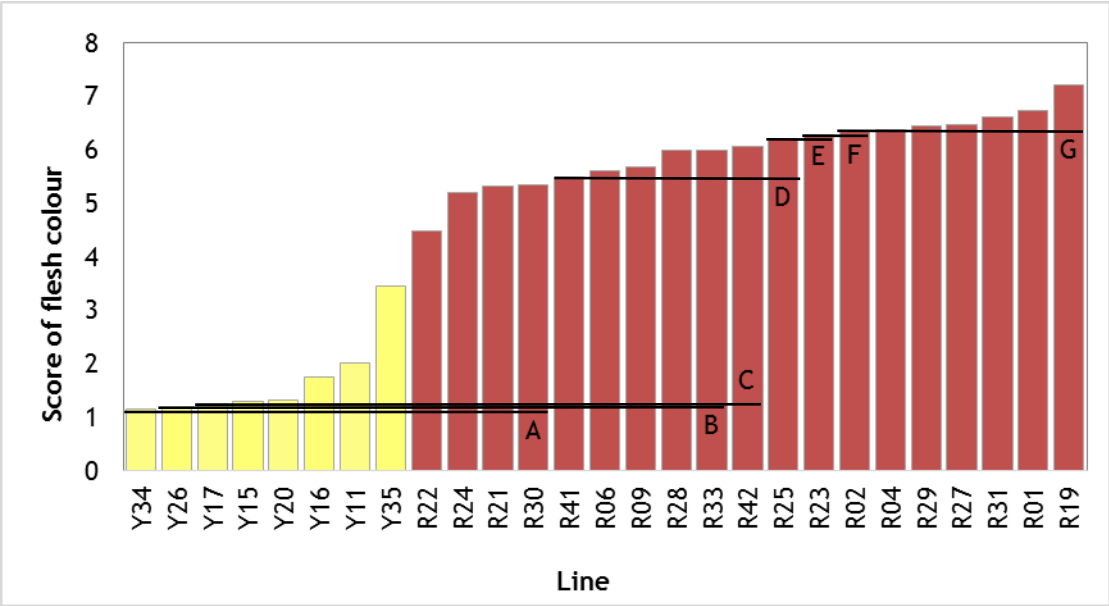

Figure 4.19: Score of flesh colour of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

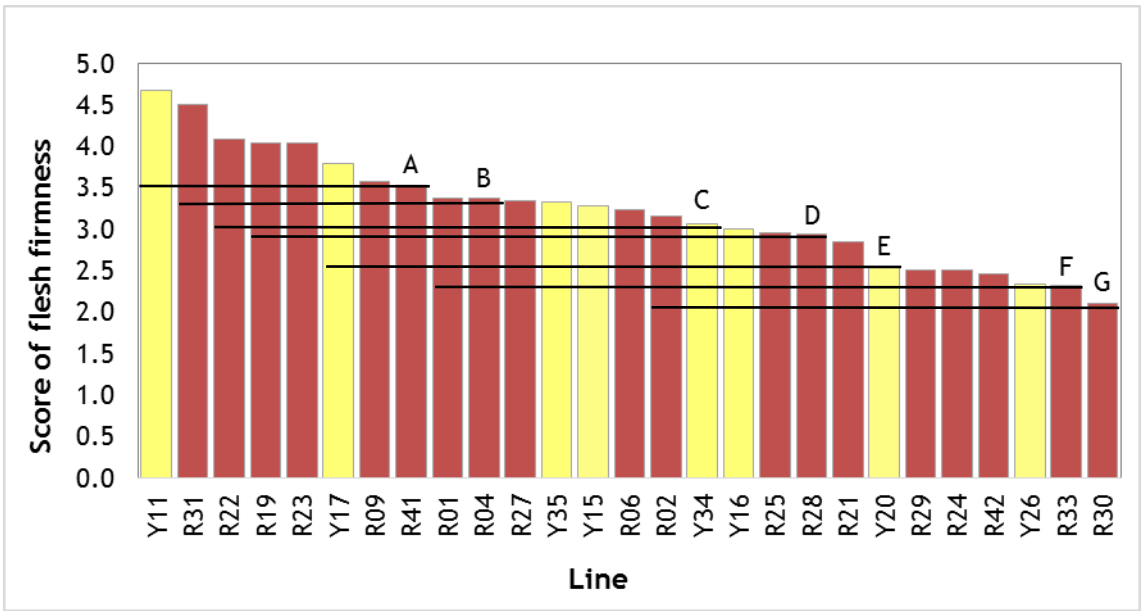

Figure 4.20: Score of flesh firmness of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

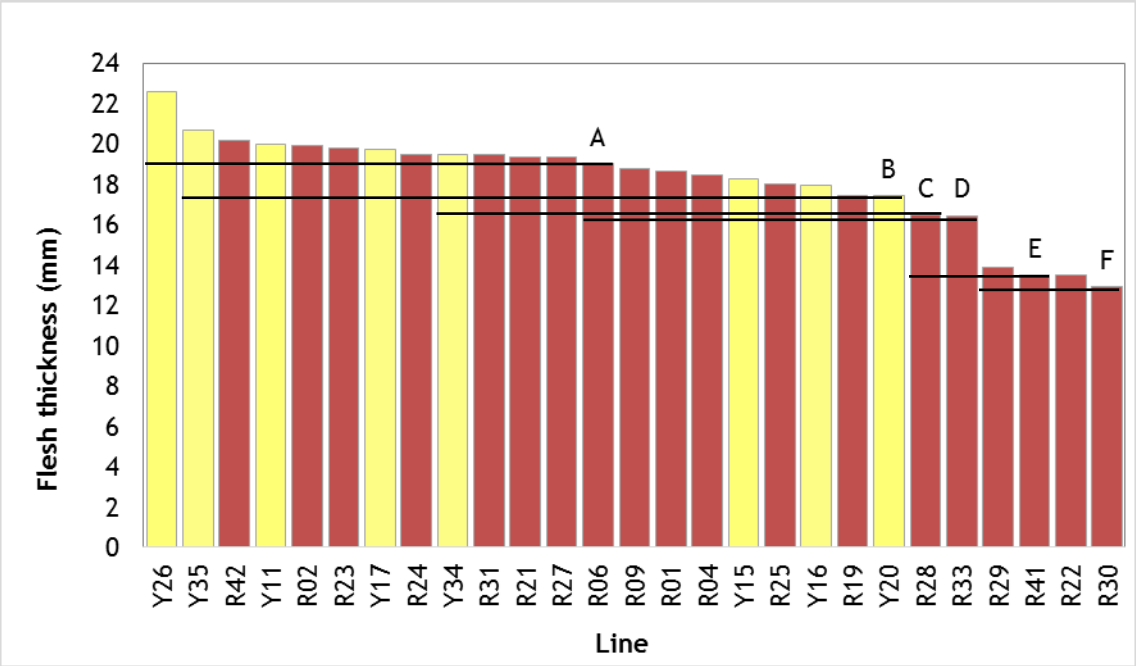

Figure 4.21: Score of flesh thickness of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

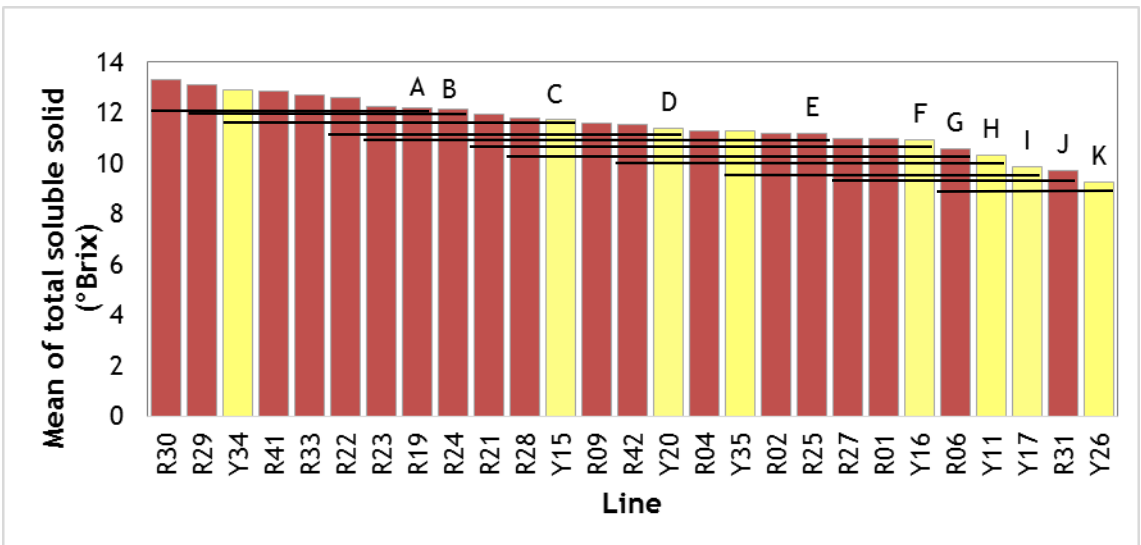

Figure 4.22: Means of total soluble solid (TSS) of 27 lines of yellow (yellow bars) and red (red bars) papaya. Means under the same line and letter are not significantly different from each other (P > 0.05)

4.12 Progress of breeding program to improve Australian commercial lines

The best three lines that were selected on the first evaluation (April 2012) were TS2, Malaysian Red 2 and Sunrise Solo. They exhibited excellent fruit eating quality (flesh flavour, firmness and thickness) and were selected to cross with Australian commercial lines 1B, RB1 and RB2. The data from all three harvests are presented in Table 4.11. Variation of the same trait in the same tree can be detected in different harvesting times, a fact noted (above) by the relatively low repeatability estimates. Sixteen crosses were established for the second phase of the breeding program (Table 4.12). F_2 populations of QDAFF lines and Australian commercial lines were also produced.

