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Final report

project

Development of PRSV-P resistant papaya genotypes by introgression of genes from wild *Carica* species

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1 Acknowledgments

We would like to thank BPI-Guimaras and BPI- Davao for being part in the extensive backcrossing program way back 2002.

We also like to thank Australian Centre for International Agricultural Research (ACIAR) for supporting the project which aims to revitalize the Philippine papaya industry which was devastated by a serious virus called as Papaya Ringspot Virus.

2 Executive summary

The project on the introgression of resistant gene(s) against PRSV-P from *Vasconcellea quercifolia* to *Carica* species in the Philippines started in 2002. Initially, the project aimed to use a male backross plant Clone 54 (*C. papaya* x *V. quercifolia*) from Australia in the backcrossing activity in transferring the PRSV-P resistance to some of the elite Philippine inbred lines. This plant exhibited fertility and had displayed considerable resistance in Australia but unfortunately, this clone succumbed to the Philippine strain of PRSV-P two months after field transplanting. This development opt the team to produce an alternative to Clone 54 that is more resistant and that can be used in the backcrossing program.

To breed papaya genotypes that are resistant to papaya ringspot virus form- P (PRSV-P), five inbred lines (4108, 4172, 5648, 5893, and 3878) developed by the Institute of Plant Breeding and five F_1 intergeneric hybrids (404, 410, 468, 469 and 507) from Australia were planted and maintained in the field last October 18, 2002. The five F_1 intergeneric hybrid lines of *C. papaya x V. quercifolia* were used as paternal parents to incorporate PRSV-P resistant gene(s) to five elite papaya inbred lines of IPB.

Although intergeneric hybrid line 410 was predominantly used in the production of BC_1, there were other four intergeneric hybrid lines (404, 468, 469, and 507) evaluated. After more than two years in the field, two hybrid lines (404 and 507) developed typical PRSV-P symptoms on the leaves. ELISA test results confirmed that intergeneric hybrid lines 404 and 507 broke down its resistance to Philippine strain of PRSV-P while lines 410, 468 and 469 remained virus- and symptom free. Two additional intergeneric hybrid lines 468 and 469 were utilized to produce more BC_1 crosses using the same recurrent parents. Slow production of BC_1 with these intergeneric hybrid lines were experienced because they are not male-stable lines. Most of the time they bear hermaphrodite and female flowers. Line 469 dominantly produces female flowers. Intergeneric hybrid line 410 is a male-stable line.

A total of fifteen (15) crosses were produced in Laguna, Davao, and Guimaras. A total of 940 fruits were harvested and 114,839 seeds were dissected. Only 1,011 embryos were rescued and only 733 were germinated and developed into plantlets. About 700 BC_1 plants were generated and screened under screenhouse condition. After three manual inoculations, plants that showed putative resistance were transplanted along with the susceptible check, 'Davao Solo' in the field.

The extensive backcrossing of the F_1 intergeneric hybrids and inbred lines has resulted in one PRSV-P resistant backcross plant (BC_1).This is a cross between inbred line 5648 and intergeneric hybrid 410. Resistance to PRSV-P was confirmed after one year in field of high disease pressure. ELISA test verified the absence of masked symptoms of the virus. This male fertile BC_1 was used for further backcrossing to generate BC_2, BC_3 and BC_4 plants.

A total of 1465 plants (256 BC_4, 147 BC_3, 379 BC_3 sib-crosses, 137 BC_2 and 546 BC_2 sib-crosses) were inoculated. Four hundred eleven or 28.05 % inoculated plants showed typical symptoms of PRSV-P, which ranged from distortion of young leaves, mosaic, chlorosis to shoe-string on older leaves.

After seven years of continuous selection and crossing, there are now sib-cross 3 (BC_3 03R 86 1 x 7-11) and backcross 4 plant (BC_4 03R 73-8) that are visually free from viral infection after 11 months of exposure in the field and this observation was then further confirmed by the result of the serological assay/ELISA.

In general, except from sib-cross 3 (BC_3 03R 86 1 x7-11) and backcross 4 plant (BC_4 03R 73-8) which are completely symptomless, there was variation in the rate of symptom development of BC_2, sib-cross 2, BC_3, sib-cross 3 and BC_4 from the control, Davao Solo. The rate of symptom development in the backcross generation was slower than that of the Davao Solo which produced severe symptoms. There were plants (BC_3 and BC_2 sib-

crosses) that remained symptom free for about 5-6 months. The difference between backcross papaya and Davao Solo was also evident in the ability of the trees to bear good quality fruit. Davao Solo produced a few small and unmarketable fruits. Backcross plants in contrast to the Davao Solo had the ability to recover from infection based on visual inspection and serological test

Fruit qualities of BC_4, BC_3, BC_3 sib-cross, BC_2 and BC_2 sib- cross were presented. Fruits were generally sweet. The observed sweetness of the fruit could be attributed to *V. quercifolia* since it is known for its high sugar levels. TSS ranged from values 11.31 to 12.83^0B. The fruit weight ranged from 540.8 – 1490.5 grams and the fruits have firm yellow orange flesh with mild papaya aroma.

Resistance/susceptibility of reconstituted 'Sinta' (cross between best BC_2 female plants and IPB, inbred line 4172) and fruit evaluation planted in farmer's field in Batangas, Philippines were also done.

3 Background

3.1 History and Importance of Disease

Papaya, a native of Central and South America, is a fast growing aborescent herb. Dioecious and hermaphrodite cultivars of this plant are grown in many tropical and sub-tropical countries for edible fruit, vitamin rich fruits and to a lesser extent also for their milky latex (Drew et al. 1998 and Drew et al. 2006). In 2005, the world production of papaya was 6.342 million metric tonnes (FAO, 2005).

Papaya is the best known member of the family Caricaceae that consists of 4 genera (*Carica, Jacaratia, Jarilla and Cylicomorpha*) and a total of 31 species (Badillo, 1971). *Carica* is the largest and most economically important genus in the family, having 22 species distributed in the neotropics from southern Mexico to Argentina and Chile. This genus is diploid and includes 8 species with a chromosome number of 18 (Storey, 1976).

Recently, Badillo (2000) has recommended dividing the genus into 2 genera: *Carica* containing *C. papaya* and *Vasconcellea* containing the other 21 species. This division into 2 genera is consistent with genetic analyses of these species using molecular markers that have shown that *C. papaya* is quite distinct from the other species (Jobin-Décor et al, 1997; Kim et al. 2002). *Vasconcellea* species, V*. cauliflora, V. pubescens, V quercifolia* and *V. stipulata* are resistant to PRSV-P (Conover, 1964; and Horovitz and Jimenez, 1967).

There are many diseases of papaya, which are considered as economically important, and the most important among these is papaya ring spot (PRS) (Purcifull, 1972) which is considered as the greatest single threat to papaya production in the world (Litz, 1985). Papaya ring spot is caused by a potyvirus called Papaya ring spot virus (PRSV-P). The importance of the said pathogen was further highlighted since all *C. papaya* cultivars and genotypes are susceptible to PRSV-P (Cook and Zettler, 1970; Magdalita et al, 1988; Manshardt and Drew, 1998).

It was first observed on the island of Oahu in Hawaii in 1945 (Lindner et. al, 1945; Holmes et al.1948). In the 1950's, diseases with similar symptoms were reported in Bangladesh, Columbia, Cuba, El Salvador, Sri Lanka, and Venezuela (Hamilton 1986). PRSV has been reported in India (Capoor and Varma 1958) and Africa (Kulkarni, 1970). It was then reported in Taiwan, Thailand (Hamilton, 1986) and in Malaysia and Australia (Thomas and Dodman, 1992).

In the Philippines, PRSV was first observed in 1982 in Silang, Cavite but was confined to a small area (Magdalita et al, 1988). By 1984 the disease had spread rapidly infecting 200 hectares of papayas with 60-100% incidence with an estimated loss of yield of P6,309,000 (Magdalita, 1988). The disease developed in epidemic proportions in Cavite, Batangas, Laguna and has also spread to Quezon, Bulacan, Rizal, Pampanga, Nueva Ecija, Tarlac, Pangasinan and Metro Manila (Opina, 1986).

3.2 Symptoms

Symptoms induced by different isolates of PRSV-P may vary in intensity, but dark green, often slightly sunken, rings are diagnostic. The number of rings on fruits can be variable, and the rings become less distinct as the fruit matures and yellows. Fruits often show uneven bumps, especially those fruits that develop after a tree is infected. Intense yellow mosaic on leaf lamina and numerous "oily" streaks on petioles could also be observed. The leaf canopy becomes smaller as the disease progresses due to the development of smaller leaves and stunting of the plant. Fruit yield and brix levels are markedly lower than fruit from healthy plants. Leaf and fruit symptoms are most intense during the cool season.

Leaves often develop a shoe-string appearance caused by the extreme reduction of leaf lamina similar to that caused by broad mites. Papaya trees of all ages are susceptible and generally will show symptoms two to three weeks after inoculation. Trees infected at a very young stage never produce fruit but rarely die because of the disease (Gonsalvez, 1993; verified 27 May 2009).

3.3 The Pathogen

3.3.1 Biology

PRSV-P is a member of the potyvirus group, with flexous, filamentous particles about 780 x 12 nm. The virus induces cylindrical (pinwheel) inclusions and amorphous inclusions in the cytoplasm of host cells. It is transmitted mechanically and by many species of aphids in a non-persistent manner. PRSV-P has a narrow host range that includes species of three dicotyledonous families: *Caricaceae, Chenopodiaceae* and *Cucurbitaceae.* The virus is serologically indistinguishable from watermelon mosaic virus-1 (WMV-1) which recently was reclassified as papaya ringspot vims-W and is of economic importance wherever cucurbits are grown (Purcifull et al, 1984).

3.3.2 Epidemiology

Papaya ringspot virus can be rapidly spread by several aphid species in a non-persistent manner. Though many cucurbits are susceptible to PRV-P, they do not serve as an important alternate host. Instead, the dominant strain in cucurbits is PRV-W. Therefore, the spread of the virus (PRV-P) into and within an orchard is primarily from papaya to papaya. The development of the disease in an orchard follows the general pattern of viruses that are spread by aphids in a non-persistent manner. The amount of primary infection increases as the distance from infected papaya trees decrease. Secondary infection spreads rapidly and an orchard can become totally infected in three to four months. This situation occurs in young orchards located close to infected plants and during periods when populations of winged aphid flights are high (Gonsalvez, 1993; verified 27 May 2009).

3.3.3 Control Measures (Non-Chemical Control)

Different strategies were tested in order to control and to manage PRSV-P. Some of these strategies are the following: application of suitable cultural practices, cross protection, genetic manipulation of the plant and breeding for tolerance/resistance against PRSV-P.

In Hawaii PRV-P has been kept out of the Puna district of Hawaii where nearly 98% of the state's commercial papaya is produced. Their success is due to the regular monitoring and rouging of infected papaya by the State Department of Agriculture in the surrounding areas, and discouraging the movement of seedlings into the Puna district (Gonsalvez, 1993; verified 27 May 2009).

Several other cultural practices are useful for reducing crop damage. The most important is to establish orchards with seedlings that are not infected with PRV-P. Secondly, new orchards should be situated as far as feasible from infected orchards. Orchards should not be established by inter-planting seedlings among trees that are infected with PRV-P. Additionally, disease incidence can be reduced by planting a non-host crop, such as corn, around the orchard and even between rows. The rationale for this, is that, aphids flying into the papaya orchard would first land and feed on the alternate crop and lose their ability to transmit the virus to papaya due to the non-persistent mode of transmission (Gonsalvez, 1993; verified 27 May 2009).

Finding a milder strain of the virus and using it to inoculate the seedlings is another way on how to minimize the effect of the severe strains. This is actually the principle of cross protection. The rationale here is to infect the papaya plants with milder form of the virus so

as to boost its resistance/tolerance and preparing it for the attack of a more virile strain of the said pathogen. But finding milder forms of PRSV-P seemed to be hard. To circumvent this difficulty, Yeh et al in 1988 conducted an artificial mutagenesis to create milder form of PRV HA, a severe strain isolated from Hawaii.

The effort to genetically engineer papaya has been motivated largely by the need to develop cultivars with resistance to papaya ring spot virus. The theoretical basis for genetically engineered resistance to viral disease was provided in the concept of pathogen-derived resistance, which was developed by Sanford and Johnston (1985). This theory relies on the fact that the relationship between parasite and host organism is intimate and intricate, and on the conjecture that deviations in timing or level of expression of genes crucial to the interaction may result in failure of parasitic relationship, i.e., host resistance. In the case of papaya and PRSV-P, the theory was tested by cloning and engineering the coat protein gene of PRSV (Quemada et al. 1990; Ling et al. 1991), so that it could be constitutively expressed when integrated into the genome of papaya cells. This was actually first accomplished by microprojectile bombardment (Fitch et al. 1992) at Cornell University (Geneva, NY), from which transgenic plants were regenerated in Hawaii, using a protocol based on embryogenic callus derived from zygotic embryos (Fitch and Manshardt, 1990),

The importance of PRSV resistance as a worldwide papaya breeding objective is revealed by the fact that projects to transform papaya with coat protein genes isolated from local PRSV strains are underway in several countries. In Taiwan, transgenic Tainung No. 2 plants show good resistance and are now undergoing field trials (Yang et al. 1996; Yeh et al. 1998). Researchers from Jamaica, Brazil and Thailand have produced PRSV-resistant transgenic plants in Cupertino with Dennis Gonsalvez, virologist at Cornell University in Geneva, NY. In Australia (Mahon et al. 1996) and Mexico, independent programs are transforming local papaya cultivars with coat protein genes of local PRSV strains.

Traditional plant breeding has been used since 1984 in an attempt to produce PRSV-P tolerant varieties to counter the disease. Papaya breeding in the Philippines started in 1981 through a research grant from PCARRD. Many of their breeding lines were lost due to rapid spread of PRSV-P in 1984. Since then, plant breeders have collected seed from surviving trees and imported seed of varieties which have been reported to have some tolerance to PRSV-P in other countries. Screening of local, imported and hybrid lines has produced no promising results as all lines have been very susceptible to the disease. Some tolerance has resulted from crosses with *Cariflora* from Florida, however these lines fall short of a commercial level of tolerance.

It was only then in 1996, when Institute of Plant Breeding (IPB) was able to produce an F_1 hybrid named 'Sinta' which is a semi-dwarf hybrid papaya that bears good quality fruits (with sweet, yellow, firm flesh). Its best attribute is its moderate tolerance to papaya PRSV-P thus can be profitably grown in PRSV-P infected area (NSIC Catalogue, 1994).

Another strategy to control/minimize the effect of PRSV-P is the use of resistant varieties. The use of disease resistant varieties is probably the cheapest and most effective method of combating the disease. There are however, many difficulties in breeding resistant varieties and one of these is the absence of source for resistance gene(s) within the *Carica* genus. All papaya cultivars and genotypes are susceptible to PRSV-P (Cook and Zettler, 1970; Magdalita et al, 1988; Manshardt and Drew, 1998).

4 Objectives

4.1 General Objectives

The aim of this project is to developed PRSV-P resistant and fertile backcross lines that could be used as genetic stocks for papaya development. Technology transfer is an important goal of this project thus, the team aims to produce elite genotypes of papaya which are PRSV-P resistant and to trial these on growers' properties.

4.2 Specific Objectives

1. *Selection of Backcross generations*. Evaluation of backcross 2 (BC_2), 3 (BC_3) and 4 (BC_4) generations, and sib-cross 2 (SbC_2) and 3 (SbC_3) generations, in the field at IPB, Los Baños. These backcross generations will be developed from the BC_1 plant that was symptomless in the field for 12 months. Germinated seedlings will be initially grown in a screenhouse where they will be inoculated 3 times with PRSV-P at 2 weeks intervals. Plants that will remain symptom free will be planted in the field and will be assessed for resistance/susceptibility to the Philippine strain of PRSV-P, for fertility, and for agronomic traits. Selected BC plants will be sib-crossed in an effort to produce plants that are homozygous for the PRSV-P resistant gene i.e carrying the RR form of the gene.
2. *DNA markers for PRSV-P resistance*. Continue laboratory work aimed at developing/screening molecular markers to identify the PRSV-P resistance gene of *V. quercifolia* to the backcross papaya.
3. *Micropropagation of elite genotypes*. Maintenance of valuable germplasm that has been produced will be essential for future projects and for commercialization of PRSV-P resistant lines. Regular transfer and subculture of in vitro collection that includes F_1's intergeneric crosses and selected backcross plants.
4. *Evaluation on Growers' properties*. An important part of this project will be to field test BC_3 and BC_4 generations on growers' properties and to develop commercial PRSV-P resistant lines. This is an important objective in terms of technology transfer of results to the papaya industries.
5. An important objective of this project is to determine how PRSV-P resistant papaya lines will be commercialized in the Philippines and to develop a commercialization strategy.

5 Methodology

5.1 Time and Place of Study

This study was conducted at the Institute of Plant Breeding, College, Laguna from Jan 2002 to June 2009.