Table 4.11: Fruit eating quality in three harvest time of the selected trees to improve Australian commercial lines

Line	Harvest time	Flesh firmness	TSS (ºBrix)	Flavour Score	Flavour
Y22-7	1	5	12	12	Khagdum
(TS2)	2	5	14	9	Solo
	3	5	14	6	Solo
	Mean	5	13	9	
R24-2	1	5	13	12	Musk
(Malaysian Red 2)	2	3	11	4	Nasturtium
	3	1	13	9	Musk
	Mean	3	12	8.3	
R29-9	1	3	13.5	12	Solo
(Sunrise Solo)	2	3	14	6	Solo
	3	1	15	12	Solo
	Mean	2.33	14	10	

Table 4.12: Crosses used to produce progeny populations in the second cycle of applied breeding

Code	Female x Male	Line details
#50	Y22 x Y26	TS2 x 1B
#51	Y15 self (tree #7)	F_2 (1B x 33-66)
#52	Y15 self (tree #8)	
#53	Y16 self (tree #4)	F_2 (7-82 x 1B)
#54	R04 self (tree #7)	F_2 (RB2 x 18-45)
#56	RB1 x R24 (tree #2)	RB1 x Malaysian Red
#57	RB1 x R24 (tree #2)	
#62	R27 (tree #5) x R24 (tree #2)	RB2 x Malaysian Red
#63	RB2 x R24 (tree #2)	
#65	RB2 x R24 (tree #2)	
#64	RB2 x R29 (tree #13)	RB2 x Sunrise Solo
#67	RB2 x R29 (tree #13)	
#68	Y26 (tree #8) x R24 (tree #2)	1B x Malaysian Red
#69	Y26 (tree #6) x R24 (tree #2)	
#70	Y26 (tree #4) x R24 (tree #2)	
#71	R41 self (tree #1)	F2 (JC2 x Vietnam Red)

Of these 16 crosses from the second breeding population, seven trees, two and five of yellow and red papaya, respectively, were selected for their flavour and overall yield. These trees were used as parental lines to backcross to commercial varieties 1B, RB1 and RB2 and to sib cross to produce new hybrids. The selected trees are detailed in Table 4.13. An evaluation of this population has not been completed. Details of futures direction are described in Chapter 6.

Table 4.13: Parent lines selected for improvement flavour of commercial papayas

Flesh colour	Line	Flower sex
Yellow Papaya	#50-1	Female
	#50-male	Male
	#68-7	Hermaphrodite
Red Papaya	#62-5	Female
	#63-5	Hermaphrodite
	#64-2	Hermaphrodite
	#56-2	Hermaphrodite

	#56-5	Female

4.13 Progress of segregating population for development of fruit quality traits in papaya

Five categories of fruit and tree attributes (flavour, skin attributes, eating quality attributes of both yellow and red-fleshed types and tree productivity traits) were identified from the 22 traits that were evaluated in order to produce wide crosses segregating populations for the identification of DNA markers. Twenty-three trees were selected from 270 because they were the most consistent in expressing each trait in the three evaluation times. The details of the five categories and selected lines are detailed in Table 4.14. Trees were crossed and progeny populations were produced but evaluating these progeny was not completed within the time frame of this chapter. Future directions of this work are described in Chapter 6.

Table 4.14 : Five categories of traits selected to produce segregating populations for the development of DNA markers

Categories	Tree selected	Details
1. Flavour		
	R29-13 (Sunrise Solo)	Solo flavour
	Y15-6 (1Bx33-66)	Nasturtium flavour
	R24-2 (Malaysian Red 2)	Musk favour
	R41-8 (JC2 x Vietnam Red)	Khag Dum flavour
	R27-5 (RB2)	No flavour
2. Skin traits:		
skin quality and skin freckle	R30-5 (Solo Linda)	Poor skin quality
	R04-2 (RB2x18-45)	Good skin quality
Skin colour	Y34-11 (2.54-14 self)	Light yellow skin colour
	R41-6 (JC2 x Vietnam Red)	Deep orange skin colour
3. Eating quality: Yellow papaya		
Flesh colour, flesh firmness, flesh thickness and TSS content	Y16-1 (7-82 x 1B)	Light yellow flesh colour, soft, thin and low TSS
	Y35-12 (2.54-12 self)	Orange skin colour, firm, thick and high TSS
Flesh colour consistency	Y17-15 (24-87 x 1B) and Y35-12 (2.54-12 self)	< 50% flesh colour consistency
	Y16-1	100% flesh colour consistency
4. Eating quality: Red Papaya		
Flesh colour	R24-1 (25-5 self)	Light orange flesh colour
	R27-5 (RB2) R4-10 (RB2 x 18-45)	Deep red flesh colour
Flesh firmness and thickness	R30-9 (Solo Linda)	Soft and thin flesh
	R27-1 (RB2)	Firm and thick flesh
TSS content	R27-2 (RB2)	Low TSS
	R29-9 (Sunrise Solo)	High TSS

Flesh colour consistency	R09-12 (25-5 x RB1)	< 50% colour consistency
	R29-9 (Sunrise Solo)	100% colour consistency
5. Production traits		
Number of side shoots	26-13 (1B)	0 side shoot
	R30-9 (Solo Linda)	High number of side shoot
Height to first marketable fruit	R27-5 (RB2)	Low
	R29-9 (Sunrise Solo)	High
Number of carpeloid fruit (Hermaphrodite tree)	R42-11 (TS2 self) or R02-13 (RB1x24-29)	Less (0 fruit)
	R04-10 (RB2x18-45)	More than 20 fruits
Peduncle length	R30-10 (Solo Linda) R09-14 (25-5 x RB1) Y17-12 (24-87x1B)	Short
	R04-10 (RB2x18-45) R27-7 (RB2) R27-11 (RB2)	Long
Yield gap:	R29-9 (Sunrise solo)	No yield gap
	Y17-15 (24-87x1B)	High yield gap

DISCUSSION:

There was a wide range in the phenotypic variations observed in these populations. Similar variations were reported in the descriptors provided by IBPGR 1988. The IBPGR work showed high phenotypic variation in leaf, flower and fruit attributes and reaction to pests and diseases. The differences of most traits between lines were quantitative and did not allow for grouping of individual varieties. This might be due to many fruit quality traits being controlled by multiple genes and low heritability.

4.14 Effect of harvesting time to fruit productivity and quality traits

Evaluation of performance of trees in different harvest times is necessary as the result showed relatively low repeatability in most traits and some seasonal effects of on fruit and tree development can be observed in this study.

Fruit productivity traits expressed relatively consistently in the first two harvesting times, but varied more in in the last of three harvests. A similar result was found in the report of Benson and Poffley (1998). In general, yield of yellow papaya was higher than red papaya. This could be because of the nature of dioecious outcrossed plants that can produce higher yields than selfed hermaphrodites which may be exhibiting inbreeding depression (Drew et al. 1998a; 1998b; Chay-Prove et al. 2000). Interestingly, some traits such as number of carpelloid fruits were observed by growers to be affected by season, but this study showed no significantly different in this trait between the three harvesting times at the one location. This suggests that environmental effects on the expression of this trait were not as important as gene expression. Yield gap varied with lines. Magdalita et al. (1998) and the The Organisation for Economic Co-operation and Development (OEDC 2005) suggested yield and yield gap varied considerably and was affected by cross pollination, environmental condition, floral character and flower

receptivity. Female sterility in hermaphrodite trees can be exacerbated in stress conditions such as high temperatures and water and nitrogen shortages (Awada and Ikeda 1957; Arkle and Nakasone 1984; Amleida et al. 2003). In Australia, variable fruit production could be due to low pollen viability and absence of pollinators especially in cooler, winter seasons. In subtropical climates, fruit set declines or may even cease during the colder winter months (Garret 1995; Allan 2002). Further study into factors affecting pollen and flower receptivity could be recommended because of the magnitude of the gaps noted in this study (up to 60% or more).

Fruit quality traits are complex and could be controlled by quantitative loci. The quality of fruits varied according to location, variety and season as well as farm practice (Elder et al. 2000a). In South Africa, fruits harvested from more mature trees, giving better quality fruit, and in particular, a higher sugar content (Department of Agriculture, Forestry and Fisheries, South Africa 2009). Uniformity of fruit size, shape and appearance of dioecious fruits were better than those from hermaphrodites (Storey 1976; Ying 2008). Fruit shape and size were controlled by 14 QTL with phenotypic effects; they were identified across six LGs with clusters of two or more QTL on LG2, LG3, LG7 and LG9 (Blas et al. 2012). These loci contain homologs to the tomato fruit QTL ovate, sun and *fw2.2* regulating fruit size and shape (Van der Knaap and Tanksley 2001).

One of the traits affected by temperature or season of harvest was flesh colour. A single gene controls colour wherein yellow is dominant over red flesh (Ying 2008; Blas et al. 2010). In the present study, lines and plants within lines were either yellow or red-fleshed, and repeatability of the attribute was relatively high. Nevertheless, colour intensity varied with the season being lowest in fruits developed in winter 2012 (the October 2012 harvest). This could be related to ripening process of papaya because fruit ripen satisfactorily between 20 and 25ºC (Akamine 1966; Broughton et al. 1977) but do not ripen at 10 and 15ºC (Nazeeb and Broughton 1978). The result of temperature to the development of flesh colour is noted in other fruit, for example in tomato so that that higher temperatures affect red color development and softening (Hall 1964).

The season when fruit developed also affected skin freckle, which is one of the important traits for fruit appearance. Eloisa and Paull (1994) suggested freckles resulted from aberrant physiology associated with a rapid growth late in the fruits' development. Both low and high temperatures in the two months before harvest increased freckle occurrence. This was supported the result of fruits developed in winter (second harvest in October 2012) had higher score of skin freckle than the other two harvest.

4.15 Correlation of traits

Even though some traits in this study were significantly related, most of them showed low degree of correlation. Only the measurement of height to first flower, first fruit and first marketable fruit were highly (positively) correlated, therefore, height to first flower can be used to determined the height at which fruit will set on the tree. Yield, number of carpelloid fruits and yield gap can be negatively correlated. However, peduncle length tended to positively associated with yield. These factors were previously predicted by the growers of north Queensland to directly affect yield (Kath, personal communication).

Interestingly, flesh colour score was positively correlated with skin colour but negatively with consistency of flesh colour. This effect was also noted by growers: viz. the darker of the skin colour, the deeper of the flesh colour. The positive relationship between skin colour and flesh

colour is also reported in other fruit, for example apricot (Ruiz and Egea 2008).

One of the important traits to improve in commercial papayas in Australia is level of TSS which is commonly refer to Brix (Papaya breeding reference group and growers, personal communication). The TSS content is usually associated with sweetness and reports of TSS range from 8% to 16% or more (Singh 1990). In the present study, TSS was generally in the range of 10 to 12%. There was no correlation between TSS and other traits in this study, which was different from the report of Hansen (2005) who reported linkage between high sugar content and skin freckle, that is, the greater the TSS the worse the skin freckle. The TSS is controlled by additive gene effects and has a high heritability. Papaya fruit lack starch reserves for post harvest conversion to soluble sugars and final sugar content is determined by the quality and quantity of translocatable sugar in the fruit at time of harvest (Chan et al. 1979; Manshardt 1992).

4.16 Applied plant breeding

To generate new varieties, expeditious evaluation and prompt decision-making are part of effective plant breeding. In the present study, parental lines used to improve eating quality in commercial Australian papayas were selected and crossing was done after the first harvest. Despite relatively low repeatability of measurements of most traits across harvest times in these plants, plants were noted that did consistently perform at high (or low) levels. It should be noted, too, that the time required to establish the subsequent generation of plants following cross-pollination did allow for repeated observation of the selected parent plants. It is likely that repeatability of measurements will improve as gene frequencies are moved by selection, re-crossing and focus on the most important traits. The wide heterogeneity within groups (see Formula 4.2) is likely to progressively decline with selection and re-crossing.

Another aim of this research was to establish segregating populations for applied plant breeding to develop DNA markers for quantitative traits. Parents for this work were selected on consistent expression of each trait in three harvests. For this parent material, trait expression was quite repeatable. Discussion here regards some concerns about future breeding plans.

As noted in the introduction to this chapter, the Australian market associates yellow flesh with round fruit and red flesh with elongate fruit. Fruit shape is determined by the sex of the plant (Storey 1938; Ming et al. 2007). The Australian consumers' association of flesh colour with fruit shape is not a fixed genetic linkage. Plant sex type also influences flesh thickness and fruit uniformity (Anh et al. 2011). Papaya has three basic sex types: female, male and hermaphrodite. Genetic control of sex of papaya has been studied since 1938 (Hofmeyr 1938; Storey 1938). It was hypothesised that papaya has three distinct sex chromosomes (X, Y and Y^h) with a lethal factor in the male- and hermaphrodite-specific region (Storey 1941). Males (XY) and hermaphrodites (XY^h) are heteromorphic and the expression of sex is controlled by Y for male and Y^h for hermaphrodite, while females (XX) are homomorphic. Any combination of YY, Y^hY or Y^hY^h lines is lethal (Ming et al. 2007). Therefore the segregation ratio of 1:1 of female to either male or hermaphrodite can be found in female crosses to either male or hermaphrodite, respectively. Selfed hermaphrodite or sib crosses between hermaphrodite flowers express 1:2 of female to hermaphrodite plants. Thus the selection of females and males to be used as parental lines for yellow papaw is ideal to meet the requirement of the market in terms of fruit shape. But for red papaya it is preferable to self or sib-cross hermaphrodite flowers to achieve a greater number of hermaphrodite fruits for evaluation and selection.

Flavour is a very complicated trait and it needs further study to be able to classify flavour scientifically. There are a number of reports in the literature discussing the volatile chemicals in papaya fruits (Flath and Forrey 1977; Idstein et al. 1985; Pino et al. 2003, Almora et al. 2004). Papaya possesses a characteristic aroma, which is due to several volatile components, such as alcohols, esters, aldehydes, and sulphur compounds (Marostica and Pastore 2007). Almost 400 volatiles profiles of various papaya cultivars were identified in more than 40 years of intensive work (Pino et al. 2003).

CONCLUSION:

The result from this study led to the selection of three lines, which were TS2, Sunrise Solo and Malaysian Red 2, to use in a breeding program to improve flavour of commercial varieties in Australia. They exhibited excellent fruit eating quality (flesh flavour, firmness and thickness) were used to pollinate Australian papaya commercial lines, 1B, RB1 and RB2.

Of the 22 evaluated traits, five groups of traits which deal with fruit flavour, skin quality, eating quality of red papaya, eating quality of yellow papaya and yield were identified for the production of segregating populations to be used for subsequently developing DNA markers for those traits. Twenty-three representative trees were selected as parental lines because they were consistent in expression of each trait of interest over the three evaluation times. Seventeen crosses were made for a future breeding program to develop improved commercial lines and for a program of MAS.

Appendix 4

Chapter 5 of Ph.D. thesis of Dr. Chat Kanchana-udomkan

This chapter provides complete experimental details of research associated with the development of molecular markers as described in this report

Chapter 5:

Application of DNA Markers to Select for Fruit Flesh Colour in Papaya

INTRODUCTION:

The majority of papaya fruits are consumed fresh as ripe fruit or when green as a component in salads. In Australia, the market clearly distinguishes types of papaya according to flesh colour, as yellow papaw or red papaya. Flesh colour of papaya is an indicator of antioxidant activity in red flesh and a source of high vitamin A in yellow flesh (World Health Organization 2007; Blas et al. 2010). In general, red papaya has better and more acceptable flavour to consumers than yellow papaya. Cross pollination between red and yellow papaya is a potential approach toward improving the flavour of yellow papaya. Yellow flesh colour was observed to be dominant over the red flesh colour in a simple Mendelian ratio (Ying 2008). Cross pollination of heterozygous yellow flesh papaya to either another heterozygous yellow or red-fleshed papaya can result in segregation of flesh colour in later progenies. However, a mixture of yellow and red flesh coloured fruit within the same plantation area is a farm management issue related to time of harvesting and fruit sorting. This may lead to a mixture of red- and yellow-flesh fruits in one sale unit (one carton) and mislabelling such that consumers may not get the fruit that they desire. This factor has resulted in loss of consumer confidence.

A breeding program to improve eating quality of both fruit types has been funded by Horticulture Innovation Australia (known as Horticulture Australia Limited) since 2005 (Hansen 2005; Drew 2005). The selection process is the most crucial procedure for a breeding program. Conventional breeding relies on phenotypic expression, in this case, flesh colour, which takes time to express and is likely greatly influenced by the environment. Fruit flesh colour is easily to identify when trees are mature and produce fruit, which can take approximately 12 months after planting. However, to identify this trait in seedling can be very useful for growers in terms of farm management. Especially, in breeding lines where red papaya may cross pollinate with yellow papaya and following generations may present mix phenotype. Subsequently, DNA markers, that select for the major genetic components of such a trait have become routinely used as an efficient tool. DNA marker assisted selection can be done at any stage of plant development, without having to wait for the desired traits to be expressed. A gene that controls yellow flesh colour, *CpCYC-b* was identified and DNA markers linked to this trait were identified (Blas et al. 2010). It is, therefore, an opportune time to take this genomic information and use it to apply to the breeding programs, to demonstrate marker assisted selection (MAS) for improving the efficiency of papaya breeding.

Australia has limited papaya genetic resources (Hansen 2005), therefore papaya germplasm from various sources was obtained and evaluated as detailed in Chapter 4. Over 300 trees of 27 lines were evaluated for traits related to production and fruit quality. Fruit flesh colour is an important trait for papaya marketing in Australia and as an indicator of nutritional benefit leading to consumer preference. It is, therefore, of great interest to breeders to be able to accurately select for flesh colour throughout the breeding process. Flesh colour in papaya is a result of the accumulation of □-carotenoids in yellow flesh or lycopene in red flesh in fruit cell chromoplast (Yamamoto 1964; Blas et al. 2009).

The location of genes controlling fruit flesh colour, *CpCYC-b*, and genes related to PRSV-P resistance on genetic maps is very important in the development of markers, for future breeding, and for an understanding of gene function. They were reported to be mapped on the same linkage group by Chen et al. (2007). They reported that the gene controlling fruit flesh colour was mapped on LG5 of a high density genetic map of papaya, which was the same linkage group where *prsv-1* was located (Dillon 2006b; 2006c; Razean Haireen 2013). Another report was that the flesh colour gene (Fcolor) was identified on LG7 of the high density AFLP map and the transgenic PRSV-P coat protein gene (PRSVCO) was also mapped to the same LG7 (Ma et al. 2004). Moreover, there were two large indels of 1,805 and 2,556 bp between the two BAC clones, one from red flesh SunUp (SH18O09) and the other from yellow flesh AU9 (DM105M02). The insertion of two Ts in red flesh papaya produced a frame-shift mutation and result in a premature stop codon in red fleshed papaya (Blas et al. 2010). Three SCAR markers were developed from the variation between the two BAC clones (Blas et al. 2010). The presence of the fragments was surprisingly found only in nontransgenic AU9 (yellow flesh) but absent in transgenic SunUp which is a red flesh papaya (Blas et al. 2010). Thus, it is possible to hypothesise that *CpCYC-b* and gene(s) related to PRSV-P resistance may locate on the same linkage group.

This approach would be to identify sequences that code for the genetic components that condition the colour. This may include components such as carotenoid, a precursor for the biosynthesis of Abscisic acid (ABA) (Cunningham and Gantt 1998; Walton and Yi 1995; Zeevaart and Creelman 1988). Carotenoid is also involved in plant defence (Ton et al. 2009; Mauch-Mani and Mauch 2005; Anderson et al. 2004).

RESEARCH AIMS:

1. To determine the transferability of published flesh colour markers of papaya to the current lines of the breeding population

2. To determine if these and other markers on the supercontig 28 on linkage group 5 are accurate in flesh colour selection within a wide population

MATERIALS AND METHODS:

5.1 Plant materials

Between 10 and 15 individuals of each of the 28 lines (330 plants in total) were used from Chapter 4 (Table 4.1). A 1 to 9 scale was used for assessing flesh colour, where 1 was yellow and 9 was red. The segregation of flesh colour score among all individuals assessed is shown in Figure 5.1. Based on the scale of flesh colour, trees were grouped as yellow flesh with a score ≤ 3 and as red flesh with a score >3. The details of the scoring system are described in Chapter 4, 4.1.1.

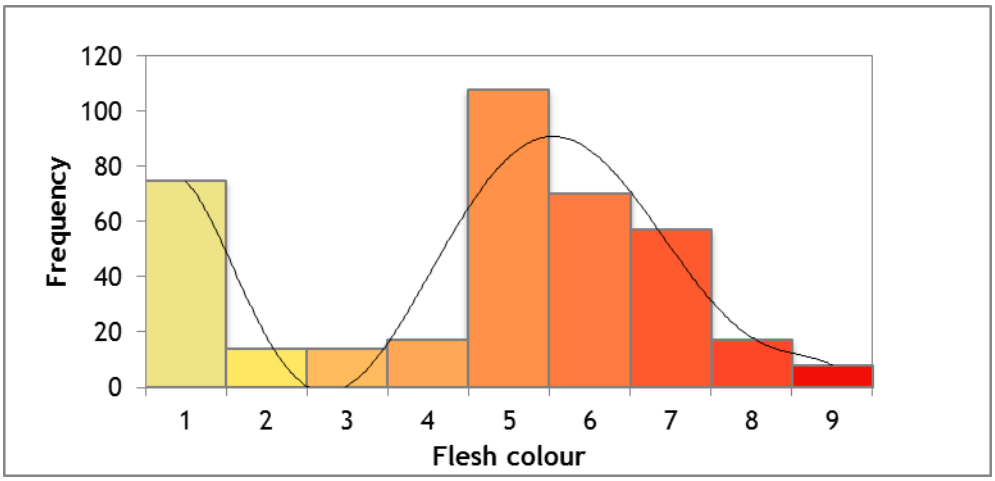

Figure 5.1: Segregation of flesh colour of all of the individuals used in this study. The scale of 1 to 9 represented the variation from yellow to red flesh

5.2 Bulked segregant analysis

Total genomic DNA was extracted separately from a leaf sample each individual using protocols described in Chapter 2, 2.1.1. DNA quantity and quality were evaluated by spectrophotometry (detailed in Chapter 2, 2.2.1). DNA samples were diluted to a 25 ng/µl working concentration and kept at -20ºC. The DNA samples were sorted according to flesh colour. Ten µl (250 ng) of DNA from each of 11 trees with a score of 1 were bulked as 'Yellow' and 10 µl (250 ng) of DNA from each of 11 trees with the score of ≥8 were bulked as 'Red' (Table 5.1). The bulked DNAs were then used for optimisation of PCR reactions and identification of candidate markers potentially associated to flesh colour based on their segregation within the population and statistical association with flesh colour.