5.2 Backcrossing

5.2.1 First backcross (BC1) to papaya

The five F1 intergeneric hybrid lines (404, 410, 468, 469 and 507) of *C. papaya x V. quercifolia* that had previously demonstrated PRSV-P resistance were used as paternal parents to incorporate PRSV-P resistant gene/genes to five elite papaya inbred lines (4108, 4172, 5648, 5893 and 3878) of IPB. Duplicate plantings were established on the islands of Davao in Mindanao, and in Guimaras in the Visayas. Pollen was transferred onto the stigma of *C. papaya*. To compensate for the low pollen viability of intergeneric hybrid line 410 which ranges from 0.4 to 2.0%, 1 female flower per 10 male flowers was used in the pollination. Cytological observations showed that there were meiotic irregularities which lead to formation of non-functional gametes explaining the observed low pollen viability of intergeneric hybrid line 410. Female flowers were bagged 3 days before and immediately after pollination. Immature embryos were rescued from immature fruits (90-120 days after pollination) and cultured *in vitro*. A total of fifteen (15) crosses were produced in Laguna, Davao, and Guimaras. A total of 940 fruits were harvested and 114,839 seeds were dissected. Only 1,011 embryos were rescued and only 733 were germinated and 700 from it developed into plantlets. Resultant plants were then screened for PRSV-P resistance in screenhouses and in the field.

5.2.2 Second, third and fourth backcross (BC2, BC3, and BC4) generations

Advancement of Backcross Generations

BC_2, BC_3 and BC_4 generations were derived from the resistant male BC_1 plant. This BC_1 resulted from cross between intergeneric hybrid (*C. papaya x V.quercifolia*) line 410 and IPB inbred line 5648. Flowers were bagged three days before and immediately after pollination. Four months after pollination or after reaching maturity, seeds were extracted from the fruit. Seeds were germinated and the resultant plants were screened for PRSV-P resistance in the screenhouse and in the field. The procedure was repeated to produce the next best backcross generations

Production of Sib-cross Plants

Male BC_2 plants and BC_3 with fewest PRSV-P symptoms were used to pollinate flower of the female BC_2 plants and BC_3 with fewest PRSV symptoms as well. Again flowers were bagged before and immediately after pollination. Upon reaching maturity, seeds were extracted from the fruit of these controlled crosses.

5.3 Evaluation of PRSV-P Resistance

5.3.1 Screenhouse and Field Evaluation

Sowing of BC4, BC3 and BC2 sib-crossed seeds.

Seeds of BC_4, BC_3, BC_3 sib-crosses (SbC_3) and BC_2 sib-crosses (SbC_2) were sown into plastic trays containing sterilized soil. At two-leaf stage, seedlings were pricked into individual 2.5 in x 2.5 in x 2.5 in polyethylene bags with holes.

Screenhouse Evaluation

Seedlings approximately one month old were evaluated for Papaya Ringspot Virus-P (PRSV-P) resistance in the screenhouse. This was done by manually inoculating the plants three times using the inoculum obtained from infected papaya plants (Davao Solo).

Field Evaluation

Plants that showed putative resistance against PRSV-P (after three manual inoculations in the screenhouse) were used in field evaluation. These plants together with the susceptible control (Davao Solo) were planted in the field (Mainit, Bay, Laguna) in the presence of a high inoculum level of PRSV-P and were exposed to the natural inoculation by aphids. Susceptibility to PRSV-P was determined by symptom expression and by serological test.

5.3.2 Serological Test

Indirect ELISA

Papaya leaf samples (0.05 g) were ground in 1ml carbonated coating buffer (1.5g Na_2CO_3; 2.93g $NaHCO_3$; 1000 ml dH_2O) using mortar and pestle. The resulting sap was pipetted to a clean ELISA plate where 100µl/well of antigen was added and was incubated overnight at 4°C. The plate was washed three times using the washing buffer (1000 ml PBS; 0.05% Tween 20). Two hundred (200) µl blocking solution (1000 ml PBS; 1% skim milk) was added in each well and was incubated for 1 hour at 37°C. After incubation, the plates were washed three times. One hundred (100) µl of antibody buffer (1000 ml PBS; 0.2% Egg albumin) was added to each well and was incubated for 2-3 hours at 37°C. After 3 hours, the plate was washed again three times using the washing buffer. One hundred (100) µl of anti-rabbit enzyme conjugate was added to each well and was incubated for 2-3 hours at 37°C. After incubation and washing of the plate, 100µl substrate (2 Nitrophenyl phosphatase (NPP) tablets/10 ml PBS) was immediately added to each well and was incubated for 30 mins. Viral concentration in each well was determined using an ELISA reader machine.

5.3.3 Molecular Characterisation

Primer

A new set of primers was designed using the WebTroll program and Vector NTI based on a previously identified tandem repeat found in the exon region of the *C. papaya* cysteine proteinase gene (Genbank No.M15203) or papain (CPAPAP). The primer is expected to amplify the 459bp region, where the perfect tandem repeat (TA)10 was located (110-1129bp). Figure 1 illustrates the SSR primer pair binding sites at the CPAPAP gene used in this study.

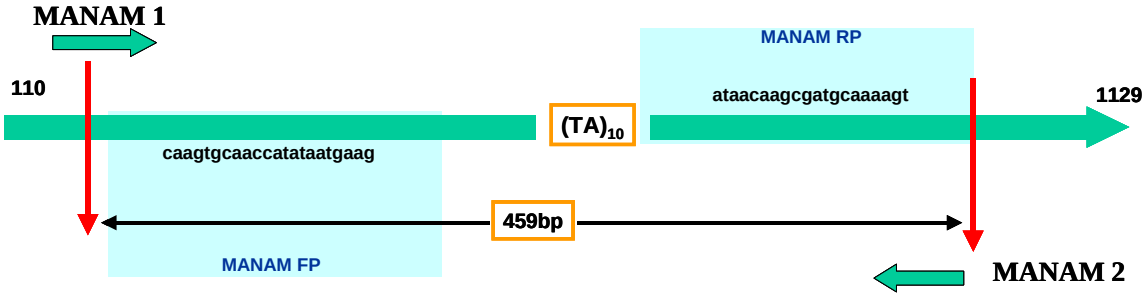

Figure 1. Illustration of MANAM SSR primers used in amplifying the 456bp region of CPAPAP gene with the $(TA)_{10}$.

Table 1. Primer combination used to identify introgression of resistance genes to backcross papaya plants from *V. quercifolia*.

Primer Combination	Primers	3'- 5' Primer sequence	Expected size
1	MANAM RP	ATAACAAGCGATGCAAAAGT	459
2	MANAM FP	CAAGTGCAACCATATAATGAAG	

The primer design involved the collection of nucleotide and peptide sequences of the selected enzymes grouped according to species, genus, family, and order. This also involves multiple sequence alignment resulted from the consensus sequence of the chosen enzymes, in which the SSR primers were designed.

Web TROLL program, http://wsmartins.net/webtroll/troll.html (Castelo et al, 2002; Martins et al., 2006), was used to detect putative simple sequence repeats and the corresponding primer candidates as designed by the Primer3 program. The primer candidates were further validated using Vector NTI and BLAST programs for properties of self and cross complementation, palindromes, repeats and energy relationships.

Aside from the designed set of primer which is based on papain, another set of primers (5 pairs which is based on papaya and *Vasconcellea*) is being screened for its usefulness in tracking the introgressed gene(s) from the backcross generations.

DNA Extraction

Total DNA was extracted from young papaya leaf sample following the revised protocol adapted from Cheung (1993). Liquid nitrogen was used in order to ease grinding using a mortar and pestle. Polyvinylpyrilidone (PVV) was added and mixed with the ground leaves. This was done to stop the phenol activity in the sample. In their oxidized forms, polyphenols, covalently bind to proteins and DNA, giving the DNA a brown color and making it useless for most research application. One thousand five hundred µl or 1.5 ml of Cheung's buffer was added (2 M NaCl; 0.2 M Tris-HCl, pH 8.0; 0.07 M EDTA and 0.2 M ß- mercaptoethanol) immediately in the ground sample. Immediate addition of buffer re-assures that the sample is in its optimum condition (pH 8-8.5) thereby minimizing the possible degradation of the DNA. The solution was transferred in sterile 1.5 ml tubes. The microcentrifuge tubes were then incubated at 65°C for 45 minutes. The tubes were centrifuged at 13,000 rpm for 10 minutes using a microcentrifuge. The resulting supernatant was pipetted to new, sterile and previously labeled 1.5 ml tubes where 125 µl of 3M sodium acetate and 500µl cold isopropanol was added. These tubes were then put in the freezer overnight. To collect the DNA pellet, centrifugation at 11,000 for 6 minutes at 4°C was done using a refrigerated microcentrifuge. The collected pellet was then washed two times with 200 µl of 70% ethanol and centrifuged at 11,000 for 6 minutes at 4°C. The pellet was air-dried and suspended in 30 µl distilled nanopure water.

Standardization of DNA Quantity and Quality

The quantity and quality of the DNA samples were checked by running them on a 1% agarose gel. Standardized DNA concentrations of 25-30 ng/µl were obtained by diluting DNA samples appropriately with sterile distilled water.

PCR Amplification

A modified protocol for Polymerase Chain Reaction (PCR) developed by Zaporteza (2004) was adopted in the molecular genotyping. PCR was done using MANAM 1/2 primers. PCR amplification was performed in a 25-ul reaction mixture containing 2 ul of DNA template (approximately 25- 30 ng of DNA) in distilled nanopure water; 10x PCR buffer (with 15 mM Mg2+), 10 mM dNTPs, 10 uM of primer (MANAM ½), and 1 unit of Taq DNA polymerase.

PCR amplification was done using the Programmable Thermal controller. An initial denaturation at 94°C for 2 min was done followed by 35 cycles that included denaturation at 94°C for 1 min, primer annealing at 51°C for 1 min, and primer extension at 72°C for 2 min. The final extension was at 72°C for 10 minutes. All amplification reactions were carried out in sterile 0.5 ml tubes. DNA from Davao Solo was used as positive control and sterile distilled water as negative control.

Gel Electrophoresis

Following amplification, PCR products were separated using electrophoresis. One hundred ml of 2% agarose gel was prepared by mixing 2 g of agarose in a dry flask containing 100 ml of 0.5X TAE buffer. The suspension was heated in a microwave oven for 6 minutes, allowed to cool, then poured into mould with a 28-well comb, and then allowed to solidify before it was placed in the electrophoresis tank with 0.5X TAE. To individual wells, 10 ul of PCR products, each mixed with 1 ul of commercial loading dye were dispensed. Electrophoresis was done for 1 hour at 100-110V. Ten ul 1 Kb+ ladder (50 ng) of 1kb DNA ladder on the first and last wells.

5.3.4 Agronomic Evaluation

Fruit Evaluation

Fruit quality was evaluated and several important parameters of the papaya fruits were measured. These were fruit weight, TSS (total soluble solids), taste, fruit length, and fruit width. Total Soluble Solids (TSS) was measured using a refractometer (0-32 °B). Fruits were allowed to ripen at room temperature (28-30 °C).

6 Achievements against activities and outputs/milestones

Objective 1: To incorporate PRSV-P resistance gene(s) to IPB papaya inbred lines

no.	activity	outputs/ milestones	completion date	Comments
1.1	Backcrossing using the pollen of BC_3 to produce BC_4 plants	BC_4 seeds	March 2008	Production of seeds was hampered due to series of typhoon that hit Los Baños, Philippines. The best trees will be utilized for pollination until it dies. This is to have as many seeds as possible (especially F_1 re-constituted 'Sinta' seeds since it is the one which will be planted in the farmer's field).
1.2	Sib-crossing of the best male and female BC_3 and BC_4 plants	BC_4 and BC_3 sib-crossed seeds	BC_3 sib-crossed seeds : March 2008; BC_4 sib-crossed seeds: Still on going, expected date of completion is July 2009	
1.3	Re-constitution of 'Sinta' papaya by pollinating the best BC_4 and BC_3 plants using pollen from IPB inbred line, 4172	Reconstituted 'Sinta' seeds	Still on-going	

PC = partner country, A = Australia

Objective 2: To select plants showing PRSV-P resistance with good agronomic traits from backcross generations

no.	Activity	outputs/ milestones	completion date	comments
2.1	Planting of BC_3 and BC_2 sib-crossed plants (that remained symptomless after three manual inoculation) in the field to assess its resistance/susceptibility against PRSV-P	Backcross generations that were mildly infected by the virus and had the ability to recover from infection	Feb. to March 2007	
2.2	Growing of BC_3 sib-cross and BC_4 seedlings in the screenhouse for three manual inoculation at two weeks interval	BC_3 sib-cross and BC_4 seedlings with putative resistance against PRSV-P after manual inoculation	June 2008	
2.3	Planting and screening of BC_3 sib-cross and BC_4 seedlings in the field	One BC_3 sib-cross and one BC_4 plants showed good resistance to the virus after 11 months in the field	Screening for resistance/susceptibility to PRSV-P is still on-going.	Screening will be done as along as the trees are still present in the field.
2.4	Serological assay for BC_2, BC_3 and BC_4 plants maintained in the field	Quantification of viral titre in the backcross plants in relation to the susceptible check, 'Davao Solo'	BC_2 - Year 2006 BC_2 sib-cross - May 22, 2007; BC_3 - Jan. 16, 2008, BC_3 sib-cross and BC_4 - April, 2009	In general, the result showed that the backcross plants have this ability to suppress the reproduction and development of the virus inside the plant.

PC = partner country, A = Australia

Objective 3: To develop/screen molecular markers that will aid in identifying plants carrying the resistance gene(s) from V. quercifolia

no.	activity	outputs/ milestones	completion date	comments
3.1	Development of a molecular marker (SSR) based on papain gene	SSR marker (MANAM 1/2)	20 May 2007	This pair of SSR marker was designed based on the papain gene.
3.2	Screening of 5 new SSR markers.	Preliminary result of tracking the introgressed resistance(s) gene from *V. quercifolia* to papaya	On-going	All of these primers are based on *Carica papaya* genome. These primers had shown polymorphism in *Carica papaya*. In addition, four of these 5 SSR markers are also polymorphic when used to *Vasconcellea* species.

PC = partner country, A = Australia

Objective 4: To hybridize other IPB inbred papaya lines with BC_1 03R (5648 x 410)

no.	activity	outputs/ milestones	completion date	Comments
4.1	Intensive pollination between inbred lines (4108, 5893, 4172 and 3878) and BC_1	Progeny of the cross between IPB inbred lines and BC_1	5 March 2008	

PC = partner country, A = Australia

Objective 5: To maintain valuable germplasm thru micropropagation

no.	activity	outputs/ milestones	completion date	Comments
5.1	*In vitro* micropropagation of elite and potentially commercial lines--- Maintenance of elite or useful genotypes for commercialization	Cultures that could be useful for future breeding program	On-going	

PC = partner country, A = Australia

Objective 6: To evaluate elite papaya lines on growers' properties

no.	activity	outputs/ milestones	completion date	Comments
6.1	F_1 re-constituted 'Sinta' (a cross between best female plant and IPB inbred lines, 4172) were planted in a farmer's field vis a vis with the original 'Sinta'	Data from fruit evaluation of F1 re-constituted 'Sinta' in comparison with the original 'Sinta'	8 Nov 2007	Based on the serological test, the F1-reconstituted 'Sinta' has also the ability to recover from infection.

PC = partner country, R = Australia

Objective 7: Commercialization of the resistant papaya genotype

no.	activity	outputs/ milestones	completion date	comments
7.1	Meeting with the possible private companies regarding the commercialization of the resistant papayas	Presentation of resistant papaya in Investor's forum last December, 2008.		East-West Seed Company expressed interest with our resistant line. Negotiations on franchising deal to follow.

PC = partner country, R = Australia

7 Key results and discussion

To breed papaya genotypes that are resistant to papaya ringspot virus form- P (PRSV-P), five inbred lines (4108, 4172, 5648, 5893, and 3878) developed by the Institute of Plant Breeding and five F1 intergeneric hybrids (404, 410, 468, 469 and 507) from Australia were maintained in the field. Table 2 shows the fruit characteristics and reactions to PRSV-P of the different inbred lines.

The five F1 intergeneric hybrid lines (404, 410, 468, 469 and 507) of *C. papaya x V. quercifolia* were used as paternal parents to incorporate PRSV-P resistant gene/genes to five elite papaya inbred lines (4108, 4172, 5648, 5893 and 3878) of IPB. A series of backcrossing and subsequent selection and evaluation of resistant lines was used in the development of resistant genotypes with good agronomic characters.

Although intergeneric hybrid line 410 was predominantly used on the production of BC1 there were other four intergeneric hybrid lines (404, 468, 469, and 507) evaluated. After more than two years in the field, two hybrid lines developed typical PRSV-P symptoms on the leaves. ELISA test results confirmed that intergeneric hybrid lines 404 and 507 broke down its resistance to Philippine strain of PRSV-P while lines 410, 468 and 469 remained virus- and symptom free (Table 3). Two additional intergeneric hybrid lines 468 and 469 were utilized to produce more BC_1 crosses using the same recurrent parents. Slow production of BC_1 with these intergeneric hybrid lines were experienced because they are not male-stable lines. Most of the time they bear hermaphrodite and female flowers. Line 469 dominantly produces female flowers. Intergeneric hybrid line 410 is a male-stable line.