Table 5.1: Individual plants (genotypes), their flesh colour score and grouping into 'Yellow' or 'Red' bulk

Line 'Yellow'	Tree Number	Flesh score	Line 'Red'	Tree Number	Flesh score
27-29 x RB2	Y11-5	1	RB1 x 18-45	R1-9	9
1B x 33-66	Y15-1	1	RB1 x 24-29	R2-3	9
	Y15-10	1	Malaysian Red 1	R23-7	8
7-82 x 1B	Y16-5	1		R23-8	9
	Y16-12	1		R23-14	9
24-87 x 1B	Y17-10	1	Malaysian Red 3	R25-10	8
JC2	Y20-1	1	RB4	R28-12	9
	Y20-9	1	RD6 self	R31-13	8
1B	Y26-11	1	2.54-12 self	R35-7	9
2.54-14 self	Y34-9	1	TS2 self	R42-11	8
	Y34-13	1		R42-14	9

5.3 PCR analysis

5.3.1 Published DNA markers for papaya flesh colour

Sequence characterised amplified region (SCAR) primers (Table 5.2), which were developed by Blas et al. (2010) for differentiation of flesh colour in papaya, were synthesised (Sigma Aldrich, Australia) and applied to amplify a product in both bulked DNAs. Primers that amplified a clear and reproducible size polymorphisms among the 'Red' and 'Yellow' bulk were further investigated for segregation among all of the individual 330 trees.

Table 5.2: SCAR primers used to differentiate papaya flesh colour (Blas et al. 2010)

Primer name	Primer sequence (5' → 3')	Flesh colour detected	Expected size
CPFC1	Forward: GACGTGTTAGTGTCCGACAA	Yellow/Red	500
	Reverse: GACCAGGAAGCAAATTTTGTAA		
CPFC2	Forward: GGACCACAGGAGCTGATTAG	Yellow	600
	Reverse: TATCTCTGCCACATGCAACC		
CPFC3	Forward: TGCAAAGAAATGGAGGGTTT	Yellow/Red	450
	Reverse: TGAAATCCTTCTGAGCCAAA		

5.3.2 The development of novel SSR and EST markers in SC28

Three SSR primer pairs, SSR28.50, SSR28.106 and SSR28.103-104) were designed on SC28 by Dr Cameron Peace (personal communication). These primers targeted microsatellite sequences as follows:

- SSR28.50 targeted a 247 bp length of just Cs and Ts in gene 28.50.
- SSR28.106 targeted a $(TTTTC)_5$ microsatellite that is in the promoter of 28.106, 387 bp upstream from that gene's start codon.
- SSR28.103-104 targeted a $(AG)_{10}$ repeatas 11.7 kb upstream of 28.105, placing it between genes 28.103 and 28.104.

Further primer pairs were designed to the putative PRSV-P diseases resistance gene LRR28.12 on SC28 using Primer 3 software (http://frodo.wi.mit.edu/ primer3; Rozen and Skaletsky 2000). Optimum primer length was 20 nucleotides, optimum melting temperature (Tm) of primers was 60ºC, optimum primer GC content was 50% and maximum self-complementarity at the 3'-end was 3.00. The primer pairs were then tested again for their self-complementarity using the Oligo Analysis Tool Kit (http://www.operon.com/tools/oligo-analysis-tool.aspx). Details of primers are shown in Table 5.3.

Table 5.3: Primers on supercontig 28 used in the flesh colour study

Primer name	Primer Sequences	Primer Tm (ºC)
SSR28.50	Forward: GCGTGCAACACTTTTCTCC	63.4
	Reverse: AAAAACTGCGTGAGATGTCG	62.8
SSR28.106	Forward: ACACCATTGTCAGCTCAACG	63.8
	Reverse: TGGTATTGGTTTCAGCATGG	63.3
SSR28.103-104	Forward: GTTGGACGCATCTACTCACG	63.3
	Reverse: CCTCTTCGACTACGCACACC	64.8
LRR28.12.1	Forward: TTCCTCCTCTTCCTCCTCCT	59.37
	Reverse: TCATTTGGTATTTCGCCAGA	59.11
LRR28.12.2	Forward: CAATCCCTCCTTTCAACCAA	59.90
	Reverse: TATTCATCTCCCGCATCCTC	60.00
LRR28.12.3	Forward: CAACAGGAGAGGGACACACA	59.70
	Reverse: TTGGGATGAACCAGAGGAAG	60.04

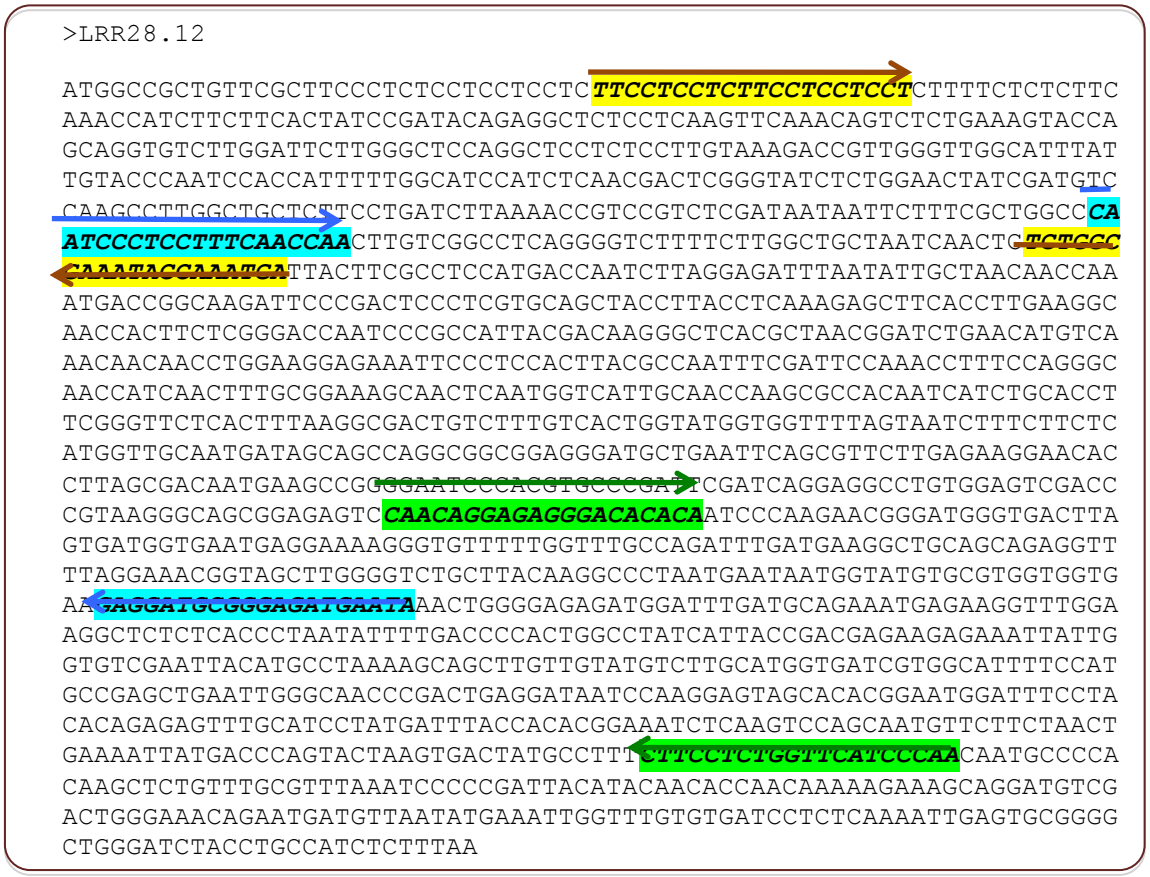

Figure 5.2: Nucleotide sequence of LRR28.12 (Zhu, personal communication) and priming site of primer LRR28.12-1 (highlighted in yellow), LRR28.12-2 (highlighted in blue) and LRR28.12-3 (highlighted in green). The arrows indicated direction of amplification for each primer, where → were forward primers and ← were reverse primers

5.3.3 PCR cycle optimisation

PCR reactions were optimised by using pooled DNA of 'Yellow' and 'Red' as the DNA templates. The components for each reaction and the PCR conditions are detailed in Chapter 2, 2.2.3. The reactions were optimised at eight different annealing temperatures using gradient function between 50 and 60ºC in MyCycler PCR machine (BioRad). The 2-step PCR cycle (detailed in Chapter 2, Figure 2.4) was applied to primer CPFC2 because it produced a dominant marker only in 'Yellow' but no amplification was detected in 'Red'.

5.3.4 DNA sequencing

The PCR products that were amplified from primers pairs were amplified in triplicate then purified and sequenced using protocols described in Chapter 2, 2.4.

5.4 Fragments and gene analysis

DNA sequences of forward and reserves strands were analysed and trimmed at Phred scores higher than 30 using 4peak software version 1.7.1 (Griekspoor and Groothuis, www.mekentosj.com). The sequences were initially aligned using the alignment tool within the NCBI database, and then by using CLUSTALW to obtain a longer sequence. Sequences for each set of primers for both flesh colours were aligned using CLUSTALW to search for dissimilarity between the alleles from the two flesh colours. The sequences were then BLASTed to NCBI and papaya databases on www.phytozome.net to search for similarity to sequences from papaya and other related crops.