A total of fifteen (15) crosses were produced in Laguna, Davao, and Guimaras. A total of 940 fruits were harvested and 114,839 seeds were dissected. Only 1,011 embryos were rescued and only 733 were germinated and developed into plantlets (Table 4).

About 700 BC_1 plants were generated and screened under screenhouse condition. After three manual inoculations, plants that showed putative resistance were transplanted along with the susceptible check, "Davao Solo" in the field. One resistant BC_1 derived from 5648 x 410 was selected from IPB crosses last 2004. Resistance to PRSV-P was confirmed after one year in field of high disease pressure. ELISA test verified the absence of masked symptoms of the vitus. This BC_1 resistant plant is a male tree and has 65% pollen fertility.

BC_2, BC_3 and BC_4 generations were developed from the said BC_1 plant that was symptomless in the field in Los Baños, Philippines for 12 months. The BC_1 was the product of hybridization between papaya inbred line, 5648 and an F_1 intergeneric hybrid, 410. Intergeneric hybrid 410 is a cross between *Carica papaya* and *Vasconcellea quercifolia* where *V. quercifolia* is the source of resistance to PRSV-P. BC_2 and BC_3 sib-crosses were developed by sib-crossing the female and male BC_2 and BC_3 plants showing with few or symptoms of PRSV-P. Selection of these female and male BC_2 and BC_3 plants was based on the ELISA test result and symptom development in the field. Sib-crossing was done in an effort to produce plants that are homozygous for the PRSV-P resistant gene i.e. plants that carry the RR form of the gene.

Germinated seedlings were initially grown in a screenhouse where they were inoculated three times at two weeks interval (Figure 2). A total of 1465 plants (256 BC_4, 147 BC_3, 379 BC_3 sib-crosses, 137 BC_2 and 546 BC_2 sib-crosses) were inoculated. Four hundred eleven or 28.05 % inoculated plants (Table 5) showed typical symptoms of PRSV-P, which ranged from distortion of young leaves, mosaic, chlorosis to shoe-string on older leaves.

Plants that remained healthy and symptom free together with the susceptible control, Davao Solo, were then planted in the field and were assessed for resistance/susceptibility to the Philippine strain of PRSV-P. PRSV-P reaction of the papaya plants at different stages of their growth and development was observed. Evaluation of backcross 2 (BC_2)

was done at National Seed Foundation (NSF) field of IPB, while backcross 4 (BC_4), backcross 3 (BC_3), sib-cross 3 (SbC_3), and sib-cross 2 (SbC_2) generations was done in the field at Mainit, Bay, Laguna, Philippines (Figure 3).

There are sib-cross 3 (BC_3 03R 86 1 x7-11) and backcross 4 plant (BC_4 03R 73-8) that are visually free from viral infection after eleven months of exposure in the field. Aside from these, another BC_4 plant (BC_4 03 73-3-7) and a male sib-cross 2 plant (BC_2 03R 37 x 53-11) exhibited good level of tolerance to PRSV-P. This BC_4 and SbC_2 plant only showed mild infection after 8 months of exposure to inoculation by viruliferous aphids in the field. Its new leaves were healthy and free from the typical symptoms caused by PRSV-P (Figure 4).

Generally, there was variation in the rate of symptom development of BC_2, sib-cross 2, BC_3, sib-cross 3 and BC_4 from the control, Davao Solo. The rate of symptom development in the backcross generation was slower than that of the Davao Solo which produced severe symptoms. There were plants (BC_3 and BC_2 sib-crosses) that remained symptom free for about 5-6 months. The difference between backcross papaya and Davao Solo was also evident in the ability of the trees to bear good quality fruit. Davao Solo produced a few small and unmarketable fruits. Backcross plants in contrast to the Davao Solo had the ability to recover from infection based on visual inspection and serological test (Figure 4 and 5).

Serological assay

ELISA tests were done to further validate the observed putative resistance of the plants against PRSV-P in the field. Prior to the serological tests, twenty-four of the twenty-seven BC_2 plants already showed infection of the virus base on the observed symptoms in the plants. However, after three and a half months of exposure to natural infection and after the serological evaluation, results revealed that only eight out of the twenty-seven plants or 29.63% tested were positive to the virus (Table 6). The mild symptom observed during the first few months after field transplanting did not progress in some plants.

For the BC_2 sib-crosses, 29 out of the 35 of the plants tested for viral concentration showed PRSV-P symptom. The first ELISA test was conducted after five months of exposure of the plants to aphid vectors using Davao Solo that had a high inoculum level of PRSVP-P as a control. Results showed that only 13 out of the 35 or 37.14 % sib-crossed plants were positive to the virus (Table 7). Few weeks after the first ELISA test, it was observed that some of the infected plants were still able to grow vigorously and were recovering from the infection. After further two months, ELISA tests were again performed to verify whether the plants had recovered from the infection. The results revealed that some of the previously infected plants had recovered from the infection. Of the thirteen plants that were infected, only eight or 23.53% had a positive ELISA result (Table 7). Young leaves of these plants were free from the typical symptoms caused by PRSV-P.

Based on the obtained values of viral concentration in the plants, it could be inferred that the viral reproduction and growth in the plant was impaired. For example, in BC_2 03R 37 x 34-1, the absorbance reading in the first and second ELISA test was 0.770 and 0.350 respectively (Table 7). These values show that there is a considerable decrease of viral concentration in the plant. This is consistent with gene silencing in response to the virus.

To further validate the above observation, serological assay/ELISA using old and new/young leaf of BC_3 papaya plants were used. Using old infected leaf, 22 out 34 or 64.71 % were present of PRSV-P but if young leaf was used, only two plants showed positive reaction to the virus with 32 or 94.12 % BC_3 plants showing absorbance reading below the threshold level. Result shows again that there was a considerable decrease in the viral titre concentration in the plant (Table 8). This decrease could be the reason why the backcross plants were able to recover from the viral infection in contrast to the susceptible check, Davao Solo.

Table 9 shows the absorbance reading of the BC_3 sib-cross plants after nine months of exposure in the field. Before ELISA, 47 out of 49 or 95.92 % of the plants showed viral symptoms, leaving only 2 plants free from viral symptoms but after performing the serological assay, only 11 out of 49 or 22.45% showed absorbance reading above the threshold level.

For the BC_4 papaya plants, 56 plants that survived in the field were subjected to ELISA as well. Among the 56 BC_4 plants, 37 or 66.07% showed absorbance reading below the threshold level with 19 or 33.93% plants positive for PRSV-P (Table 10).

Serological assay using leaves from F1 re-constituted 'Sinta' plants after 1 year and 8 months in the field planted side-by side with the original 'Sinta' was also performed. Table 11 shows that prior ELISA, all sample plants were positive to the virus based on visual inspection but after performing the test, the result shows that among the 26 plants, 8 or 30.77 % showed negative reaction to the virus. The noticeable decrease in the viral titre concentration showed that the re-constituted 'Sinta' had inherited the gene(s) from the BC_2 plants which could be the reason of its good performance in the field which is said to be a hot-spot for PRSV-P.

Fruit Evaluation

Fruit qualities of BC_4, BC_3, BC_3 sib-cross, BC_2 and BC_2 sib cross were presented in Table 12. Fruits were generally sweet. The observed sweetness of the fruit could be attributed to *V. quercifolia* since it is known for its high sugar levels. TSS ranged from values 11.31 to 12.83 ^{0}B. The fruit weight ranged from 540.8 – 1490.5 grams and the fruits have firm yellow orange flesh with mild papaya aroma.

More than 500 F_1 re-constituted 'Sinta' papaya , which is actually composed of four lines (BC_2 03R -31 x 4172, BC_2 03R -37 x 4172, BC_2 03R -38 x 4172 and BC_2 03R -78 x 4172) were planted in a grower's field side by side with the original 'Sinta' and was screened for its resistance/susceptibility against PRSV-P (Figure 6).

Fruit qualities of F1 re-constituted 'Sinta' were presented in Table 13. Fruits were not that sweet just like the original 'Sinta'. The low TSS values observed that ranged from 9.80-10.20 ^{o}B could be attributed to frequent rainfall during the time of evaluation. It is said that the sweetness of the fruits are generally low during wet season as compare when it is harvested during summer season. The fruit weight ranged from 1098.41- 1257.85 grams and the fruits have firm yellow orange flesh (Figure 7) with mild papaya aroma just like the backcross fruits.

Table 2. Reaction to papaya ringspot virus type (PRSV-P) and fruit characteristics of selected inbred lines developed at IPB

Inbred line	PRSV-P Reaction	Flesh color	Ht to flower (cm)	TSS (ºB)
4108	Moderately tolerant	Red orange	67.30	10.8
4172	Moderately tolerant	Red orange	121.35	11.6
4308	Susceptible	Red orange	74.54	11.1
5648	Moderately tolerant	Yellow orange	71.46	11.8
5893	Moderately tolerant	Red orange	73.70	11.0
3878	Susceptible	Yellow orange	67.54	11.4

Table 3. Enzyme Linked Immunosorbent Assay (ELISA) of two-year old intergeneric hybrids maintained in Bay, Laguna.

Line	Mean Absorbance (405nm)	PRSV Reaction
401	0.514	Resistant
404	1.035	Susceptible
410	0.514	Resistant
468	0.405	Resistant

469	0.477	Resistant
507	0.882	Susceptible
Healthy control	0.376	Negative
Healthy control	1.363	Positive
Threshold Value	0.783	

Table 4 Total seed and embryo yield of different backcrosses produced in Laguna, Davao and Guimaras

Crosses	Fruits harvested	Seeds dissected	Embryos rescued	Embryos germinated
4106 x 410	139	2,330	168	132
4106 x 401	2	0	0	0
4106 x 468	8	6	6	6
4108 x 404	10	6	0	0
4172 x 410	117	1,871	126	60
4172 x 468	9	0	0	0
4172 x 404	28	22	15	8
5648 x 410	338	67,887	452	342
5648 x 401	38	9,990	0	0
5648 x 468	35	3,686	7	0
5648 x 404	62	15,0572	81	76
5893 x 410	87	9,233	61	60
5893 x 404	30	3,550	12	5
5893 x 468	4	674	0	0
3878 x 410	35	520	83	44
Total	940	114,839	1,011	733
Percentage		0.88%	73%	

Figure 2. Screenhouse evaluation of papaya plants against PRSV-P, (A) before inoculation and (B) after inoculation.

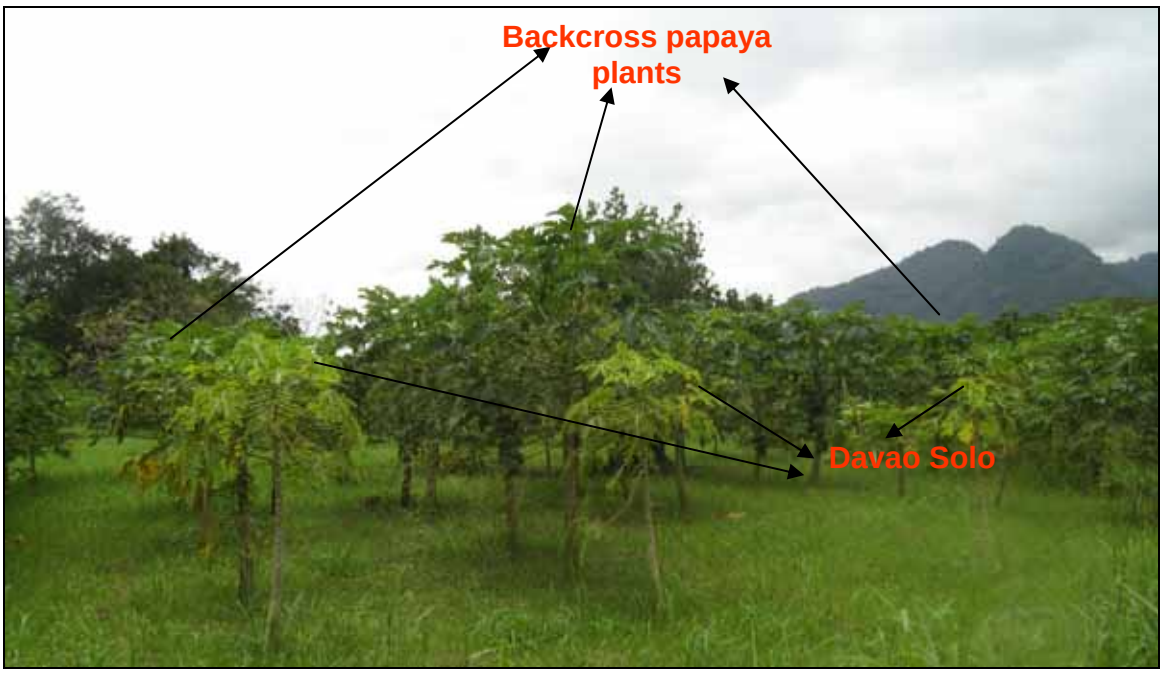

Figure 3. Field evaluation of the selected papaya lines in Mainit, Bay, Laguna

Table 5. Proportion of plants infected with PRSV-P after three manual inoculations with PRSV-P.

Papaya Populations	Total no. of plants before inoculation	Number of infected plants after three inoculations at two weeks intervals	% Infection
BC_4	256	10	28.05
BC_3	147	51	
BC_3 sib-crosses	379	33	
BC_2	137	38	
BC_2 sib-crosses	546	279	
Total	1465	411	

Figure 4. Backcross plants showing good resistance against PRSV-P under field condition in comparison to the susceptible check, Davao Solo. (A and B) BC_4 03R 73-3-7 remained symptom free for about 8 months and showed only few spots on the fruits, (C and D; E and F) BC_4 03R 73-3-8 and BC_3 03R 86 1x 7 -11 respectively. These plants are still symptom free after 11 months of exposure to the inoculation of the viruliferous aphids in the field, (G) a male BC_2 sib-cross plant, BC_2 03R 37 x 53- 11, remained symptomless after 8 months in the field, and (H) susceptible check, Davao Solo showing severe symptom.

Sib-cross plant
BC_2 03R 37 x 53

Healthy young leaf

Infected old leaf

Heavily infected Davao Solo
Positive control

Figure 5. Papaya plants after seven months of exposure to natural infection (A) Female sib-cross plant showing good recovery from the infection (new leaves were healthy and the plant was still productive) and (B) Davao Solo plant, heavily infected by PRSV-P showing fruitless tree with disfigure leaf canopy

Table 6. Enzyme Linked Immunosorbent Assay (ELISA) of BC_2 generations planted in National Seed Foundation field, IPB.

Sample	Sex	Symptom	Absorbance 405 nm	PRSV-P Reaction	Selection
4172 (-) Control		-	0.235	Absent	-
4172 (+) control	Female	Severe	1.350	Present	-
5648-1	Female	+	0.608	Present	-
5648-2	Female	+	0.705	Present	-
BC_2 03R-31	Female	+	0.346	Absent	Selected
BC_2 03R-34	Male	+ (Mild)	0.237	Absent	Selected
BC_2 03R-37	Female	+	0.301	Absent	Selected
BC_2 03R-38	Female	+	0.228	Absent	Selected
BC_2 03R-42	Female	+	0.263	Absent	Selected
BC_2 03R-47	Female	+	0.859	Present	-
BC_2 03R-50	Female	+	0.422	Absent	-
BC_2 03R-51	Female	+ (Mild)	0.588	Present	-
BC_2 03R-52	Female	+	0.406	Absent	-
BC_2 03R-53	Male	+	0.478	Present	-
BC_2 03R-55	Female	+	1.698	Present	-
BC_2 03R-56	Male	+	1.180	Present	-
BC_2 03R-59	Female	+	0.354	Absent	-
BC_2 03R-60	Male	+	0.350	Absent	Selected
BC_2 03R-61	Female	+	0.543	Present	-
BC_2 03R-66	Female	+	0.347	Absent	Selected
BC_2 03R-73	Male	+	0.276	Absent	Selected
BC_2 03R-74	Female	+ (Mild)	0.403	Absent	Selected
BC_2 03R-78	Female	+	0.286	Absent	Selected
BC_2 03R-79	Female	+	0.252	Absent	Selected
BC_2 03R-83	Male	+	0.911	Present	-
BC_2 03R-86	Male	+ (Mild)	0.397	Absent	Selected
BC_2 03R-96	Female	+	0.480	Present	-
BC_2 03R-100	Female	+	0.359	Absent	-
BC_2 03R-101	Female	-	0.218	Absent	-
BC_2 03R-102	Female	-	0.218	Absent	-
BC_2 03R-103	Male	-	0.263	Absent	-
Threshold value	0.470				

Table 7. Enzyme Linked Immunosorbent Assay of BC_2 sib-cross plants maintained in the IPB field.