Correlation between marker and flesh colour were analysed and calculated for percentage of association using Equation 5.1.

$$\text{Percentage of association (\%)} = 100 - \frac{\text{number of mismatchs between marker and expression x 100}}{\text{total number of plants}}$$

Equation 5.1: Calculation of percentage of association between marker and trait

RESULTS:

5.5 DNA polymorphism between yellow and red bulked DNAs

5.5.1 Amplicon size differentiation using published DNA markers linked to flesh colour

Three primers successfully amplified products from both bulked DNAs. Fragments of 399 bp, 566 bp and 316 bp were amplified from primer pairs CPFC1, CPFC2 and CPFC3, respectively (Figure 5.3). The CPFC2 marker was dominant, present in the yellow bulk and absent in the red bulk. The other two markers, CPFC1 and CPFC3, were dominant but present in both bulks.

When the 2-step PCR cycle was applied to the samples with the CPFC2 primers, two distinct alleles appeared at this locus of 600 bp and 900 bp, which were correlated with yellow and red flesh, respectively (Figure 5.4).

5.5.2 Sequence polymorphism among amplicons

Sequence comparision of the CPFC1 fragment amplified from the 'Yellow' and 'Red' bulks uncovered base substitutions at nucleotides 182 and 193 (Figure 5.5). A similar result was found comparing the CPFC3 sequence among the bulks. Base substitutions were observed at nucleotides 124, 131, 154, 219 and 256. Additionally, a single base insertion was present at 214 bp in the yellow bulk DNA (Figure 5.6).

5.5.3 Sequence alignment of amplified markers

The BLAST results showed a 98-100% identity for each of the three loci-generated sequences among the bulks to two *C. papaya* BAC clones, DM105M02 (accession number GQ478572) and SH18O09 (accession number GQ478573) (Table 5.4). The yellow flesh related sequences were most similar to DM105M02, and the red flesh associated sequences were most similar to SH18O09. All of the sequences queried covered only 0% of the target sequences, but of these, 98-100% were similar to the database sequence fragments.

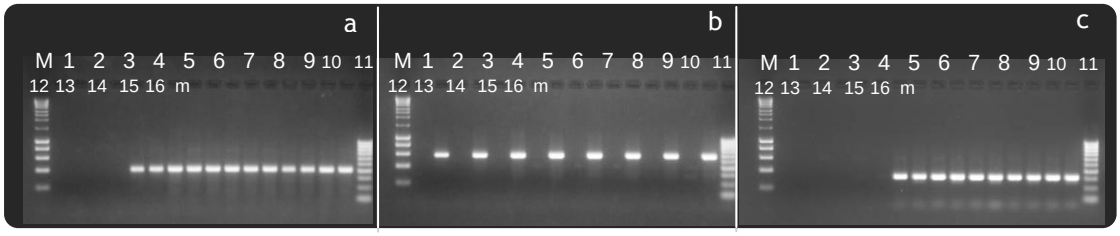

Figure 5.3: Optimisation of PCR using published DNA markers linked to flesh colour (a-CPFC1; b-CPFC2; and c-CPFC3) in bulk DNAs of 'Red' (lanes which were indicated in odd number) and 'Yellow' (lane which were indicated in even number). The reactions were performed at eight different annealing temperatures

M and m are DNA molecular weight markers, HyperLadder I and IV (Bioline), respectively. Lane 1, 2 at 65ºC; lane 3,4 at 64ºC; lane 5,6 at 62ºC; lane 7,8 at 59ºC; lane 9, 10 at 55ºC; lane 11, 12 at 53ºC; lane 13, 14 at 51ºC, lane 15, 16 at 50ºC

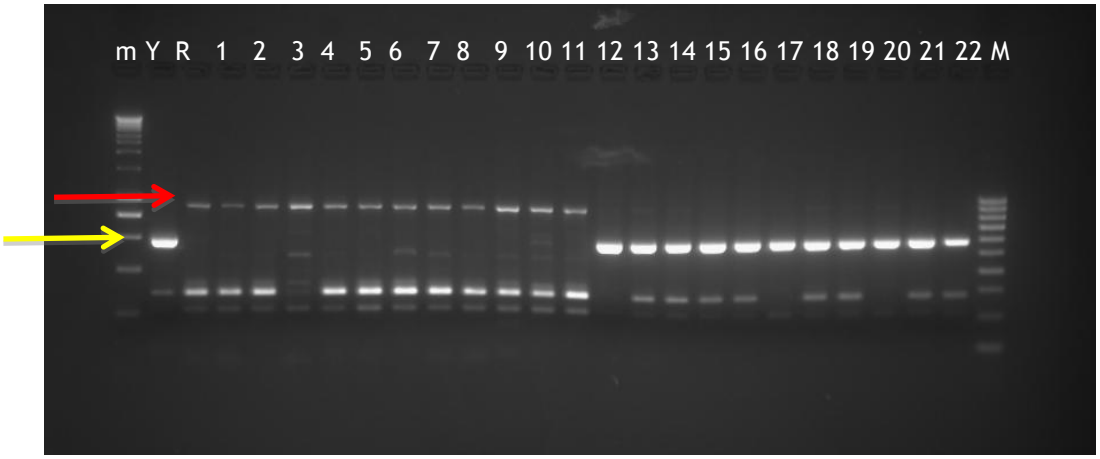

Figure 5.4: DNA markers associated with yellow (yellow arrow) and red (red arrow) flesh colour in individuals of each bulked DNA. Lane Y and R are bulked DNA of yellow and red fleshed fruit, respectively. Lane 1-11 are individual DNAs of red flesh colour used in 'Red Bulk'. Lane 12-22 are individual DNAs of yellow flesh colour used in 'Yellow Bulk'. Lane m amd M are DNA molecular weight marker HyperLadder I and IV (Bioline), respectively

```
CPFC1-Yellow      GTCCGACAAAAAAATTTAAATTTTACAAATTGATTAAGTGTCTAATTAAATATTTTATTA    60
CPFC1-Red         GTCCGACAAAAAAATTTAAATTTTACAAATTGATTAAGTGTCTAATTAAATATTTTATTA    60
                  ************************************************************

CPFC1-Yellow      AATTAAAAAATTTATTAAACGTTTGATTAGATATTTCGTTTCGTTCATTAAAACTACATA    120
CPFC1-Red         AATTAAAAAATTTATTAAACGTTTGATTAGATATTTCGTTTCGTTCATTAAAACTACATA    120
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                  ************************************************************

CPFC1-Yellow      ATTAAAAATGACGATATCACAATTACGCCAACTAAAAATTTTATTGAAATTATGTTGTAA    180
CPFC1-Red         ATTAAAAATGACGATATCACAATTACGCCAACTAAAAATTTTATTGAAATTATGTTGTAA    180
                  ************************************************************

CPFC1-Yellow      ACAACTTATTGAGCATCTAACTAATTAAAATTTCATAATTAAAAATTCAAACAATTATTT    240
CPFC1-Red         ATAACTTATTGAACATCTAACTAATTAAAATTTCATAATTAAAAATTCAAACAATTATTT    240
                  * ********** ********************************************

CPFC1-Yellow      GAGTCACTGGAGAAATAAAATAAAATATGTCTTATATTAAGTGTTAAAATAAAAAATTTA    300
CPFC1-Red         GAGTCACTGGAGAAATAAAATAAAATATGTCTTATATTAAGTGTTAAAATAAAAAATTTA    300
                  ************************************************************

CPFC1-Yellow      ATAAAAATATAATTAATTATATAACCTGTACTAGATTCGAACTGGAAATGATGTTATATT    360
CPFC1-Red         ATAAAAATATAATTAATTATATAACCTGTACTAGATTCGAACTGGAAATGATGTTATATT    360
                  ************************************************************

CPFC1-Yellow      ATGTCATAAAAATTTAAAGTTTAAATTATTACAAAATTT   399
CPFC1-Red         ATGTCATAAAAATTTAAAGTTTAAATTATTACAAAATTT   399
                  ***************************************
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Figure 5.5: Alignment of DNA sequences of marker CPCF1 in bulked DNA of yellow (CPFC1-Yellow) and red (CPFC1-Red)

```
CPFC3-Yellow      TTGCAAAGAAAGGAGGGTTTCTATAACATCATCAGGGTCTTTGTTTGTGACACATAATGA    60
CPFC3-Red         TTGCAAAGAAAGGAGGGTTTCTATAACATCATCAGGGTCTTTGTTTGTGACACATAATGA    60
                  ************************************************************

CPFC3-Yellow      ATTGTATCATCTAGTTGACTGACAGAAGCAATTCCCATGGACCATGCAGGTTATGATGTT    120
CPFC3-Red         ATTGTATCATCTAGTTGACTGACAGAAGCAATTCCCATGGACCATGCAGGTTATGATGTT    120
                  ************************************************************

CPFC3-Yellow      TTCCATGTTTGTTTCTGCAAAGAACATTGTTATGCATTGACTGGCATTTACTTGACTACA    180
CPFC3-Red         TTCTATGTTTATTTCTGCAAAGAACATTGTTATACATTGACTGGCATTTACTTGACTACA    180
                  *** ****** ********************** ************************

CPFC3-Yellow      TGTTATACTTAACTGTCCTGGTGATCTTTTAGGTATTAGGGCATTTGATGCAAGATTAAT    240
CPFC3-Red         TGTTATACTTAACTGTCCTGGTGATCTTTTAGG-ATTACGGCATTTGATGCAAGATTAAT    239
                  ******************************** **** ********************

CPFC3-Yellow      CATCTTTCCTCTTCAGGATATTTCTTTTCTCATCAACCCAACAAGAAGTTAATTTGTTTG    300
CPFC3-Red         CATCTTTCCTCTTCAAGATATTTCTTTTCTCATCAACCCAACAAGAAGTTAATTTGTTTG    299
                  *************** ******************************************

CPFC3-Yellow      GCTCAGAAGGATTTCA    316
CPFC3-Red         GCTCAGAAGGATTTCA    315
```

Figure 5.6: Alignment of DNA sequences of marker CPCF3 in bulked DNA of yellow (CPFC3-Yellow) and red (CPFC3-Red)

Table 5.4: BLAST result of nucleotide sequences for CPFC1, CPFC2 and CPFC3 markers in bulked DNA of yellow and red fleshed papaya on NCBI database

Marker	*C. papaya* clone BAC DM105M02, complete sequence Accession #GQ478572.2				*C. papaya* clone BAC SH18O09, complete sequence, Accession #GQ478573.2			
	Max score	Total score	E value	Ident	Max score	Total score	E value	Ident
CPFC1-Yellow	739	739	0.0	100%	728	728	0.0	99%
CPFC1-Red	726	726	0.0	99%	737	737	0.0	100%
CPFC2-Yellow	1046	1046	0.0	100%	520	520	7e-150	99%
CPFC3-Yellow	425	478	2e-121	99%	545	545	1e-157	98%
CPFC3-Red	409	462	2e-116	98%	573	573	5e-166	99%

The alignment of BAC clones DM105M02, SH18O09 and all the sequences from the CPFC primer set as listed on Table 5.2 were aligned to each other by using DM105M02 as the template. The query of SH18O09 covered 89% of DM105M02. The BAC clones showed 99% identity with an E value of 0.00. Each CPFC fragment aligned to each BAC clones at a different place. CPCF2-Yellow aligned 100% to DM105M02, but it was located at the insertion/deletion between the sequence alignments of the two BAC clones.

5.5.4 Amplification using novel markers from SC28

All primers as listed in Table 5.3 were successfully amplified in bulked DNA of both red and yellow-fleshed papaya. Base substitutions were found in SSR-28.50 but the other two SSR markers did not contain any sequence polymorphisms between the two bulks at those loci (Figure 5.7, 5.8 and 5.9).

The set of primers for 28.12, which targeted gene LRR28.12, successfully amplified a fragment in both bulk DNAs that were 100% identical (Figure 5.10, 5.11 and 5.12). Due to the overlapping of each primer pair (Figure 5.2), the nucleotide sequences of 28.12.1, 28.12.2 and 28.12.3 were aligned and of the contigs 28.12-Red for the 'Red' bulk DNA and 28.12-Yellow for the 'Yellow' bulk DNA were assembled and confirmed to be 100% identical. Interestingly, a 137 bp insertion, from position 1257 to 1394 bp was detected in this sequence when compared to the sequence of LRR28.12 sourced from the papaya genome database deduced amino acids remained the same due to the insertion of intron 1 was detected (Figure 5.13).

```
28.50-YELLOW      ------CTCTTTTTTCGGTCATGCACCATACTCACTTCCTCCTCCTCCTCCTCCTTCTTC    54
28.50-RED         ACACAACTCTTTTTTCGGTCATGCACCATACTCACTTCCTCCTCCTCCTCCTCCTTCTTC    60
                        **************************************************
```

```
28.50-YELLOW      TTTTTCTTCTTCTTCTCCTTCTCCTTCTTTTTCTTCTTCTTCTTCTCCTTCTCCTTTTTT    114
28.50-RED         TTTTTCTTCTTCTTCTCCTTCTCCTTCTTTTTCTTCTTCTTCTTCTCCTTCTCCTTTTTT    120
                  ************************************************************

28.50-YELLOW      TCTTCTTCTTCTTCTCCTTTTTTTTCTTCTTCTTCTTTTCCTTCTTTTCATCTTCATCTT    174
28.50-RED         TCTTCTTCTTCTTCTCCTTTTTTTTCTTCTTCTTCTTTTCCTTCTTTTCATCTTCATCTT    180
                  ************************************************************

28.50-YELLOW      CTTCTTCTCCTTCTTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTTTTCTTCTCTTCT    234
28.50-RED         CTTCTTCTCCTTCTTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTTTTCTTTCTTCT    240
                  ************************************************** ******

28.50-YELLOW      TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT------------------          273
28.50-RED         TCTTCTTCTTCTTCTTCTTTTTCTTCTTCTTCTTCTTCTTGGCGGCAGCGCGTGCCG      298
                  ****************** ******************
```

Figure 5.7: Alignment of DNA sequences of marker 28.50 in bulked DNA of yellow (28.50-Yellow) and red (28.50-Red) papayas. Blue colour fonts show the target microsatellite sequences

```
28.103-YELLOW     GATCGGGATTGAGAGGACATCTTTGAATGGTCTGGATCCCTTCTCACAATGGACGACCAC    60
28.103-Red        --TCGGGATTGAGAGGACATCTTTGAATGGTCTGGATCCCTTCTCACAATGGACGACCAC    58
                    ************************************************************

28.103-YELLOW     GATTTAATTTCACTTATATATAAAAAATTATGCTTTTACAAAAAAGAGAGGACAAGAAAT    120
28.103-Red        GATTTAATTTCACTTATATATAAAAAATTATGCTTTTACAAAAAAGAGAGGACAAGAAAT    118
                  ************************************************************

28.103-YELLOW     CCTAAGAGAGAGAGAGAGAGAGA   143
28.103-Red        CCTAAGAGAGAGAGAGAGAG-     140
                  **********************
```

Figure 5.8: Alignment of DNA sequences of marker 28.103 in bulked DNA of yellow (28.103-Yellow) and red (28.103-Red) papayas. Blue colour fonts show the target microsatellite sequences

```
28.106-Yellow     TTCAATGCAGTGTACGACAGAATTACCCACAAGACAAGATGGACAAATCTTTCATTGTTG
```

```
28.106-Red          TTCAATGCAGTGTACGACAGAATTACCCACAAGACAAGATGGACAAATCTTTCATTGTTG
                    ************************************************************

28.106-Yellow       CTGACTTGACAATAGTGTATGTATAAGCAGAAAAACATGGTGAATGATCATTTTCTTTTC
28.106-Red          CTGACTTGACAATAGTGTATGTATAAGCAGAAAAACATGGTGAATGATCATTTTCTTTTC
                    ************************************************************

28.106-Yellow       TTTTCTTTTCTTTTCTTCTTATTTTCGTTTTTCAGAGCTGGACCCTGAAGGTATTAAAGT
28.106-Red          TTTTCTTTTCTTTTCTTCTTATTTTCGTTTTTCAGAGCTGGACCCTGAAGGTATTAAAGT
                    ************************************************************

28.106-Yellow       ATTGGTTTTCACCTGTACTAAGATTAGAAGTAGATCATCCATGCTGAAACCAATACCA
28.106-Red          ATTGGTTTTCACCTGTACTAAGATTAGAAGTAGATCATCCATGCTGAAACCAATACCA
                    ************************************************************
```

Figure 5.9: Alignment of DNA sequences of marker SSR28.106 in bulked DNA of yellow (28.106-Yellow) and red (28.106-Red) papayas. Blue colour fonts show the target microsatellite sequences

```
28.12.1-Red         ----------CCTCCTCCTCTTTTCTCTCTTCAAACCATCTTCTTCACTATCCGATACA   49
28.12.1-Yellow      TTCCTCCTCTTCCTCCTCCTCTTTTCTCTCTTCAAACCATCTTCTTCACTATCCGATACA   60
                              *********************************************

28.12.1-Red         GAGGCTCTCCTCAAGTTCAAACAGTCTCTGAAAGTACCAGCAGGTGTCTTGGATTCTTGG   109
28.12.1-Yellow      GAGGCTCTCCTCAAGTTCAAACAGTCTCTGAAAGTACCAGCAGGTGTCTTGGATTCTTGG   120
                    ************************************************************

28.12.1-Red         GCTCCAGGCTCCTCTCCTTGTAAAGACCGTTGGGTTGGCATTTATTGTACCCAATCCACC   169
28.12.1-Yellow      GCTCCAGGCTCCTCTCCTTGTAAAGACCGTTGGGTTGGCATTTATTGTACCCAATCCACC   180
                    ************************************************************

28.12.1-Red         ATTTTTGGCATCCATCTCAACGACTCGGGTATCTCTGGAACTATCGATGTCCAAGCCTTG   229
28.12.1-Yellow      ATTTTTGGCATCCATCTCAACGACTCGGGTATCTCTGGAACTATCGATGTCCAAGCCTTG   240
                    ************************************************************

28.12.1-Red         GCTGCTCTTCCTGATCTTAAAACCGTCCGTCTCGATAATAATTCTTTCGCTGGCCCAATC   289
28.12.1-Yellow      GCTGCTCTTCCTGATCTTAAAACCGTCCGTCTCGATAATAATTCTTTCGCTGGCCCAATC   300
                    ************************************************************

28.12.1-Red         CCTCCTTTCAACCAACTTGTCGGCCTCAGGGGTCTTTTCTTGGCTGCTAATCAACTCTCT   349
28.12.1-Yellow      CCTCCTTTCAACCAACTTGTCGGCCTCAGGGGTCTTTTCTTGGCTGCTAATCAACTCTCT   360
                    ************************************************************

28.12.1-Red         GGCGAAATACCAAATGA 366
28.12.1-Yellow      GGCGAAATACCAAATGA 377
                    *****************
```

Figure 5.10: Alignment of DNA sequences of marker 28.12.1 in bulked DNA of red (28.12.1-Red) and yellow fleshed (28.12.1-Yellow) papayas

```
28.12.2-Red         ----------------------------------------------------------AC   2
28.12.2-Yellow      CAATCCCTCCTTTCAACCAACTTGTCGGCCTCAGGGGTCTTTTCTTGGCTGCTAATCAAC   60
                                                                             **

28.12.2-Red         TCTCTGGCGAAATACCAAATGATTACTTCGCCTCCATGACCAATCTTAGGAGATTTAATA   62
28.12.2-Yellow      TCTCTGGCGAAATACCAAATGATTACTTCGCCTCCATGACCAATCTTAGGAGATTTAATA   120
                    ************************************************************

28.12.2-Red         TTGCTAACAACCAAATGACCGGCAAGATTCCCGACTCCCTCGTGCAGCTACCTTACCTCA   122
28.12.2-Yellow      TTGCTAACAACCAAATGACCGGCAAGATTCCCGACTCCCTCGTGCAGCTACCTTACCTCA   180
                    ************************************************************

28.12.2-Red         AAGAGCTTCACCTTGAAGGCAACCACTTCTCGGGACCAATCCCGCCATTACGACAAGGGC   182
28.12.2-Yellow      AAGAGCTTCACCTTGAAGGCAACCACTTCTCGGGACCAATCCCGCCATTACGACAAGGGC   240
                    ************************************************************

28.12.2-Red         TCACGCTAACGGATCTGAACATGTCAAACAACAACCTGGAAGGAGAAATTCCCTCCACTT   242
28.12.2-Yellow      TCACGCTAACGGATCTGAACATGTCAAACAACAACCTGGAAGGAGAAATTCCCTCCACTT   300
                    ************************************************************

28.12.2-Red         ACGCCAATTTCGATTCCAAACCTTTCCAGGGCAACCATCAACTTTGCGGAAAGCAACTCA   302
28.12.2-Yellow      ACGCCAATTTCGATTCCAAACCTTTCCAGGGCAACCATCAACTTTGCGGAAAGCAACTCA   360
                    ************************************************************

28.12.2-Red         ATGGTCATTGCAACCAAGCGCCACAATCATCTGCACCTTCGGGTTCTCACTTTAAGGCGA   362
28.12.2-Yellow      ATGGTCATTGCAACCAAGCGCCACAATCATCTGCACCTTCGGGTTCTCACTTTAAGGCGA   420
                    ************************************************************