Sample	Sex	1st ELISA TEST*		2nd ELISA TEST**	
		Absorbance 405 nm	PRSV-P Reaction	Absorbance 405 nm	PRSV-P Reaction
Threshold Value		0.540	--	0.424	--
410-1	Male	0.362	Absent	0.201	Absent
410-2	Male	0.281	Absent	0.204	Absent
BC_1 (03R)-1	Male	0.421	Absent	0.219	Absent
BC_1 (03R)-2	Male	0.335	Absent	0.244	Absent
5648	Female	0.751	Present	0.223	Absent
Davao Solo	Hermaphrodite	2.251	Present	1.261	Present
BC_2 03R 96 x 60-1	Female	0.400	Absent	0.248	Absent

BC_2 03R 96 x 60-2	Male	1.305	Present	0.250	Absent
BC_2 03R 96 x 6 -3	Male	0.279	Absent	0.280	Absent
BC_2 03R 37 x 34-1	Female	0.770	Present	0.350	Absent
BC_2 03R 37 x 34-2	Male	0.319	Absent	0.245	Absent
BC_2 03R 37 x 34-3	Male	0.744	Present	0.483	Present
BC_2 03R 37 x 34-4	Male	0.252	Absent	0.237	Absent
BC_2 03R 37 x 34-5	Male	0.286	Absent	0.239	Absent
BC_2 03R 37 x 34-6	Male	0.255	Absent	"Dead"	--
BC_2 03R 37 x 34-7	Female	0.307	Absent	0.292	Absent
BC_2 03R 37 x 34-9	Female	0.312	Absent	0.394	Absent
BC_2 03R 37 x 34-10	Female	0.861	Present	0.370	Absent
BC_2 03R 96 x 86-1	Female	0.241	Absent	0.215	Absent
BC_2 03R 96 x 86-2	Female	0.346	Absent	0.246	Absent
BC_2 03R 96 x 86-3	Male	0.751	Present	0.452	Present
BC_2 03R 96 x 86-4	Female	0.313	Absent	0.296	Absent
BC_2 03R 96 x 86-5	Female	0.462	Absent	0.337	Absent
BC_2 03R 96 x 86-6	Female	0.388	Absent	0.263	Absent
BC_2 03R 96 x 86-7	Female	0.618	Present	0.437	Present
BC_2 03R 96 x 86-8	Male	0.286	Absent	0.223	Absent
BC_2 03R 37 x 53-1	Female	0.268	Absent	0.244	Absent
BC_2 03R 37 x 53-2	Female	0.249	Absent	0.242	Absent
BC_2 03R 37 x 53-3	Male	0.865	Present	0.438	Present
BC_2 03R 37 x 53-4	Female	0.344	Absent	0.247	Absent
BC_2 03R 37 x 53-5	Male	0.934	Present	0.618	Present
BC_2 03R 37 x 53-6	Male	0.358	Absent	0.262	Absent
BC_2 03R 37 x 53-7	Female	0.290	Absent	0.405	Absent
BC_2 03R 37 x 53-8	Male	0.362	Absent	0.840	Present
BC_2 03R 37 x 53-9	Female	1.133	Present	0.272	Absent
BC_2 03R 37 x 53-10	Male	0.950	Present	0.618	Present
BC_2 03R 37 x 53-11	Male	0.391	Absent	0.250	Absent
BC_2 03R 37 x 53-12	Female	0.540	Present	0.274	Absent
BC_2 03R 37 x 53-13	Female	0.343	Absent	0.414	Absent
BC_2 03R 37 x 53-14	Female	0.588	Present	0.600	Present
BC_2 03R 37 x 53-15	Female	0.602	Present	0.325	Absent

* *1st ELISA test after five months of exposure to natural infection*
2nd ELISA test after seven months of exposure to natural infection

Table 8. Enzyme Linked Immunosorbent Assay (ELISA) of BC_3 papaya plants using old and new/young leaf planted in Mainit, Bay, Laguna.

Sample	Sex	New Leaf	PRSV-P Reaction	Old Leaf	PRSV-P Reaction
Negative Control	-	0.286	Absent	-	
410-1	Male	0.336	Absent	0.411	Absent
410-2	Male	0.435	Absent	0.365	Absent
BC_1 (03R)	Male	0.278	Absent	0.401	Absent
Davao Solo (+)	Hermaphrodite	0.801	Present	-	-
5648	Female	0.432	Absent	1.097	Present
BC_3 03R 34-1	Male	0.268	Absent	0.321	Absent
BC_3 03R 34-2	Male	0.321	Absent	0.882	Present
BC_3 03R 86-1	Female	0.413	Absent	1.205	Present

BC_3 03R 86-2	Male	0.436	Absent	0.545	Absent
BC_3 03R 86-4	Female	0.266	Absent	1.304	Present
BC_3 03R 86-5	Female	0.327	Absent	1.372	Present
BC_3 03R 86-6	Female	0.260	Absent	1.103	Present
BC_3 03R 86-7	Male	0.251	Absent	0.493	Absent
BC_3 03R 73-11	Male	0.239	Absent	0.921	Present
BC_3 03R 73-2[1]	Female	0.293	Absent	1.067	Present
BC_3 03R 73-3[1]	Male	0.306	Absent	0.438	Absent
BC_3 03R 73-4[1]	Male	0.272	Absent	2.193	Present
BC_3 03R 73-5[1]	Male	0.261	Absent	1.783	Present
BC_3 03R 73-6[1]	Female	0.268	Absent	0.877	Present
BC_3 03R 73-7[1]	Male	0.366	Absent	0.384	Absent
BC_3 03R 73-8[1]	Female	0.428	Absent	1.493	Present
BC_3 03R 73-9[1]	Female	0.305	Absent	0.355	Absent
BC_3 03R 73-10[2]	Female	0.399	Absent	0.716	Present
BC_3 03R 73-11[2]	Female	0.215	Absent	0.316	Absent
BC_3 03R 73-12[2]	Male	0.235	Absent	0.314	Absent
BC_3 03R 73-13[2]	Male	0.239	Absent	0.271	Absent
BC_3 03R 73-14[2]	Male	0.253	Absent	1.039	Present
BC_3 03R 73-15[2]	Male	0.306	Absent	1.173	Present
BC_3 03R 73-16[2]	Male	0.231	Absent	0.239	Absent
BC_3 03R 73-17[2]	Female	0.795	Present	1.189	Present
BC_3 03R 73-18[2]	Male	0.436	Absent	0.803	Present
BC_3 03R 73-19[2]	Female	0.311	Absent	0.338	Absent
BC_3 03R 73-20[2]	Male	0.365	Absent	1.493	Present
BC_3 03R 73-21[2]	Male	0.231	Absent	0.305	Absent
BC_3 03R 73-22[2]	Male	0.913	Present	1.934	Present
BC_3 03R 73-23[2]	Male	0.226	Absent	0.446	Absent
BC_3 03R 73-24[1]	Female	0.255	Absent	0.794	Present
BC_3 03R 73-25[1]	Male	0.309	Absent	0.586	Present
BC_3 03R 73-26[1]	Male	0.266	Absent	1.273	Present
Threshold value	0.571				

Table 9 . Enzyme Linked Immunosorbent Assay of BC_3 sib-cross plants after nine months in the field

Sample	Sex	Symptom	Absorbance reading 405 nm	PRSV-P Reaction
410 (-)	Male	-	0.192	Absent
Davao Solo (+)	Hermaphrodite	+	0.662	Present
BC_3 03R 4 x 2 -1	Female	+	0.255	Absent
BC_3 03R 4 x 2 -8	Male	+	0.222	Absent
BC_3 03R 4 x 2 -12	Female	+	0.322	Absent
BC_3 03R 4 x 2 -13	Male	+	0.193	Absent
BC_3 03R 4 x 2 -16	Female	+	0.358	Absent
BC_3 03R 4 x 2 -19	Female	+	0.280	Absent
BC_3 03R 73 6 x 5- 1	Female	+	0.341	Absent
BC_3 03R 73 6 x 5- 2	Male	+	0.378	Absent
BC_3 03R 73 6 x 5- 3	Male	+	0.331	Absent

BC_3 03R 73 6 x 5- 4	Male	+	0.307	Absent
BC_3 03R 73 6 x 5- 5	Male	+	0.450	Present
BC_3 03R 73 6 x 5- 6	Male	+	0.291	Absent
BC_3 03R 73 6 x 5- 7	Male	+	0.390	Present
BC_3 03R 73 6 x 5- 8	Male	+	0.301	Absent
BC_3 03R 73 6 x 5- 9	Male	+	0.331	Absent
BC_3 03R 73 6 x 5- 10	Male	+	0.522	Present
BC_3 03R 73 6 x 7- 1	Female	+	0.235	Absent
BC_3 03R 73 6 x 7- 2	Female	+	0.235	Absent
BC_3 03R 73 6 x 7- 3	Male	+	0.432	Present
BC_3 03R 73 6 x 7- 4	Male	+	0.493	Present
BC_3 03R 73 6 x 7- 5	Female	+	0.351	Absent
BC_3 03R 73 6 x 7- 6	Female	+	0.468	Present
BC_3 03R 73 6 x 7- 7	Female	+	0.193	Absent
BC_3 03R 73 6 x 7- 8	Female	+	0.333	Absent
BC_3 03R 73 6 x 7- 9	Male	+	0.319	Absent
BC_3 03R 73 6 x 7- 10	Male	+	0.578	Present
BC_3 03R 73 6 x 7- 11	Female	+	0.342	Absent
BC_3 03R 73 6 x 7- 12	Female	+	0.357	Absent
BC_3 03R 73 6 x 7- 13	Male	+	0.380	Absent
BC_3 03R 73 6 x 7- 14	Male	+	0.192	Absent
BC_3 03R 86 1 x 7-1	Female	+	0.425	Present
BC_3 03R 86 1 x 7-5	Female	+	0.224	Absent
BC_3 03R 86 1 x 7-6	Female	+	0.205	Absent
BC_3 03R 86 1 x 7-7	Male	+	0.336	Absent
BC_3 03R 86 1 x 7-10	Female	+	0.308	Absent
BC_3 03R 86 1 x 7-11	Female	-	0.214	Absent
BC_3 03R 86 1 x 7-14	Female	+	0.215	Absent
BC_3 03R 86 1x 7-15	Male	-	0.238	Absent
BC_3 03R 86 1x 7-16	Male	+	0.502	Present
BC_3 03R 86 1x 7-17	Female	+	0.262	Absent
BC_3 03R 86 1x 7-18	Female	+	0.248	Absent
BC_3 03R 86 1x 7-19	Male	+	0.385	Present
BC_3 03R 86 1x 7-20	Female	+	0.236	Absent
BC_3 03R 86 5 x 2- 1	Female	+	0.243	Absent
BC_3 03R 86 5 x 2- 2	Female	+	0.203	Absent
BC_3 03R 86 5 x 2- 3	Male	+	0.235	Absent
BC_3 03R 86 5 x 2- 4	Male	+	0.408	Present
BC_3 03R 86 5 x 2- 5	Female	+	0.258	Absent
BC_3 03R 86 5 x 2- 6	Male	+	0.258	Absent
Threshold Value	0.383			

Table 10. Enzyme Linked Immunosorbent Assay of BC_4 plants after nine months of exposure in the field

Sample	Sex	Symptom	Absorbance reading 405 nm	PRSV-P Reaction
410	Male	-	0.205	Absent
Davao Solo (+)	hermaphrodite	+	0.628	Present
BC_4 03R 34 - 1- 6	Male	+	0.292	Absent
BC_4 03R 34 - 1- 8	Female	+	0.195	Absent
BC_4 03R 34 - 1- 9	Female	+	0.433	Present
BC_4 03R 34 - 1- 14	Male	+	0.241	Absent
BC_4 03R 34 - 1- 16	Female	+	0.252	Absent
BC_4 03R 73-1-1	Male	+	0.244	Absent
BC_4 03R 73-1-4	Female	+	0.281	Absent
BC_4 03R 73-1-5	Female	+	0.228	Absent
BC_4 03R 73-1-7	Female	+	0.245	Absent
BC_4 03R 73-1-10	Female	+	0.201	Absent
BC_4 03R 73-1-11	Male	+	0.253	Absent
BC_4 03R 73-1-12	Male	+	0.238	Absent
BC_4 03R73-1-13	Female	+	0.268	Absent
BC_4 03R73-1-15	Female	+	0.597	Present
BC_4 03R 73-1-16	Female	+	0.283	Absent
BC_4 03R 73-1-17	Male	+	0.225	Absent
BC_4 03R 73-1-18	Female	+	0.362	Absent
BC_4 03R 73-3-2	Female	+	0.434	Present
BC_4 03R73-3-3	Female	+	0.572	Present
BC_4 03R 73-3-5	Female	+	0.214	Absent
BC_4 03R 73-3-7	Female	-	0.256	Absent
BC_4 03R 73-3-8	Female	-	0.246	Absent
BC_4 03R 73-3-10	Female	+	0.341	Absent
BC_4 03R 73-3-11	Female	+	0.297	Absent
BC_4 03R 73-3-12	Female	+	0.543	Present
BC_4 03R 73-3-13	Male	+	0.419	Present
BC_4 03R 73-3-14	Male	+	0.185	Absent
BC_4 03R 73-3-18	Female	+	0.764	Present
BC_4 03R 73-3-21	Female	+	0.680	Present
BC_4 03R 73-3-22	Female	+	0.407	Absent
BC_4 03R 73-3-23	Female	+	0.350	Absent
BC_4 03R 73-3-24	Male	+	0.466	Present
BC_4 03R 73-3-25	Female	+	0.467	Present
BC_4 03R 73-3-26	Female	+	0.439	Present
BC_4 03R 73-14-1	Female	+	0.598	Present
BC_4 03R 73-14-2	Female	+	0.281	Absent
BC_4 O3R 73-14-3	Female	+	0.458	Present
BC_4 03R 73-14-6	Female	+	0.584	Present
BC_4 03R 73-14-7	Male	+	0.184	Absent

BC_4 03R 73-14-12	Male	+	0.214	Absent
BC_4 03R 73-14-13	Female	+	0.249	Absent
BC_4 03R 73-14-14	Female	+	0.257	Absent
BC_4 03R 73-14-18	Female	+	0.253	Absent
BC_4 03R 73 - 16- 1	Female	+	0.233	Absent
BC_4 03R 73 - 16- 3	Female	+	0.276	Absent
BC_4 03R 73 - 16- 4	Male	+	0.575	Present
BC_4 03R 73 - 16- 6	Female	+	0.750	Present
BC_4 03R 73 - 16- 7	Female	+	0.430	Present
BC_4 03R 73 - 16- 8	Female	+	0.520	Present
BC_4 03R 73 - 16- 9	Female	+	0.344	Absent
BC_4 03R 73 - 16- 15	Male	+	0.249	Absent
BC_4 03R 73 - 16- 17	Female	+	0.359	Absent
BC_4 03R 86 - 2 - 1	Female	+	0.412	Present
BC_4 03R 86 - 2 - 2	Female	+	0.232	Absent
BC_4 03R 86 - 2 - 4	Male	+	0.332	Absent
BC_4 03R 86 - 2 - 6	Male	+	0.305	Absent
Threshold value	0.381			

Table 11. Enzyme Linked Immunosorbent Assay (ELISA) of F1 re-constituted Sinta, a cross between female BC_2 plant and hermaphrodite IPB inbred line, 4172 maintained in a farmer's field in Batangas for about one year and eight months.

Lines	Absorbance 405 nm		Absorbance 405 nm	
	Infected Old Leaf	PRSV-P Reaction	Young Leaf	PRSV-P Reaction
410 (-)	--	--	0.383	Absent
Davao Solo (+)	2.268	Present	--	--
Sinta (Original)	2.221	Present	1.735	Present
BC_2 03R 37 x 4172-2	3.356	Present	2.629	Present
BC_2 03R 37 x 4172-3	2.117	Present	0.791	Present
BC_2 03R 37 x 4172-4	3.470	Present	1.416	Present
BC_2 03R 37 x 4172-5	3.242	Present	2.107	Present
BC_2 03R 37 x 4172-7	1.383	Present	0.922	Present
BC_2 03R 37 x 4172-8	1.052	Present	0.499	Absent
BC_2 03R 37 x 4172-9	3.438	Present	0.487	Absent
BC_2 03R 37 x 4172-10	2.622	Present	2.104	Present
BC_2 03R 37 x 4172-15	2.235	Present	1.381	Present
BC_2 03R 37 x 4172-17	2.460	Present	3.480	Present
BC_2 03R 37 x 4172-18	3.500	Present	2.807	Present
BC_2 03R 38 x 4172-7	2.611	Present	0.770	Present
BC_2 03R 38 x 4172-10	1.901	Present	0.408	Absent
BC_2 03R 38 x 4172-17	3.349	Present	3.167	Present
BC_2 03R 31 x 4172-3	3.498	Present	0.404	Absent
BC_2 03R 31 x 4172-11	1.933	Present	1.660	Present
BC_2 03R 31 x 4172-14	2.416	Present	0.482	Absent
BC_2 03R 31 x 4172-15	3.247	Present	2.695	Present
BC_2 03R 31 x 4172-16	3.388	Present	1.150	Present
BC_2 03R 31 x 4172-18	2.932	Present	1.813	Present

BC_2 03R 31 x 4172-19	2.571	Present	1.085	Present
BC_2 03R 78 x 4172-11	2.384	Present	1.071	Present
BC_2 03R 78 x 4172-13	2.929	Present	1.205	Present
BC_2 03R 78 x 4172-17	3.088	Present	0.740	Absent
BC_2 03R 78 x 4172-18	2.594	Present	0.746	Absent
BC_2 03R 78 x 4172-19	1.006	Present	0.609	Absent
BC_2 03R 78 x 4172-20	2.037	Present	0.923	Present
Threshold Value	0.766			

Table 12. Fruit qualities of three backcross generations (BC_2, BC_3 and BC_4) evaluated in IPB experimental field

Line and Generation	Number of fruits evaluated	Average Fruit weight (g)	Flesh Color	Aroma	Average TSS (°B) value	Taste	Flesh Firmness	Fruit length (cm)	Fruit width (cm)
BC_4 03R 34-1	7	733.69	Yellow orange	mild	11.54	Not so sweet-Sweet	Firm	12.04	11.54
BC_4 03R 73-1	11	879.21	Yellow orange	mild	12.25	Not so sweet-Sweet	Firm	12.31	12.46
BC_4 03R 73-3	20	907.08	Yellow orange	mild	11.45	Not so sweet-Sweet	Firm	11.93	12.77
BC_4 03R 73-14	7	859.76	Yellow orange	mild	11.83	Not so sweet-Sweet	Firm	11.84	12.27
BC_4 03R 73-16	17	743.20	Yellow orange	mild	12.53	Not so sweet-Sweet	Firm	11.79	11.81
BC_3 03R 73	13	868.69	Yellow orange	mild	11.58	Not so sweet-Sweet	Firm	12.68	12.33
BC_3 03R 86 1 x 7	9	1145.53	Yellow orange	mild	11.31	Not so sweet-Sweet	Firm	13.63	13.42
BC_3 03R 86 4 x 2	2	1490.50	Yellow orange	mild	11.50	Not so sweet-Sweet	Firm	16.20	14.8
BC_2 03R -37	-	964.20	Yellow orange	mild	12.00	Sweet	Firm	12.00	13.2
BC_2 03R -38	-	585.50	Yellow orange	mild	12.00	Sweet	Firm	9.30	11.00
BC_2 03R -74	-	540.80	Yellow orange	mild	12.00	Sweet	Firm	10.00	10.70
BC_2 03R -78	-	1266.10	Yellow orange	mild	12.83	Sweet	Firm	12.50	13.90
BC_2 03R -96	-	653.40	Yellow orange	mild	11.33	Not so sweet-Sweet	Firm	11.00	11.70
BC_2 03R 34 x 37	1	597.80	Yellow orange	mild	12.40	Sweet	Firm	10.00	10.80
BC_2 03R 37 x 53	4	755.80	Yellow orange	mild	12.20	Sweet	Firm	10.10	12.40
BC_2 03R 96 x 86	2	723.10	Yellow orange	mild	12.50	Sweet	Firm	10.10	11.90
Davao Solo	3	573.93	Yellow orange	strong	10.10	Not sweet	Firm	11.60	10.03

Table 13. Fruit qualities of F1 re-constituted 'Sinta' in comparison to the original 'Sinta' planted in a farmer's field.

Line	Number of fruits evaluated	Average Fruit weight (g)	Flesh Color	Aroma	Average TSS (^oB) value	Taste	Flesh Firmness	Fruit length (cm)	Fruit width (cm)
BC_2 03R 31 x 4172	34	1257.85	Yellow orange	mild	9.80	Not sweet	Firm	19.50	12.03
BC_2 03R 37 x 4172	39	1160.95	Yellow orange	mild	10.20	Not so sweet	Firm	18.24	11.46
BC_2 03R 38 x 4172	30	1098.41	Yellow orange	mild	10.10	Not so sweet	Firm	19.23	11.03
BC_2 03R 78 x 4172	39	1312.49	Yellow orange	mild	9.94	Not so sweet	Firm	19.41	12.11
Sinta	10	1062.51	Yellow orange	mild	11.00	Not so sweet	Firm	18.07	11.17

Figure 6. Photos of F1 re-constituted 'Sinta' a cross between female BC_2 plant and hermaphrodite IPB inbred line, 4172 planted in a farmer's field in Batangas for about 1 year and 8 months.

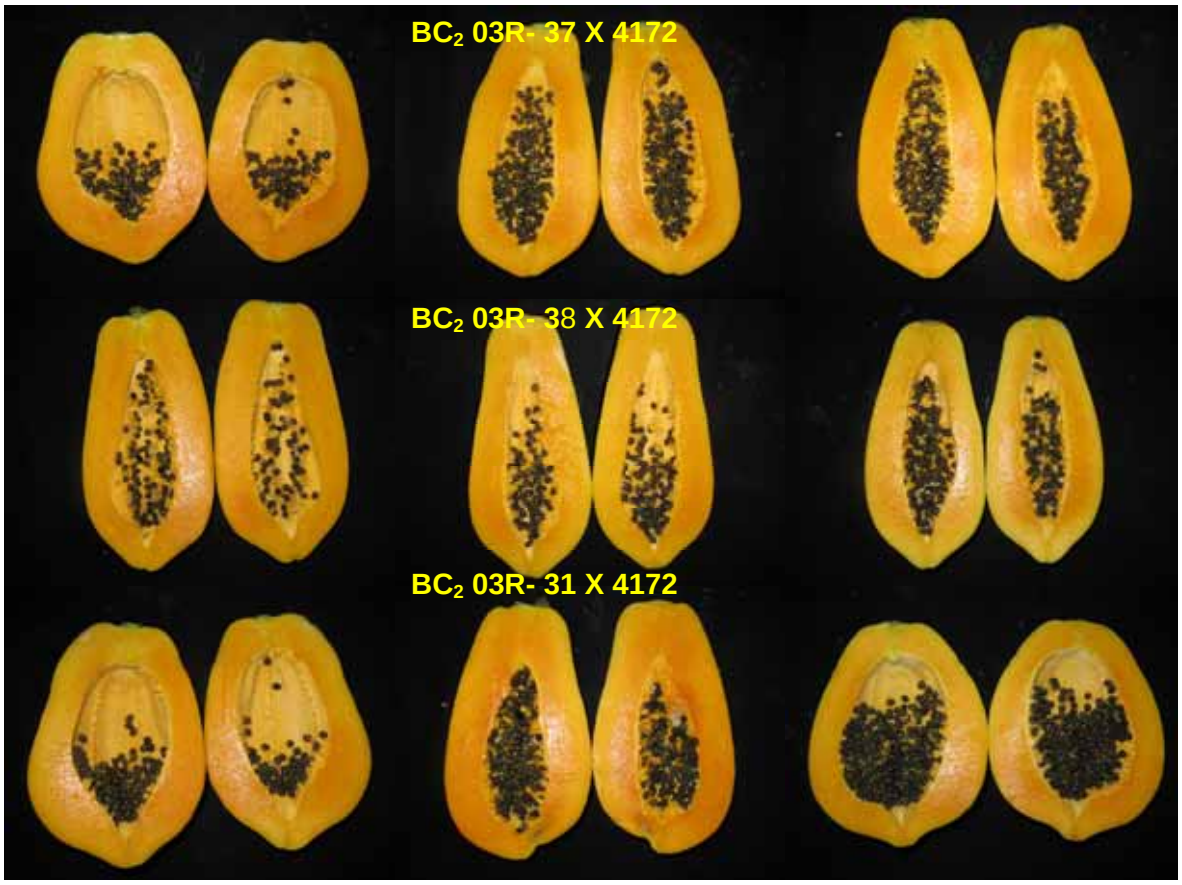

Figure 7. Flesh of re- constituted 'Sinta'.

Molecular Characterization

Verification of the introgression of resistance gene(s) was done by PCR using a new set of Primers, MANAM 1/2. This marker is the result of continuous attempt to develop SSR

marker that could be used to fingerprint the introgression of the resistant trait from *V. quercifolia* to the BC_2 and F_1 intergeneric hybrid lines.

Initial results showed that the *V. quercifolia,* (lines 9-10, Fig. 8) produced 4 distinct bands with sizes at 150 (designated as Q150), 200 (designated as Q200), 300 and 800 bp as estimated in gel electrophoresis. BC_2 (Figure 8, lines 2-8) and BC_2 sib-cross plants (Figure 9) that showed high tolerance against PRSV-P produced a very distinct band at around 500 bp and a faint band at around 100 bp. The F_1 intergeneric hybrids (lines 11-18) had very distinct band patterns, which are more or less, a combination of band patterns from parental V. quercifolia and C. papaya. F_1 intergeneric hybrids contained the ubiquitous H400, the distinguishing Q150, Q200 bands introgressed from *V. quercifolia* and the serendipitous H100 band, which is observable in the *C. papaya* hybrids, which show resistance to PRSV-P. The F1 hybrids identified to have shown resistance to PRSV-P lines were confirmed by ELISA, and had the presence of the H100 band. The 100bp band (H100) was found to be common in resistant lines of the intergeneric hybrids and BC_2 lines that showed high tolerance against PRSV-P. However, the BC_2 resistant lines did not produce the Q150 and Q200 bands, and V. quercifolia did not produce the H100 bands.

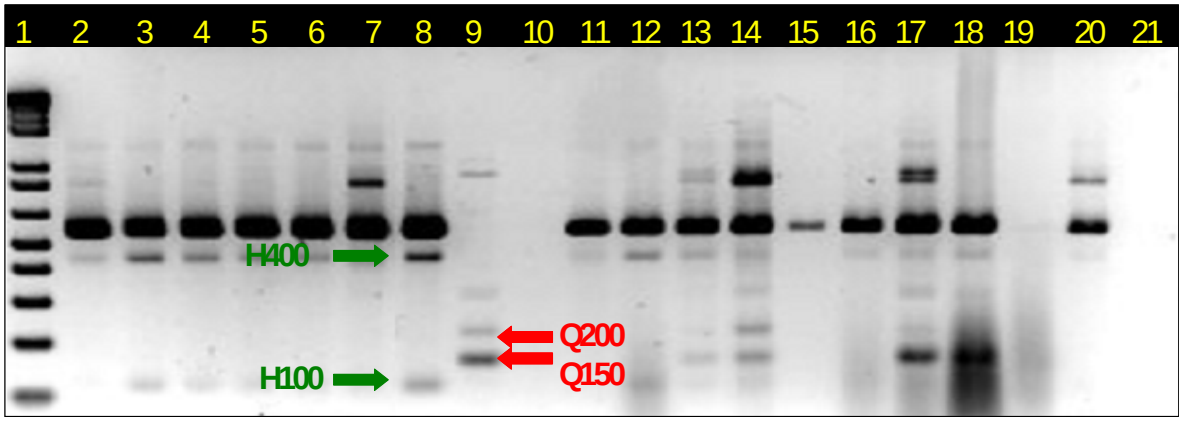

Figure 8. DNA banding pattern following Polymerase Chain Reaction of total genomic DNA from *V. quercifolia*, F_1 intergeneric hybrid, and C. papaya plants using SSR FP1 & RP2 primer. Well 2-8 (BC_2 plants), 9-10 (V. quercifolia), 11-18 (F1 intergeneric hybrids), 19 (BC_1 plants), 20 (Davao Solo) and 21 (negative control). The green (hybrids) and red (*V. quercifolia*) arrows represent the polymorphic bands submitted for sequencing.

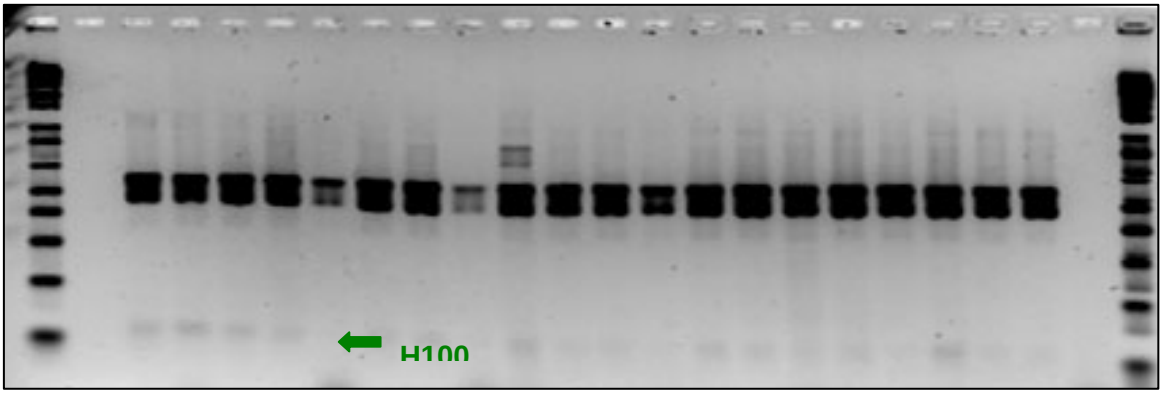

Figure 9. DNA banding pattern following polymerase chain reaction of total genomic DNA from sib-cross plants exhibiting PRSV-P resistance showing the H100 polymorphic band.

DNA Sequencing

Prior to DNA sequencing, it was hypothesized that the H100 band observed in the backcross plants is a fragment of that Q150 band. This Q150 band is consistently present in the resistant F1 intergeneric hybrids 410, 468, and 469 and is assumed to be the band that indicates the resistance against PRSV-P. If this hypothesis was found to be true, this would explain the good resistance observed on the BC_2 and BC_2 sib-cross plants.

To prove that H100 is the marker showing PRSV-P resistance, the purified PCR products from the hybrid plants (labeled as H100 and H400) and *V. quercifolia* (labeled as Q150 and Q200) were submitted for sequencing. Using the Vector NTI, the forward and complementary sequences were assembled to determine the overlapping portions of the fragments.

Results revealed that Q150 and Q200 have high sequence similarity, 85 % (Figures 10 and 11). Q150 and Q200 bands were common in the *V. quercifolia* and the PRSV-P resistant F_1 intergeneric lines.

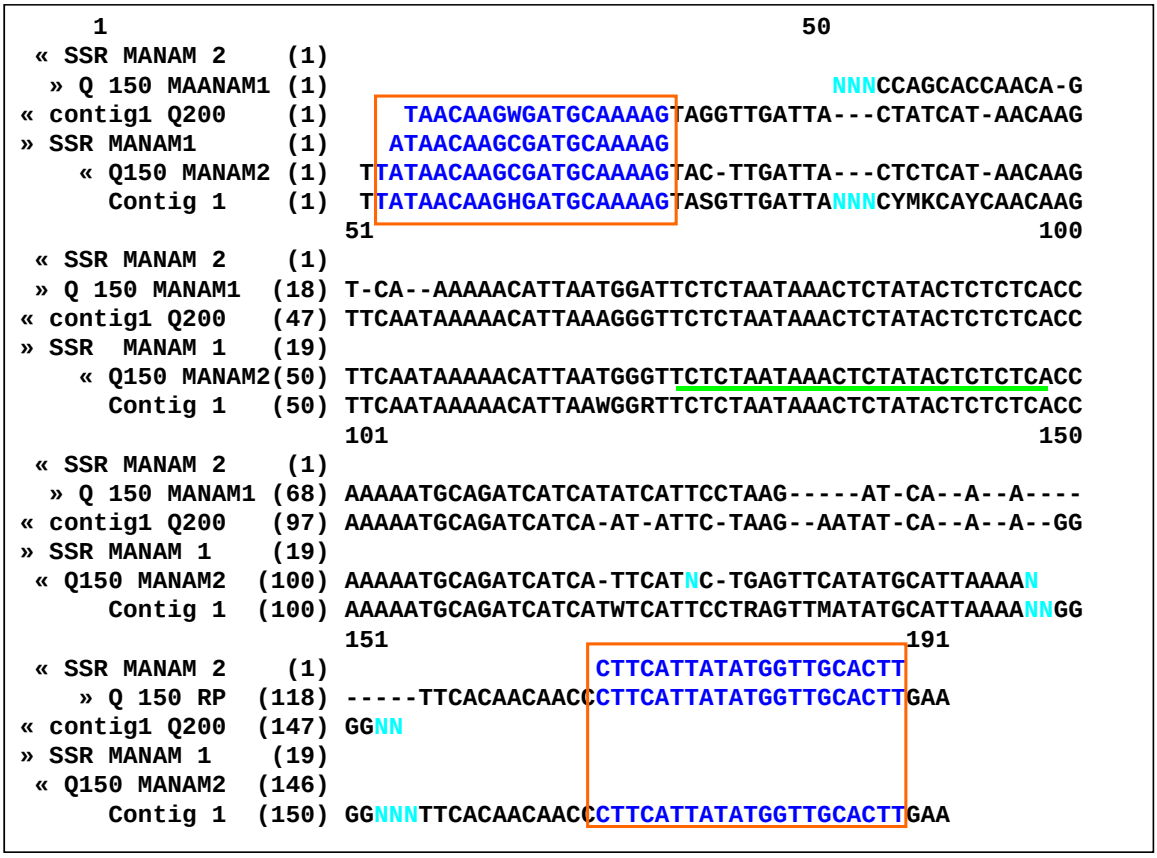

Figure 10. Diagram of contig assembly and multiple sequence alignment of Q150 and Q200 (contig 1) illustrating that Q200 is a dimerized form of Q150

In general, initial results of molecular evaluation showed that the old primers (Cpy) used in the previous experiments could only detect transfer of resistance up to F_1 intergeneric hybrids only. While the new set of primers (MANAM ½) could be used to fingerprint the introgression of resistance from *V. quercifolia* up to the backcross generations.

This primer MANAM ½ however was based only on the identified tandem repeat found in the exon region of the C. papaya cysteine proteinase gene (Genbank No.M15203) or papain (CPAPAP). A more reliable and accurate primer designs could be made if it is based on *the C. papaya* and Vasconcellea genome.