28.12.2-Red         CTGTCTTTGTCACTGGTATGGTGGTTTTAGTAATCTTTCTTCTCATGGTTGCAATGATAG   422
28.12.2-Yellow      CTGTCTTTGTCACTGGTATGGTGGTTTTAGTAATCTTTCTTCTCATGGTTGCAATGATAG   480
                    ************************************************************

28.12.2-Red         CAGCCAGGCGGCGGAGGGATGCTGAATTCAGCGTTCTTGAGAAGGAACACCTTAGCGACA   482
28.12.2-Yellow      CAGCCAGGCGGCGGAGGGATGCTGAATTCAGCGTTCTTGAGAAGGAACACCTTAGCGACA   540
                    ************************************************************

28.12.2-Red         ATGAAGCCGGGGAATCCCACGTGCCCGATTCGATCAGGAGGCCTGTGGAGTCGACCCGTA   542
28.12.2-Yellow      ATGAAGCCGGGGAATCCCACGTGCCCGATTCGATCAGGAGGCCTGTGGAGTCGACCCGTA   600
                    ************************************************************

28.12.2-Red         AGGGCAGCGGAGAGTCCAACAGGAGAGGGACACACAATCCCAAGAACGGGATGGGTGACT   602
28.12.2-Yellow      AGGGCAGCGGAGAGTCCAACAGGAGAGGGACACACAATCCCAAGAACGGGATGGGTGACT   660
                    ************************************************************

28.12.2-Red         TAGTGATGGTGAATGAGGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAG   662
28.12.2-Yellow      TAGTGATGGTGAATGAGGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAG   720
                    ************************************************************

28.12.2-Red         CAGAGGTTTTAGGAAACGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTA   722
28.12.2-Yellow      CAGAGGTTTTAGGAAACGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTA   780
                    ************************************************************

28.12.2-Red         TGTGCGTGGTGGTGAAGAGG------      742
28.12.2-Yellow      TGTGCGTGGTGGTGAAGAGGATGCGG     806
                    ********************
```

Figure 5.11: Alignment of DNA sequences of marker 28.12.2 in bulked DNA of red (28.12.2-Red) and yellow fleshed (28.12.2-Yellow) papayas

```
28.12.3-Red         -------------------------AAGAACGGGATGGGTGACTTAGTGATGGTGAATGA   35
28.12.3-Yellow      CAACAGGAGAGGGACACACAATCCCAAGAACGGGATGGGTGACTTAGTGATGGTGAATGA   60
                                             **********************************

28.12.3-Red         GGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAGCAGAGGTTTTAGGAAA   95
28.12.3-Yellow      GGAAAAGGGTGTTTTTGGTTTGCCAGATTTGATGAAGGCTGCAGCAGAGGTTTTAGGAAA   120
                    ************************************************************

28.12.3-Red         CGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTATGTGCGTGGTGGTGAA   155
28.12.3-Yellow      CGGTAGCTTGGGGTCTGCTTACAAGGCCCTAATGAATAATGGTATGTGCGTGGTGGTGAA   180
                    ************************************************************

28.12.3-Red         GAGGATGCGGGAGATGAATAAACTGGGGAGAGATGGATTTGATGCAGAAATGAGAAGGTT   215
28.12.3-Yellow      GAGGATGCGGGAGATGAATAAACTGGGGAGAGATGGATTTGATGCAGAAATGAGAAGGTT   240
                    ************************************************************

28.12.3-Red         TGGAAGGCTCTCTCACCCTAATATTTTGACCCCACTGGCCTATCATTACCGACGAGAAGA   275
28.12.3-Yellow      TGGAAGGCTCTCTCACCCTAATATTTTGACCCCACTGGCCTATCATTACCGACGAGAAGA   300
                    ************************************************************

28.12.3-Red         GAAATTATTGGTGTCGAATTACATGCCTAAAAGCAGCTTGTTGTATGTCTTGCATGGTAG   335
28.12.3-Yellow      GAAATTATTGGTGTCGAATTACATGCCTAAAAGCAGCTTGTTGTATGTCTTGCATGGTAG   360
                    ************************************************************

28.12.3-Red         GAAACACTCTCTTCATTTTTCCATTATATACAAATATTTTTCATTATATAGGATGTCTTT   395
28.12.3-Yellow      GAAACACTCTCTTCATTTTTCCATTATATACAAATATTTTTCATTATATAGGATGTCTTT   420
                    ************************************************************

28.12.3-Red         AAACTGTCTGAATCGGTGAGCGTTTCTCTCATCAAGACAATACGGAATTTAGATGGTTTG   455
28.12.3-Yellow      AAACTGTCTGAATCGGTGAGCGTTTCTCTCATCAAGACAATACGGAATTTAGATGGTTTG   480
                    ************************************************************

28.12.3-Red         TTTATGATCTTCAGGTGATCGTGGCATTTTCCATGCCGAGCTGAATTGGGCAACCCGACT   515
28.12.3-Yellow      TTTATGATCTTCAGGTGATCGTGGCATTTTCCATGCCGAGCTGAATTGGGCAACCCGACT   540
                    ************************************************************

28.12.3-Red         GAGGATAATCCAAGGAGTAGCACACGGAATGGATTTCCTACACAGAGAGTTTGCATCCTA   575
28.12.3-Yellow      GAGGATAATCCAAGGAGTAGCACACGGAATGGATTTCCTACACAGAGAGTTTGCATCCTA   600
                    ************************************************************

28.12.3-Red         TGATTTACCACACGGAAATCTCAAGTCCAGCAATGTTCTTCTAACTGAAAATTATGACCC   635
28.12.3-Yellow      TGATTTACCACACGGAAATCTCAAGTCCAGCAATGTTCTTCTAACTGAAAATTATGACCC   660
                    ************************************************************

28.12.3-Red         AGTACTAAGTGACTATGCCTTTCTTCCTCTGGTTCATCCCAA      677
28.12.3-Yellow      AGTACTAAGTGACTATGCCTTTCTTCCTCT-----------      690
                    ****************************
```

Figure 5.12: Alignment of DNA sequences of marker 28.12.3 in bulked DNA of red (28.12.3-Red) and yellow fleshed (28.12.3-Yellow) papayas

```
LRR28.12          16 LLLFSLFKPSSSLSDTEALLKFKQSLKVPAGVLDSWAPGSSPCKDRWVG
                     LLLFSLFKPSSSLSDTEALLKFKQSLKVPAGVLDSWAPGSSPCKDRWVG
                     LLLFSLFKPSSSLSDTEALLKFKQSLKVPAGVLDSWAPGSSPCKDRWVG
28.12-Red          2 cccttctactttctgaggccatactcagcgggtgttgcgttctagctgg
                     ttttcttacccctcacacttataactatccgttacgccgcccgaaggtg
                     cccttccaattaactagtccgcagtgaaaatcgttgtacctttactgtc

LRR28.12          65 IYCTQSTIFGIHLNDSGISGTIDVQALAALPDLKTVRLDNNSFAGPIPP
                     IYCTQSTIFGIHLNDSGISGTIDVQALAALPDLKTVRLDNNSFAGPIPP
                     IYCTQSTIFGIHLNDSGISGTIDVQALAALPDLKTVRLDNNSFAGPIPP
28.12-Red        149 attactaatgaccagtgatgaaggcgtggccgcaagccgaattggcacc
                     tagcaccttgtataacgtcgctatactcctcatactgtaaactcgctcc
                     tttcaccttcctcccgtctatctcacgtttttacctcttttctcactt

LRR28.12          114 FNQLVGLRGLFLAANQLSGEIPNDYFASMTNLRRFNIANNQMTGKIPDS
                      FNQLVGLRGLFLAANQLSGEIPNDYFASMTNLRRFNIANNQMTGKIPDS
                      FNQLVGLRGLFLAANQLSGEIPNDYFASMTNLRRFNIANNQMTGKIPDS
28.12-Red         296 taccggcagcttggacctggacagttgtaaacaataagaacaagaacgt
                      taattgtggtttccaatcgatcaaatcctcatggtatcaaatcgatcac
                      ccatcccgttcgtttactcaaattccccgcttgattttccagccgtccc

LRR28.12          163 LVQLPYLKELHLEGNHFSGPIPPLRQGLTLTDLNMSNNNLEGEIPSTYA
                      LVQLPYLKELHLEGNHFSGPIPPLRQGLTLTDLNMSNNNLEGEIPSTYA
                      LVQLPYLKELHLEGNHFSGPIPPLRQGLTLTDLNMSNNNLEGEIPSTYA
28.12-Red         443 cgccctcagcccggacttgcacctccgcacagcaataaacgggactatg
                      ttatcataatatagaatcgctcctgagtctcatatcaaatagatcccac
                      cggatccagtctaccccgaacgaaaagcgagtgcgacccgaaatcctcc

LRR28.12          212 NFDSKPFQGNHQLCGKQLNGHCNQAPQSSAPSGSHFKATVFVTGMVVLV
                      NFDSKPFQGNHQLCGKQLNGHCNQAPQSSAPSGSHFKATVFVTGMVVLV
                      NFDSKPFQGNHQLCGKQLNGHCNQAPQSSAPSGSHFKATVFVTGMVVLV
28.12-Red         590 atgtactcgaccctgaccagctacgccttgctgtctagagtgagaggtg
                      atacactagaaatggaatagagaaccacccccgcatacctttcgttttt
                      tctcatcgcctatcagactttccagaaatatgttctggtctcttggtaa

LRR28.12          261 IFLLMVAMIAARRRRDAEFSVLEKEHLSDNEAGESHVPDSIRRPVESTR
                      IFLLMVAMIAARRRRDAEFSVLEKEHLSDNEAGESHVPDSIRRPVESTR
                      IFLLMVAMIAARRRRDAEFSVLEKEHLSDNEAGESHVPDSIRRPVESTR
28.12-Red         737 atccaggaaggaccagggtagcgagccagaggggtcgcgtaaacggtac
                      ttttttcttccggggacatgttaaaatgaaacgacatcactggctaccg
                      cttcgtagaacggggttaccttggactcctacgaccgctgcggtgggct

LRR28.12          310 KGSGESNRRGTHNPKNGMGDLVMVNEEKGVFGLPDLMKAAAEVLGNGSL
                      KGSGESNRRGTHNPKNGMGDLVMVNEEKGVFGLPDLMKAAAEVLGNGSL
                      KGSGESNRRGTHNPKNGMGDLVMVNEEKGVFGLPDLMKAAAEVLGNGSL
28.12-Red         884 agaggtaaagacacaagaggtgagaggaggtgtcgtaaggggggtgagat
                      agggacagggcaacaagtgattttaaaagttgtcattacccattgaggt
                      gccagccgagactcgcggtcagggtgagttttgatgggtaagtaactcg