Testing of several new sets of primers is also in progress. These new sets of primers are based on *C. papaya*. They are being evaluated for their efficiency in tracking the introgressed gene(s) from *V. quercifolia* to the backcross papaya plant.

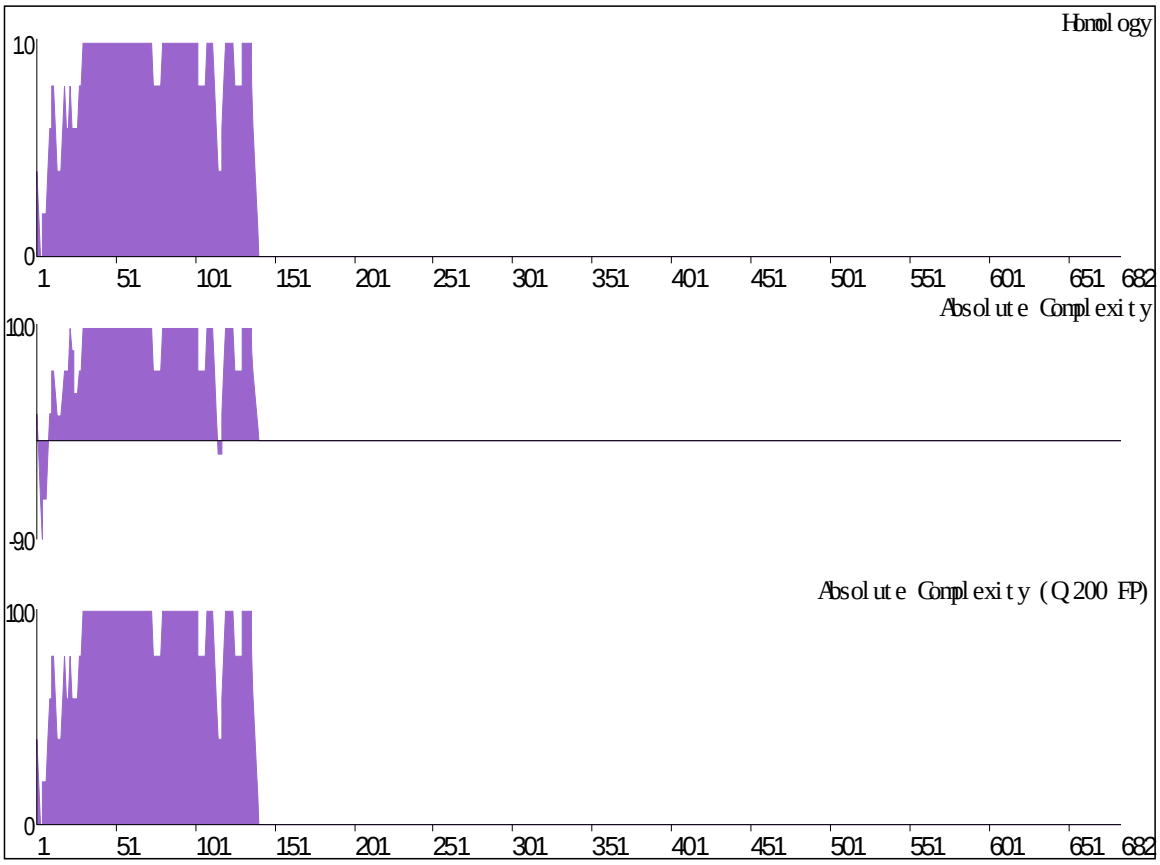

Figure11. Sequence alignment showing Q150 and Q200 as dimmers with 85 % sequence similarity.

DNA sequencing results also revealed that there was a noted 53% similarity between the H100 band and Q150 and Q200 (Figure 11). However, to have a more accurate findings, cloning of the H100 prior to sequence analyses is better suggested to determine the exact nucleotide sequence.

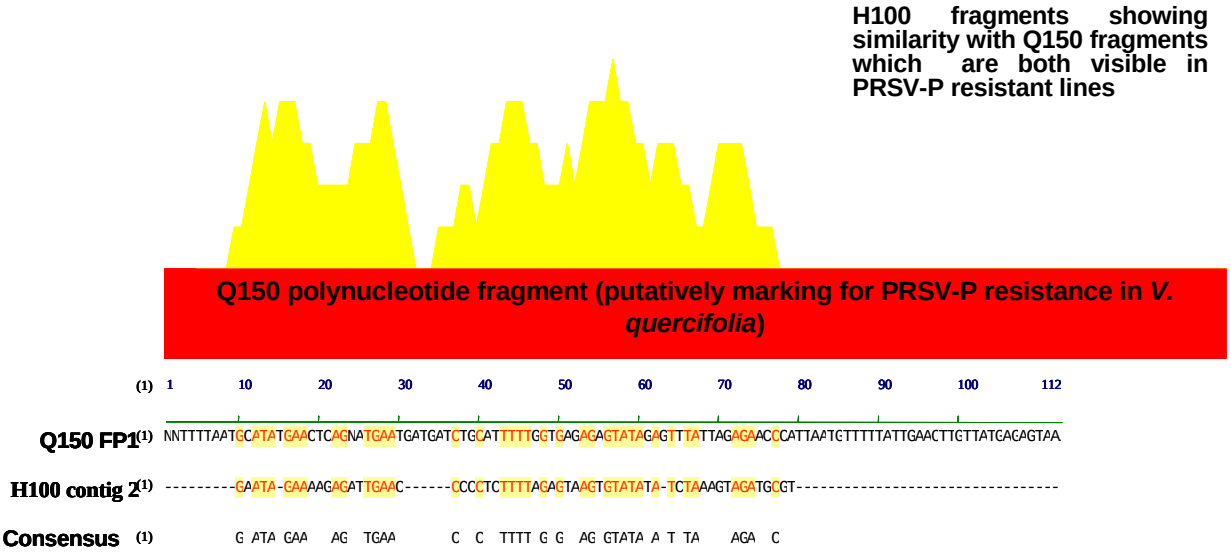

Figure 12. Diagram of sequence alignments of Q150 and H100 polynucleotide sequences isolated from V. quercifolia (Q150) and hybrid lines (H100) with ELISA confirmed PSRV-P resistance. The homology and absolute complexity of H100 (contig 2) against the Q150 is at 53%.

8 Impacts

8.1 Scientific impacts – now and in 5 years

The project was able to produce the first resistant PRSV-P papaya from conventional breeding. It is actually a major breakthrough and the first to be reported after 50 years of research work on papaya gene introgression. The production of new genotype will have a tremendous impact to future breeding program for papaya. Thus, this papaya genotype is expected to revitalize the weakened Philippine papaya industry.

Collaboration with other project was done. This project aims to combine the two noble characters (resistance to papaya ringspot virus and delayed ripening traits) to papaya, thus increasing the shelf life of the crop.

8.2 Capacity impacts – now and in 5 years

The team was able to purchase a generator for the laboratory from the project funds. Purchasing this equipment was in response to what we had experienced last September, 2006 after Typhoon "Milenyo" where we didn't have electricity for about two months. Most of the cultures being maintained were contaminated after that dreadful incidence. This generator will not only be useful to the project since other projects whose cultures where housed in the laboratory will also benefit from the said equipment.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

The development of PRSV-P resistant genotypes will restore the papaya industry in Luzon and the other regions of the Philippines where the industry has been destroyed by the virus. New papaya genotypes that will enrich and develop the industry will be made available. Outputs from the project would be of large benefit to the growers because the industry will be restored, the production will increase, more farmers will be planting papaya, the total hectarage will expand, and, that will lead to a considerable increase in farmer' income.

8.3.2 Social impacts

Before the occurrence of PRSV-P, papaya was a major cash crop. The direct effect of this notorious disease is the devastation of the papaya industry in so many growing areas in the Philippines that led to tremendous economic losses. The damage had caused loss of quality income or reduction in family income of papaya growers. Thus, to support the need of the whole family, another member of the family frequently the wife or one of the children who may have been a school drop out due to financial problems often have to work away from their families. The improvement in papaya production through the use of a PRSV-P resistant line should reduce such social stresses on the family unit.

8.3.3 Environmental impacts

There will be no concerns related to potential-cross contamination of other papayas in the surrounding environment because the PRSV-P resistant lines that will be produced in this project will a product of conventional plant breeding. The resistant gene(s) will be incorporated into the acceptable papaya cultivar by cross pollination and it will come from another wild papaya species, not from totally unrelated organisms (eg. bacterium). No other foreign genes will be introduced into the expected PRSV-P resistant line.

Another important contribution to protection of the environment is that PRSV-P resistant lines will require reduced application of pesticides, thus, they are more environment friendly.

8.4 Communication and dissemination activities

The team has regularly disseminated information on the progress of the project through publications, presentation of research through posters and oral presentations in scientific meetings and interviews.

9 Conclusions and recommendations

9.1 Conclusions

The disease had a major impact on the level of production of papaya in the Philippines. Control of the disease through this project is expected to have a relatively high pay-off. The good performance of the backcross plants against the notorious Papaya Ringspot virus could mean the restoration of the Philippine papaya industry (especially to those regions highly devastated by the disease).

In addition, the development of a variety which is resistant to t PRSV-P has potential to reduce the unit cost of papaya production substantially. It is also expected that the resistant papayas will have a potential impact on yield; increase in yield will also be substantial.

The restoration of an important cash crop would have obvious economic benefits for Filipino farmers. As papaya is an export crop for the Philippines, its economy would also benefit from a successful outcome of this project.

9.2 Recommendations

It would be best to know the genetic or the amount and distribution of genetic variations within and among populations of the pathogen. Defining the genetic structure of population is a logical first step in studies of pathogen population genetics (i.e. virus, fungi) because the genetic structure reflects its evolutionary history and its potential to evolve.

Relating this to the newly developed resistant papaya genotype, knowing the pathogen population structure is important in breeding stable forms of resistance and developing of strategies for deploying resistant varieties

Thus, it is recommended to test the resistance/susceptibility of this resistant papaya to the different regions here in the Philippines. By doing this, one can know whether the plants are resistant to all possible strains of PRSV-P here in the Philippines and if it's not, this will also give ideas on how to properly deploy the papaya plants thus prolonging its resistance to the said virus.

Continuous study about the population structure of plant pathogens is also essential because pathogens continuously evolve or constantly adapt to changes in their environment for survival. Thus it is not enough to focus only on creating resistant varieties. Understanding the diversity of the pathogen is also a critical factor for it to be effective.

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10.2 List of publications produced by project

10.2.1 Publications

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10.2.2 Paper Presentations/Lectures

Siar, S.V., Beligan, G.A., Sajise, A.G.C., Laurena, A.C., Drew, R.A. and O'Brien, C.M. 2008. Field Screening of BC_3 and BC_2 Sib-Cross Papaya Plants Introgressed with PRSV-P Resistance. Poster paper presented in the 39th Annual Scientific Meeting of Pest Management Council of the Philippines held in Austurias Hotel. May 6-9. 75th Annual Scientific Meeting of National Research Council of the Philippines (NRCP) held in Manila Hotel, Philippines. March 12.

Siar, S.V., Sajise, A.G.C., Beligan, G.A., Laurena, A.C., Drew, R.A. and O'Brien, C.M. 2007. Performance of the Second Backcross (BC_2) Generation of Papaya against Papaya Ringspot Virus (PRSV-P) and Evaluation of Agronomic Traits. Poster paper presented in the 29th Annual Scientific Meeting of National Academy of Science and Technology (NAST) with a theme: "A progressive Philippines Anchored in Science: Building A Culture Of Science In the Philippines" held in Manila Hotel, Philippines. July 11-12.

Siar, S.V., A.G.C., Laurena, Sajise, A.C., Beligan, G.A., and Tan Gana N.H. 2007. SSR Primer Pairs "MANAM 1/2" Confirmed Introgression of Papaya Ringspot Virus Resistance (PRSV-P) from *Vasconcellea spp.* to *Carica papaya. Poster paper* presented in the 29th Annual Scientific Meeting of National Academy of Science and Technology (NAST) with a theme: "A progressive Philippines Anchored in Science: Building a Culture of Science in the Philippines" held in Manila Hotel, Philippines. July 11-12.

Siar, S.V., Sajise, A.G.C., Beligan, G.A., Laurena, A.C., Tan Gana, N.H., Drew, R.A. and O'Brien, C.M. 2007. Agronomic, Serological, and Molecular Evaluation of BC2 papaya Introgressed with PRSV-P Resistance. Oral paper presented in the 38th Annual Scientific Meeting of Pest Management Council of the Philippines with a theme: " The Role of Pest Management in harnessing Biodiversity to Support Eco-Tourism" held in Bohol Tropics Resort, Tagbiliran City, Bohol, Philippines. March 20-23.

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Drew, R.A., Siar, S.V., O'Brien, C.M. and Sajise, A.G.C. 2004. Development of PRSV-P Resistant Papaya Genotypes by Introgression of Genes from wild *Vasconcellea* species. Oral paper presented at Australian Society for Horticulture Science and the New Zealand Society for Horticulture Science joint conference at the Hyatt Coolum, Sunshine Coast. Queensland, Australia.September 1-3.

Sajise A.G.C., Siar, S.V., and Sangalang, J.B. 2004. Cross Compatibility of Elite Papaya Inbred Lines to an Intergeneric Hybrid of *Carica papaya L.* x *Vasconcellea quercifolia* (Saint-Hil) *Hieron*. Poster paper presented at the Australian Society for Horticulture Science and the New Zealand Society for Horticulture Science joint Conference at the Hyatt Coolum, Sunshine Coast, Queensland, Australia. September 1-3 and the 34th International Crop Science Conference at the Brisbane Convention Scientific Conference of Crop Science Society of the Philippines. Insular Waterfront Hotel, Davao City. March 8-12.

10.2.3 Awards

2008 2nd Best Poster. Field Screening of BC_3 and BC_2 Sib-Cross Papaya Plants Introgressed with PRSV- P Resistance in the 75th Annual Scientific Meeting of National Research Council of the Philippines (NRCP) held in Manila Hotel, Philippines. March 12.

2005 Grand Prize Winner. Philippine Emerging start-Ups Open (PESO) Challenge (A technology) Innovation Business Plan Competition.

2004 PAARFI R and D Research Award. Cross Compatibility of Elite Papaya Inbred Lines to an Interspecific Hybrid of *Carica papaya L. x Carica Quercifolia* (Saint-Hil) Hieron, Naga City. July 26.

2004 Finalist Best paper Upstream Research Category. Cross Compatibility of Elite Papaya Inbred Lines to an Interspecific Hybrid of *Carica papaya L. x Carica Quercifolia* (Saint-Hil) *Hieron*, 34th Annual Scientific Conference Of Crop Science Society of the Philippines. Insular Waterfront Hotel, Davao City,March 8-12.