LRR28.12          359 GSAYKALMNNGMCVVVKRMREMNKLGRDGFDAEMRRFGRLSHPNILTPL
                      GSAYKALMNNGMCVVVKRMREMNKLGRDGFDAEMRRFGRLSHPNILTPL
                      GSAYKALMNNGMCVVVKRMREMNKLGRDGFDAEMRRFGRLSHPNILTPL
28.12-Red        1031 gtgtagcaaagatgggaaacgaaacgaggtgggaaatgactccaataccc
                      gccaacttaagtgtttagtgataatggagtacatggtggtcacattcct
                      gttcgcagtttgcgggggggggtaggatattaagagtagctctttgcag

LRR28.12          408 AYHYRREEKLLVSNYMPKSSLLYVLH
                      AYHYRREEKLLVSNYMPKSSLLYVLH
                      AYHYRREEKLLVSNYMPKSSLLYVLH
28.12-Red        1178 gtctccggattgtatacaaatttgtc
                      caaaggaaatttcaatcaggttatta
                      cttcaaagaagggtcgtaccggtcgt
```

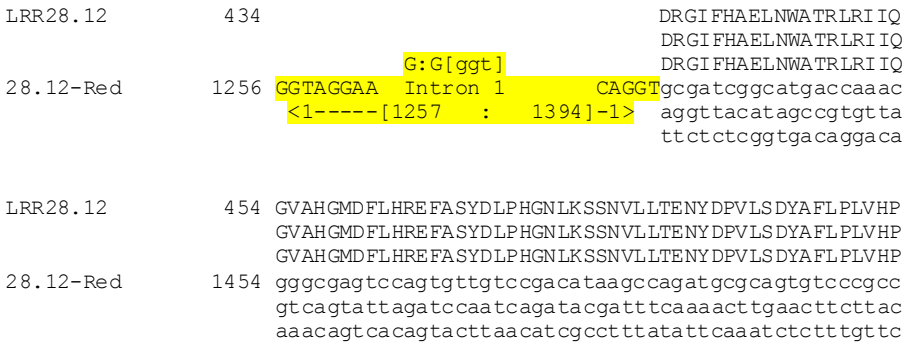

Figure 5.13: The result of Genewise comparison between the sequence of deduced amino acid of LRR28.12 and assembled DNA sequences of marker 28.12 of bulked DNA of red flesh (28.12-Red) papaya

5.6 Segregation of CPFC2 in population

Only marker CPFC2 was co-dominant and size polymorphic among the bulks, therefore, only this locus marker was investigated further in the current population of 28 lines. Figure 5.4 shows the newly developed markers at 900 and 600 bp, which were indicated by red and yellow arrows, respectively. The 900 bp marker was potentially linked to red flesh colour and was designated as CPCF2-R. The marker at 600 bp was a candidate to link to yellow flesh colour and was designated as CPCF2-Y. When screened for consistent association among phenotype (colour) and genotype (marker allele), there was a mismatch rate for CPCF2-R and CPCF2-Y of 2 and 6, repectively. This equated to a 97% and 93% association to red and yellow flesh, respectively.

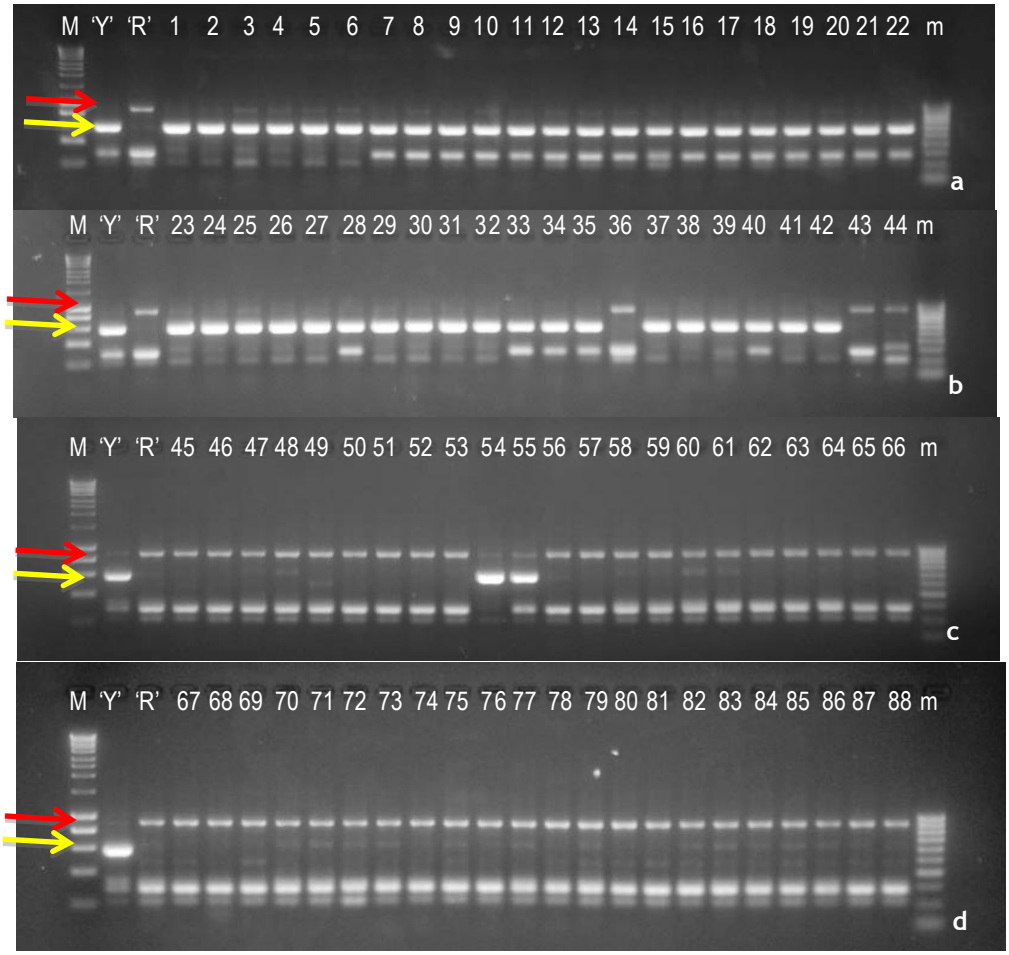

Figure 5.14: Segregation of CPCF2-R (→) and CPCF2-Y (→) in current breeding lines. Lane 'Y' and 'R' are bulked DNA of yellow and red fleshed papayas, respectively. Lane 1-44 represent individual DNAs of yellow fleshed papaya. Lane 45-88 are individual DNAs of red fleshed papayas. Lane m amd M are DNA molecular weight marker HyperLadder I and IV (Bioline), respectively. Lane 36, 43 and 44 were mismatches of the presence of marker CPCF2-R in yellow fleshed papayas. Lane 54 and 55 were mismatches of the presence of marker CPCF2-Y in red flesh papayas

DISCUSSION:

The current study demonstrated transferability of three SCAR markers developed by Blas et al. (2010) for papaya fruit flesh colour to wide range of papaya genotypes. CPFC1 and CPFC3 were reported to be co-dominant markers that can differentiate between yellow and red flesh colour. Although SCAR marker CPFC1 was reported to have approximately 98% identity to fruit flesh colour genes (Blas et al. 2010), this marker was non-polymorphic between the two colour bulk DNAs used in this study. Conversely, the CPFC2 was polymorphic and quite accurately discriminated between the yellow (93%) and red (97%) fruit bearing plants with. A small number of plants were misclassified with the CPFC2 marker most likely due to the chance of recombination and hence loss of linkage between the actual gene(s) conditioning the colour trait and the marker developed. CPFC1, which was reported to be tightly linked marker, located 580 bp away from the target gene, was still not 100% accurate due to the extremely high recombination rate (Blas et al. 2010). Data of F_2 mapping populations indicated the local recombination rate in the *CpCYC-b* region were more than 100-fold and 82-fold higher than the genome average when using KD × 2H94 (n = 219) and AU9 × SunUp (n = 54), respectively. High-density genetic mapping placed the flesh colour locus near the end of LG5 (Ma et al. 2004; Chen et al. 2007; Blas et al. 2009) indicating its position near the telomere, which is commonly associated with plant recombination hot spot (Mézard 2006).

Also, it is likely that colour is conditioned by multiple quantitative genes with varying functional influence. Therefore, it is possible that in some individuals, the additional genic component is not captured leading to a mismatch between trait and prediction based on the CPFC2 marker itself. Additionally, the colour trait is likely influenced greatly by environmental factors. There are several reports showing intensity of flesh colour of papaya varied in different temperature, which may relate to ripening process (Akamine 1966; Broughton et al. 1977; Nazeeb and Broughton 1978). This could be due to the expression of fruit flesh colour, which is affected by environment (result from Chapter 4). However, this hypothesis needs to be confirmed in a different segregating population when it becomes available.

Currently, there is no report of QTL study on flesh colour in papaya, however, there are reports of QTL to flesh colour in other fruit crops. The study of flesh colour in sweet cherry indicated the trait was controlled by one major gene with another two minor QTLs. The major QTL explained the attribute for 84.7% (Sooriyapathirana et al. 2010). Kinkade and Foolad (2013) reported two major QTLs, lyc7.1 and lyc12.1, involved in flesh colour in tomato. The lyc12.1 increased the lycopene content of ripe tomato fruits by 52-70%. Flesh colour in tomato is regulated by carotenoid biosynthesis pathway, which is the same pathway as flesh colour in papaya (Skelton et al. 2006).

Both the newly developed markers (SSR and EST) were located at the other end of LG5 from *CpCYC-b* locus. This could be a reason for the primers from SC28 to unable to detect variation of DNA between the two flesh colours in these regions, as they are located too far away from each other.

CONCLUSION:

To improve eating quality of papaya is the main goal of thepapaya breeding program. Fruit flesh colour is one of the important traits for breeders, consumers and the Australian market. Marker

assisted selection can speed up a breeding program by enabling selection for traits at the seedling stage. This research developed DNA markers linked to red flesh, CPFC2-R, and yellow flesh, CPFC2-Y. These markers identifed the trait at 95.75% in 330 breeding lines, where CPFC2-R and CPFC2-Y showed 96.64% and 93.48% homology to red and yellow fleshed plants, respectively. These markers will be validated in future research in a segregating population to confirm the linkage between the trait and the markers.