11 Appendixes

11.1 Appendix 1: Davao Solo showing typical symptoms produced by the virus

Severely infected Davao solo with symptoms ranging from distortion of young leaves, mosaic, chlorosis to shoe-stringed on older leaves

11.2 Appendix 2: F1 intergeneric hybrid line, 410. A cross between *Carica papaya* and *Vasconcllea quercifolia*

Grafted F1 Intergeneric Hybrid Line, 410

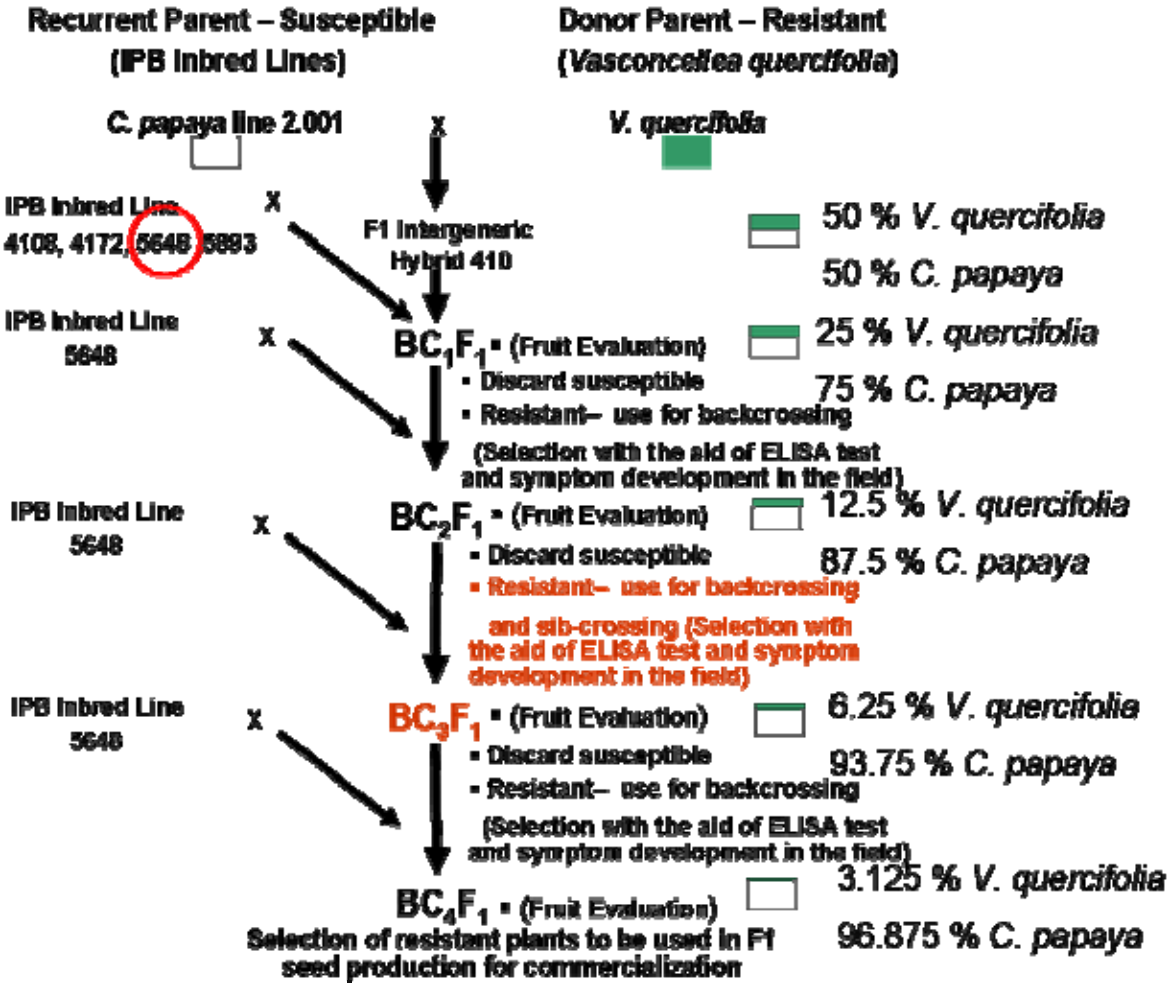
11.3 Appendix 3. Some of the IPB Inbred lines used in the backcrossing program

PARENTAL PAPAYA INBRED LINES

PRSV-P RESISTANT LINES OF INTERGENERIC

11.4 Appendix 4. Method used in producing PRSV-P papaya genotype with good agronomic characteristics

Development of PRSV-P resistant genotypes with good agronomic traits thru backcrossing



11.5 Appendix 5: Pictures of backcross papaya plants that showed good resistance against Papaya Ringspot virus (PRSV-P)

PRSV Resistant Male BC1 Papaya Plant (5648 x 410)

65% pollen fertility

BC2 Resistant Male Plant

BC2 Resistant Remale Plant

Symptomless BC2 fruits

Field Testing of BC3 Plants

BC_3 papaya lines:

- 5648 x BC_2 03R - 34
- 5648 x BC_2 03R - 73
- 5648 x BC_2 03R - 86

• These plants were used to produce the fourth backcross (BC_4) generation of papaya and BC_3 sib-cross plants.

BC_3 03R 73-19

BC_3 03R 73-9

BC_3 03R 86 -1

BC_4 03R 73 - 3 - 8

— free from infection after 11 months of exposure to PRSV-P in the field

BC_4 03R 73 - 3 - 7

— 8 months free from infection

— few spots were observed after 9 months of exposure to PRSV-P in the field

11.6 Appendix 6. Backcross papaya plants after exposure to natural exposure in the field.

Figure 1. Papaya plants after exposure to natural infection by aphids. (A) female BC_3 tree and (2) BC_2 sib-cross plant showing with few or no symptoms of PRSV-P; (C) Davao Solo plant, heavily infected by PRSV-P showing severe chlorosis with disfigure leaf canopy; (D and E) fruits produced by BC_3 and BC_2 sib-cross plants and (F) few unmarketable fruits produced by Davao Solo plant.

11.7 Appendix 7. Abstracts submitted to different scientific meetings.

Agronomic, Serological, and Molecular Evaluation of BC2 Papaya Introgressed with PRSV-P Resistance

Simeona V. Siar[1], Andres Godwin C. Sajise[1], Gil A. Beligan[1], Antonio C. Laurena[2], Neil H. Tan Gana[2], Roderick A. Drew[3] and Christopher M.O'Brien[3]

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In line with the objective of developing the first PRSV-P resistant papaya plants through conventional breeding, second backcross (BC_2) generations were evaluated and characterized using serological, agronomic and molecular techniques. The BC_2 is a product of introgressing the PRSV-P trait from the papaya wild type *Vasconcellea quercifolia* to *Carica papaya*.

It was observed after three and a half months of exposure to natural infection, twenty-seven out of the thirty BC_2 plants already showed infection of the virus base on visual inspection but after serological evaluation, results revealed that only ten out of the thirty plants tested were positive to the virus. The mild symptom observed during the first few months after field transplanting did not progress in some plants, which proved the result of the serological test.

Fruit quality evaluation of the selected BC_2 plants was the focus of the agronomic aspects of this study. In general, the fruit weight ranged from 519.5- 1266.1 grams. Fruits have firm yellow orange flesh, with mild papaya aroma. The TSS (^{0}B) values ranged from 11.33 - 12.83, which corresponded as "sweet" in the sensory assessment.

Simple sequence repeats (SSRs) was used as molecular markers to fingerprint and to confirm introgression of the resistant trait from V. quercifolia to the F_1 intergeneric hybrid lines and BC_2 plants. Results indicated the introgression of the PRSV resistance trait from *V. quercifolia* to *C. papaya*. The fingerprints showed marked divergence of polymorphic band patterns between resistant and susceptible F1 intergeneric hybrid lines and BC_2 progenies. The method successfully differentiated *C. papaya* genotypes from its wild relative, *V. quercifolia.*

The promising result of the BC_2 generation produced by conventional breeding could provide a sustainable approach in the restoration of the Philippine papaya industry previously devastated by the disease.

Keywords: Papaya Ringspot Virus Type P (PRSV-P), backcrossing, ELISA, Simple sequence repeats (SSRs)

Performance of the Second Backcross (BC2) Generation of Papaya Against Papaya Ringspot Virus (PRSV-P) and Evaluation of Agronomic Traits

Simeona V. Siar[1], Andres Godwin C. Sajise[1], Gil A. Beligan[1], Antonio C. Laurena[1], Roderick A. Drew[2] and Christopher M.O'Brien[2]

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Performance against papaya ringspot virus (PRSV-P) of the second backcross (BC2) generation of papaya from backcrossing the resistant BC_1 plants to its recurrent parent, 5648, was studied. BC_1 is the product of hybridization between papaya inbred line, 5648 and F1 intergeneric hybrid, 410. Intergeneric hybrid 410 is a cross between *Carica papaya* x *Vasconcellea quercifolia* where *V. quercifolia* is the source of resistance to PRSV-P.

A total of 137 BC_2 plants were manually inoculated three times at two-week intervals in the screenhouse. Fifty-two plants or 38 % showed typical symptoms (ranging from chlorosis to shoe-stringed leaves) of papaya ringspot virus after inoculations. Surviving healthy plants (27 plants) were transplanted in the field and were exposed to aphid vectors from susceptible papaya (inbred 4172, and Davao Solo) plants that had a high inoculum level of PRSV-P. Leaves were collected from all the twenty-seven plants and were assayed for virus concentration. ELISA test result showed that only four out of twenty-seven plants tested were positive to the virus. It was also observed that there were another four BC_2 plants that already showed mild infection of the virus base on visual inspection but were negative after performing the ELISA test. The mild symptom during the first few months after field transplanting did not progress in those four plants, which explained the result of the serological test.

Evaluation of some agronomic characters was also done. The BC_2 plants were also routinely checked to know how the backcross plants perform against the susceptible lines 4172 and Davao Solo. Difference between BC_2 plants and the susceptible check were highly evident in terms of severity of symptom on the leaves and on the number of observed spots in the fruit. The promising result could provide the basis for restoration of the papaya industry in those regions that have been devastated by disease in the Philippines.

Keywords: Papaya ringspot virus (PRSV-P), backcrossing, intergeneric hybrid, inbred lines, ELISA

SSR PRIMER PAIRS "MANAM 1/2" CONFIRMED INTROGRESSION OF PAPAYA RINGSPOT VIRUS RESISTANCE (PRSV-P) FROM VASCONCELLEA SPP. TO CARICA PAPAYA

Simeona V. Siar[1*], Gil A. Beligan[1], Antonio C. Laurena[2] , and Neil H. Tan Gana 2

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Resistance to papaya ringspot virus (PRSV-P) from *Vasconcellea quercifolia*, a wild relative of papaya was introgressed into IPB-developed inbred lines. A resistant back cross (BC_1) plant was obtained from the hybridization between papaya inbred line, 5648 and F1 intergeneric hybrid 410. Intergeneric hybrid 410 is a cross between *Carica papaya* x *Vasconcellea quercifolia*. To confirm introgression of the resistant gene from V. quercifolia, genetic fingerprinting of BC_1, IPB inbred lines, F_1 intergeneric hybrid lines and V. quercifolia via simple sequence repeats (SSRs) as the choice molecular marker technology was conducted. SSRs or simple sequence repeats are composed of a few base pairs (1-6 bp in length) and the repeat units are generally found in the non-coding regions of the DNA called introns.

C. papaya nucleotide sequences were downloaded from the GenBank database and analyzed for the presence of SSRs using several programs namely the Tandem Repeat Finder and WebTROLL. Nine repeat motifs were identified and several primer pairs were designed based on conserved sequences flanking the SSRs. All the polymorphic bands generated by V. quercifolia were characterized by the separation of PCR

fingerprints/amplicons and nucleotide sequencing. Sequences were aligned and compared to the *C. papaya* sequences.

Results indicated the introgression of the PRSV resistance trait from *V. quercifolia* as exhibited by BC_1 5648 x 410. The polymorphic bands generated differentiated the *C. papaya* genotypes from its wild relative, *V. quercifolia*. Two primer pairs (MANAM1/2) were identified as markers that can be used in screening resistant progenies of the succeeding backcross generations.

Keywords: Papaya ringspot virus (PRSV-P), molecular markers, introgression, SSRs, primers

Field Screening and Fruit Evaluation of BC3 and BC2 Sib-Crossed PAPAYA Plants Introgressed with PRSV- P Resistance

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Field performance of BC_3 and BC_2 sib-cross plants against papaya ringspot virus (PRSV-P) was evaluated. BC_3 plants are the product of introgressing the PRSV-P trait from the papaya wild type *Vasconcellea quercifolia* to *Carica papaya*. BC_2 sib-crosses were developed by sib-crossing selected female and male BC_2 plants. Selection was based on ELISA test result and symptom development in the field.

A total of 634 plants (88 BC_3, and 546 BC_2 sib-crosses) were inoculated three times at two weeks interval in the screenhouse. Three hundred twenty-five (46 BC_3 and 279 BC_2 sib-cross) or 51.3% showed typical symptoms, which ranged from distortion of young leaves, mosaic, chlorosis to shoe-stringed on older leaves. Plants that remained healthy and symptom free together with the susceptible check, Davao Solo, were then transplanted in the field and were assessed for resistance/susceptibility to the Philippine strain of PRSV-P. A total of 81 BC_2 sib-crosses and 34 BC_3 plants were planted in Mainit, Bay, Laguna. Initial results showed that, there was variation in the rate of symptom development in BC_3 and BC_2 sib- crosses from the control. Davao Solo produced severe symptoms after 1-2 months of transplanting in the field while there were BC_3 and BC_2 sib-cross plants that remained symptom free for about 7-8 months. The difference between backcross papaya and Davao solo was also evident in the ability of the trees to bear good quality fruit. Davao Solo produced a few small and unmarketable fruits. Backcross plants in contrast to the Davao Solo had the ability to recover from early infection based on visual inspection and serological test (ELISA).

The promising result of the BC_3 and BC_2 sib-cross plants produced by conventional breeding could provide a sustainable approach in the restoration of the Philippine papaya industry previously devastated by the disease.

Keywords: Papaya ringspot virus (PRSV-P), backcrossing, ELISA, resistance/ susceptibility

11.8 Appendix 8. Posters presented in different scientific meetings.

Performance of the Second Backcross (BC_2) Generation of Papaya Against Papaya Ringspot Virus (PRSV-P) and Evaluation of Agronomic Traits

Simeona V. Siar[1], Andres Godwin C. Sajise[1], Gil A. Beligan[1], Antonio C. Laurena[1], Roderick A.Drew[2] and Christopher M. O'Brien[2]

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INTRODUCTION

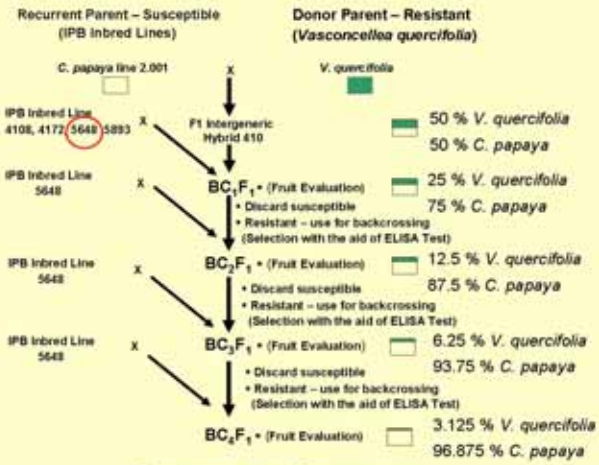
Papaya, a native of Central and South America, is a fast growing aborescent herb. Dioecious and hermaphrodite cultivars of this plant are grown in many tropical and sub-tropical countries for edible fruit, vitamin rich fruits and to a lesser extent also for their milky latex (Drew et al. 1998 and Drew et al. 2006). In 2005, the world production of papaya was 6.342 million metric tons (FAO, 2005).

The most important disease that could threaten papaya production is the Papaya Ringspot Virus (PRSV-P). In Philippines, PRSV-P has devastated papaya production in some regions on the island of Luzon and the classical example of which is the death of the whole papaya industry in Cavite. Now, the disease was recently reported to be in Mindanao where large papaya plantations are located. In Australia, PRSV-P has been confined to the Southeast corner of the state, however this still represent a severe threat to the industry in North Queensland. These shows the importance of developing a strategy that can control the said disease. The use of resistant varieties is probably the cheapest and the most effective method of combating the disease.

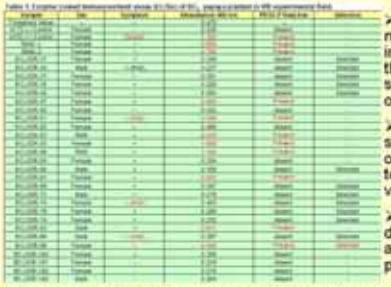
Development of PRSV-P resistant papaya inbred lines with good agronomic traits is the main objective of this study. Performance against papaya ringspot virus (PRSV-P) of the second backcross (BC_2) generation of papaya from backcrossing the resistant BC_1 plants to its recurrent parent, 5648, was studied. BC_1 is the product of hybridization between papaya inbred line, 5648 and F1 intergeneric hybrid, 410. Intergeneric hybrid 410 is a cross between *Carica papaya* x *Vasconcellea quercifolia* where *V. quercifolia* is the source of resistance to PRSV-P.

MATERIALS AND METHODS

Development of PRSV-P Resistant Genotypes with Good Agronomic Traits thru Backcrossing



RESULTS AND DISCUSSION



- After three and a half months of exposure to natural infection, twenty - four out of the twenty - seven BC_2 showed mild infection based of visual inspection.
- But after doing the serological test, only eight out of the twenty-seven plants tested were positive to the virus.
- Mild symptom that develop during the first few months after field testing did not progressed in some plants.

Table 2. Fruit qualities of five BC_2 populations evaluated in IPB experimental field.

BC_2 Populations	Parameters: Fruit wt. (g)	TSS 0B	Taste	Flesh Firmness	Fruit Length	Fruit Width
BC2 03R - 96	653.4	11.33	Sweet - not sweet	Firm	11.00	11.70
BC2 03R - 78	1266.1	12.83	Sweet w/ after taste	Firm	12.50	13.90
BC2 03R - 74	540.8	12.00	Sweet	Firm	10.00	10.70
BC2 03R - 36	585.6	12.00	Sweet	Firm	9.30	11.00
BC2 03R - 37	964.2	11.00	Sweet	Firm	12.00	13.20

- Fruits are generally sweet except for BC_2 03R- 78 which has an aftertaste.
- TSS values ranged from 11- 12.83 ^{0}B. This sweetness could be attributed to *V. quercifolia* since it is known for its high sugar levels.
- Fruit weight ranged from 540.8 – 1266.1 grams.
- Fruits have firm yellow orange flesh, with mild papaya aroma.

- Results of the 1st ELISA test showed that thirteen out of the thirty-five sib-cross plants were positive to the virus.
- After two months, ELISA test was done again. The results showed that some of the previously infected plants had recovered from the infection. From thirteen infected plants it was down to seven only.
- Young leaves were healthy and were free from the typical symptom caused by PRSV-P.

Male Sib-cross plant BC_2 03R 37 x 53 | Female Sib-cross plant BC_2 03R 37 x 53 | Infected old leaf | Heavily Infected Davao Solo (Positive Control)

Figure 1. Papaya plants after seven months of exposure to natural infection. (A) Male sib-cross plant which remained symptom and virus free based on visual inspection and serological test; (B) Female sib-cross plant showing good recovery from the infection (new leaves were healthy and the plant was still productive) and (C) Davao solo plant, heavily infected by PRSV-P showing fruitless tree with disfigure leaf canopy.

CONCLUSION

Backcross plants were showing good resistance against papaya ringspot virus as compare with Davao Solo (positive control). Difference between BC_2 plants and the susceptible check were highly evident in terms of severity of symptom on the leaves and the ability of the tree to bear fruits of good quality. These promising result could provide the basis for restoration of the papaya industry in those regions that have been devastated by disease in the Philippines.

ON GOING ACTIVITIES

- Field testing of BC_3 progenies
- Field testing of BC_2 sib-crosses
- Field screening of Re-constituted Sinta (BC_2 plant crossed to an inbred line,4172)(to test performance *vis a vis* with Sinta)
- Development of molecular primer for the molecular studies and characterization of resistant populations

LITERATURE CITED

DREW RA, O'BRIEN and MAGDALITA PM (1998). Development of interspecific Carica hybrids. Acta Hort. 461: 285-292

DREW RA, SIAR SV, O'BRIEN CM, MAGDALITA PM, SAJISE AGC (2006). Breeding for Papaya Ringspot virus resistance on *Carica papaya* via hybridization with *Vasconcellea quercifolia*. Australian Journal of Experimental Agriculture 46: 413-418

FAOSTAT data. 2005. http://faostat.fao.org/faostat/

ACKNOWLEDGEMENT : This study is a part of the project entitled "Development of PRSV-P resistant papaya genotypes by introgression of genes from wild Carica species" funded by the Australian Center for International Agricultural Research (ACIAR).

SSR PRIMER PAIRS "MANAM 1/2" CONFIRMED INTROGRESSION OF PAPAYA RINGSPOT VIRUS RESISTANCE (PRSV-P) FROM *VASCONCELLEA* SPP. TO *CARICA PAPAYA*

[redacted]

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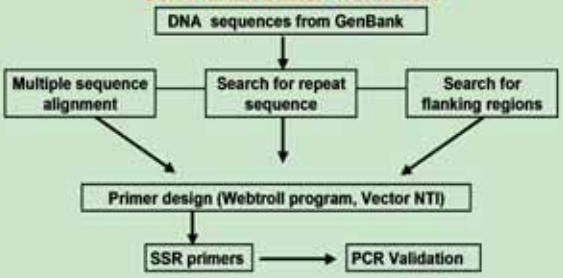
INTRODUCTION

Resistance to papaya ringspot virus (PRSV-P) from *Vasconcellea quercifolia*, a wild relative of papaya was introgressed into IPB-developed inbred lines. A resistant backcross (BC)$_1$ plant was obtained from the hybridization between papaya inbred line, 5648 and F1 intergeneric hybrid 410. Intergeneric hybrid 410 is a cross between *Carica papaya* x *Vasconcellea quercifolia*.

A new set of SSR primers were designed and used to fingerprint and evaluate the introgression of the resistance trait from *V. quercifolia* to the BC$_1$, BC$_2$ and F1 intergeneric hybrid lines.

MATERIALS AND METHODS

SSR PRIMER DESIGN WORKFLOW



RESULTS AND DISCUSSIONS

- A new set of primers were designed using the WebTroll program and VectorNTI based on the previously identified tandem repeat found in the exon region of the *C. papaya* cysteine proteinase gene (Genbank No. M15203) or papain (CPAPAP). These primers are expected to amplify the 459bp region, where the perfect tandem repeat (TA)$_{16}$ was located (110-1129bp). Figure 1 illustrates the SSR primer pair binding sites at the CPAPAP gene used in this study.

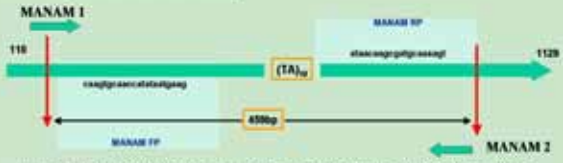


Figure 1. Illustration of MANAM SSR primers used in amplifying the 459bp region of CPAPAP gene with the (TA)$_{16}$

DNA FINGERPRINTING

- The *V. quercifolia*, (lines 9-10, Fig. 2) produced 4 distinct bands with sizes at 150 (designated as Q150), 200 (designated as Q200), 300 and 800 bp as estimated in gel electrophoresis. The putatively resistant BC$_2$ plants (lines 2-8) produced a very distinct band at around 500 bp and a faint band at around 100 bp. The F1 intergeneric hybrids (lines 11-18) had a very distinct band patterns which are more or less a combination of band patterns from parental *V. quercifolia* and *C. papaya*.
- F1 intergeneric hybrids contained the ubiquitous H400, the distinguishing Q150, Q200 bands introgressed from *V. quercifolia* and the serendipitous H100 band which is observable in the *C. papaya* hybrids which show resistance to PRSV-P.
- The F1 hybrids identified to have shown resistance to PRSV-P lines were confirmed by ELISA, and had the presence of the H100 band.
- The 100bp band (H100) was found to be common in ALL RESISTANT LINES OF THE INTERGENERIC HYBRIDS, BC$_1$, and BC$_2$. However, the BC$_2$ resistant lines did not produce the Q150 and Q200 bands, and *V. quercifolia* did not produce the H100 bands.

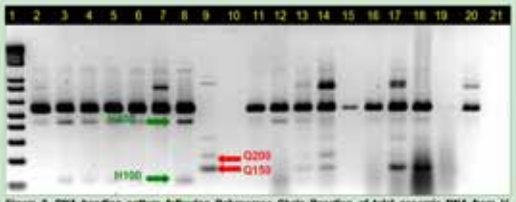


Figure 2. DNA banding pattern following Polymerase Chain Reaction of total genomic DNA from *V. quercifolia*, F1intergeneric hybrid, and *C. papaya* plants using SSR MANAM 1 & MANAM 2 primer; Well 2-8 (BC$_2$ plants), 9-10 (*V. quercifolia*), 11-18 (F1 intergeneric hybrids), 19 (BC$_1$ plants), 20 (Davao Solo) and 21 (negative control). The green (hybrids) and red (*V. quercifolia*) arrows represent the polymorphic bands submitted for sequencing.

DNA SEQUENCING

- Q150 and Q200 have high sequence similarity, 85 % (Figure 3). Q150 andQ200 bands were common in the *V. quercifolia* and the PRSV-P resistant *C. papaya* intergeneric lines.

Figure 3. Diagram of contig assembly and multiple sequence alignment of Q150 and Q200 (contig 1) illustrating that Q200 is a dimerized form of Q150.

- There was a noted 53% similarity between the H100 band and Q150 and Q200 (Figure 4). However, to have a more accurate findings, cloning of the H100 prior to sequence analyses is better suggested to determine the exact nucleotide sequence.

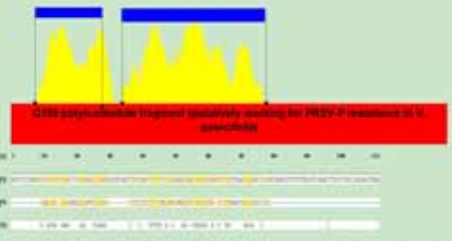


Figure 4. Diagram of sequence alignments of Q150 and H100 polynucleotide sequences isolated from *V. quercifolia* (Q150) and hybrid lines (H100) with ELISA confirmed PSRV-P resistance. The homology and absolute complexity of H100 (contig 2) against the Q150 is at 53%.

CONCLUSIONS

- The old set of primers could only detect transfer of resistance up to F1 intergeneric lines only.
- While the new sets of primers (MANAM1/2) could be used to fingerprint the introgression of resistance from *V. quercifolia* up to the backcross generations.

ACKNOWLEDGEMENT : This study is a part of the project entitled "Development of PRSV-P resistant papaya genotypes by introgression of genes from wild Carica species" funded by the Australian Center for International Agricultural Research (ACIAR).

Field Screening and Fruit Evaluation of BC_3 and BC_2 Sib-Crossed Plants Introgressed with PRSV-P Resistance

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ABSTRACT

Field performance of BC_3 and BC_2 sib-cross plants against papaya ringspot virus (PRSV-P) and its fruit quality were evaluated. BC_3 plants are the product of introgressing the PRSV-P trait from the papaya wild type *Vasconcellea quercifolia* to *Carica papaya*. BC_2 sib-crosses were developed by sib-crossing selected female and male BC2 plants. Selection was based on ELISA test result and symptom development in the field.

A total of 634 plants (88 BC_3, and 546 BC_2 sib-crosses) were inoculated three times at two weeks interval in the screenhouse. Three hundred twenty-five (46 BC_3 and 279 BC_2 sib-cross) or 51.3% showed typical symptoms, which ranged from distortion of young leaves, mosaic, chlorosis to shoe-stringed on older leaves. Plants that remained healthy and symptom free together with the susceptible check, Davao Solo, were then transplanted in the field and were assessed for resistance/susceptibility to the Philippine strain of PRSV-P. A total of 81 BC_2 sib-crosses and 34 BC_3 plants were planted in Mainit, Bay, Laguna. Initial results showed that, there was variation in the rate of symptom development in BC_3 and BC_2 sib- crosses from the control. Davao Solo produced severe symptoms after 1-2 months of transplanting in the field while there were BC_3 and BC_2 sib-cross plants that remained symptom free for about 7-8 months. The difference between backcross papaya and Davao solo was also evident in the ability of the trees to bear good quality fruit. Davao Solo produced a few small and unmarketable fruits. Backcross plants in contrast to the Davao Solo had the ability to recover from early infection based on visual inspection and serological test (ELISA).

Fruit qualities of BC_3, BC_2 sib-cross lines and Davao Solo planted in field borders were evaluated. In general, the fruit weight of the backcross lines ranged from 834.53 – 754.92 grams in contrast with Davao Solo's 202.67 grams. Fruits have firm yellow orange flesh, with mild papaya aroma. The TSS (°B) values of BC_3, BC_2 sib-cross lines and Davao Solo were 10.0, 12.2 and 9.3 respectively which correspond to sweet taste for backcross lines and not so sweet for Davao Solo.

The promising result of the BC_3 and BC_2 sib-cross plants produced by conventional breeding could provide a sustainable approach in the restoration of the Philippine papaya industry previously devastated by the disease.

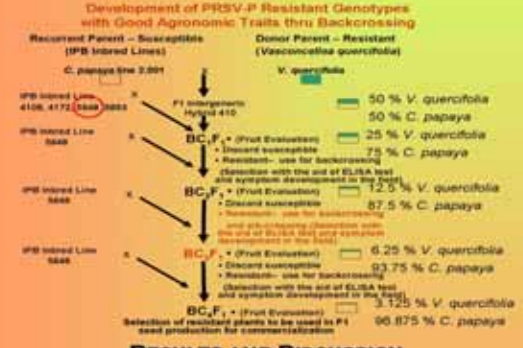
INTRODUCTION

Papaya, a native of Central and South America, is a fast growing arborescent herb. Dioecious and hermaphrodite cultivars of this plant are grown in many tropical and sub-tropical countries for edible fruit, vitamin rich fruits and to a lesser extent also for their milky latex (Drew et al. 1998 and Drew et al. 2006). In 2005, the world production of papaya was 6.342 million metric tons (FAO, 2005).

The most important disease that could threaten papaya production is the Papaya Ringspot Virus (PRSV-P). In Philippines, PRSV-P has devastated papaya production in some regions on the island of Luzon and the classical example of which is the death of the whole papaya industry in Cavite. Now, the disease was recently reported to be in Mindanao where large papaya plantations are located. In Australia, PRSV-P has been confined to the Southeast corner of the state, however this still represent a severe threat to the industry in North Queensland. These shows the importance of developing a strategy that can control the said disease. The use of resistant varieties is probably the cheapest and the most effective method of combating the disease.

Development of PRSV-P resistant papaya inbred lines with good agronomic traits via traditional breeding is the main objective of this study. Field performance against papaya ringspot virus (PRSV-P) and fruit qualities of BC_3 and BC_2 sib-crossed were evaluated. BC_3 plants are the product of introgressing the PRSV-P trait from the papaya wild type *Vasconcellea quercifolia* to *Carica papaya*. BC_2 sib-crosses were developed by sib-crossing selected female and male BC_2 plants. Selection was based on ELISA test result and symptom development in the field.

MATERIALS AND METHODS



RESULTS AND DISCUSSION

Figure 1. Papaya plants after exposure to natural infection by aphids: (A) female BC_3 tree and (B) BC_2 sib-cross plants showing with few or no symptoms of PRSV-P; (C) Davao solo plant, heavily infected by PRSV-P showing severe chlorosis with disfigure leaf canopy; (D and E) fruits produced by the BC_3 and BC_2 sib-cross plants and (F) two unmarketable fruits produced by Davao Solo plant.

- Results showed that, there was variation in the rate of symptom development in BC_3 and BC_2 sib-cross plants from the control.
- Davao Solo produced severe symptoms after 1-2 months of exposure in the field while there were BC_3 and BC_2 sib-cross plants that remained symptom free for about 7-9 months (Figure 3).
- The difference between backcross papaya and Davao Solo was also evident in the ability of the trees to bear good quality fruit (Figure 1).
- Davao Solo produced few unmarketable fruits.

Table 1. Enzyme Linked Immunosorbent Assay (ELISA) of BC_2 sib-cross (xBC2) papaya plants maintained in Mainit, Bay, Laguna

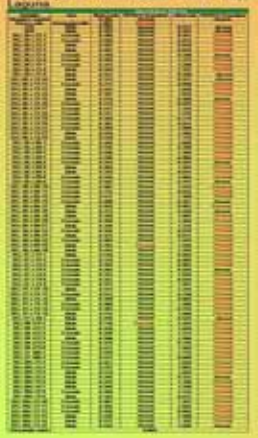


Table 2. Enzyme Linked Immunosorbent Assay (ELISA) of BC_3 papaya plants maintained in Mainit, Bay, Laguna

[1] BC_3 plants after eight months of exposure to the natural inoculations of viruliferous aphids in the field.
[2] plants after six months of exposure in the field.

Figure 2. Serological test using young and old leaf of BC_2 papaya plants maintained in Mainit, Bay Laguna. Negative control (well A1-A2), positive control (well A3-A4), BC_3 necrocyoung leaf (well A9-D6), BC_3 sib-cross healthy leaf (well D7- D10), BC_3 disinfected leaf (well D11-G8) BC_2 sib-cross infected leaf (well G9-G12).

Figure 3. Male BC_3 sib-cross plants showing putative resistance against PRSV-P. (A) line BC_3 03R 37 x 86-10, after seven months of exposure in the field and (B) line BC_3 37 x 73-15, after eight months of exposure in the filed with high disease pressure.

- Selection of the best backcross plants was based on ELISA test result and symptom development in the field (Figure 3, Table 1 and 2).
- Backcross plants in contrast to the Davao Solo had the ability to recover from early infection based on visual inspection and serological test (ELISA).
- The mild symptom observed during the first few months after field transplanting did not progressed.
- Based on the obtained values of viral concentration in the plants, it could be inferred that the viral reproduction and growth in the plant was impaired. For example, in $BC_2$03R 80 x 73-13 the absorbance readings obtained using healthy and infected leaves were, 0.248 and 1.586 respectively (Table 1).
- These values show that there is a considerable decrease of viral concentration in the plant thus enabling the plants to recover from the infection.

Table 3. Fruit quality evaluation of BC_3 and BC_2 sib-cross papaya plants in comparison with the fruits of susceptible check, Davao Solo

Lines	Fruit Wt. (g)	Peel Color	Flesh Color	Aroma	Fruit Length	Fruit Width	Firmness	Taste	TSS (°B)
BC_3	834.53	Yellow Orange	Yellow Orange	Mild	13.40	12.30	Firm	Not so Sweet	10.00
BC_2 sib-cross	754.92	Yellow Orange	Yellow Orange	Mild	12.00	11.10	Firm	Sweet	12.20
Davao Solo	202.67	Yellow Orange	Yellow Orange	Mild	9.40	8.03	Firm	Not so Sweet	9.30

- In general, the fruits from the backcross lines are sweeter than Davao solo fruits. This trait could be attributed to *V. quercifolia* since it is known for its high sugar content.
- Backcross lines have bigger and heavier fruit compare to Davao Solo. The fruit weight in backcross lines ranged from 754.92 – 834.53 grams while in Davao Solo, 202.67.
- Fruits have firm, yellow orange flesh with mild aroma.

CONCLUSION

Backcross plants produced by conventional breeding were showing good resistance against papaya ringspot virus compared with Davao Solo (positive control). This promising result could provide a sustainable approach for the restoration of the papaya industry in those regions that have been devastated by disease in the Philippines.

ON- GOING AND FUTURE ACTIVITIES

- Micropropagation of selected BC_3 sib-cross plants.
- Evaluation and screening of BC_4 plants.
- Evaluation and screening of BC_3 sib-cross plants.
- Continue to introgress PRSV-P resistance gene(s) to other IPB inbred lines and Davao Solo

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DREW RA, O'BRIEN and MAGDALITA PM (1998). Development of interspecific Carica hybrids. Acta Hort. 461: 285-292

DREW RA, SIAR SV, O'BRIEN CM, MAGDALITA PM, SAJISE AGC (2006). Breeding for Papaya Ringspot virus resistance on *Carica papaya* via hybridization with *Vasconcellea quercifolia*. Australian Journal of Experimental Agriculture 46: 413-418

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