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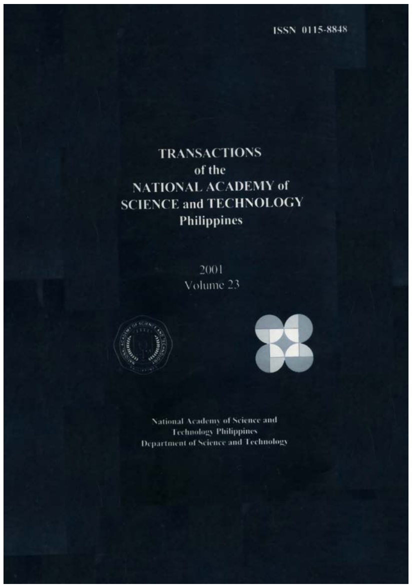

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AGRICULTURAL SCIENCES DIVISION

APPROACHES IN DEVELOPING RINGSPOT VIRUS RESISTANT PAPAYA

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ABSTRACT

Papaya ringspot virus (PRSV-P) is a major disease of papaya in the Philippines. PRSV-P infected plants exhibit leaf mottling, reduced lamina, tapering of stem, oil streaks on petioles and ringspots on the fruits. Screening available germplasm revealed that resistance is not found within the cultivated Carica papaya species. Three approaches are being utilized to develop PRSV-P resistant papaya. Intraspecific hybridization produced a moderately tolerant variety and identified tolerant genetic stocks. Interspecific hybridization, done by crossing papaya with PRSV-P resistant wild relatives (e.g. C. cauliflora, C. quercifolia, C. pubescens), produced resistant hybrids. However, sterility and hybrid breakdown hamper the backcrossing procedure to transfer the resistance trait to papaya. Genetic engineering is another technique being pursued and this works on the principle of coat protein-mediated resistance. Coat protein gene from the local virus isolate has been cloned and inserted into Davao solo papaya via microprojectile bombardment. Putative transformants now regenerating.

International collaboration has played an important role in our efforts to develop PRSV-P resistant papaya. Details of the progress made using intraspecific and interspecific hybridization and genetic engineering are discussed.

INTRODUCTION

Papaya ringspot virus (PRSV-P) is a serious disease of papaya in most papaya growing countries. It was reported in Hawaii in 1945, in El Salvador, Puerto Rico, Cuba and Venezuela in the 1950's and then a little later in India, Bangladesh and Sri Lanka. It is widespread in Central and South America. Taiwan reported its presence in 1975, the Philippines in 1984, Thailand in 1986, Australia in 1991 and Malaysia in 1992. PRSV-P was responsible for the decline in papaya production in Southern Luzon where papaya is a regular member of the multiple cropping system. PRSV-P symptoms were first observed in Silang, Cavite in 1982 and was confirmed to be PRSV in 1984, the year when an outbreak occurred at an epidemic level. The disease is now widespread in the island of Luzon and has been detected in other papaya growing islands like Mindoro, Palawan, Marinduque, Leyte and Negros Oriental.

PRSV-P infected papaya exhibits leaf mottling, reduced lamina, tapering of stem, oil streaks on petioles and ringspots on the fruits. PRSV-P infects papaya at all stages of growth. If infected at the seedling stage, leaves are mottled, the plant loses vigor and succumbs to the disease before reaching the flowing stage. If infected at a later stage, the stem tapers and the plant exhibits all the symptoms enumerated earlier. Disease management strategies include quarantine restriction, sanitation or the cutting down and proper disposal of infected plants, planting of barrier crops around the plantation, cross protection and planting in isolation.
The papaya breeding project at the Institute of Plant Breeding started in 1981 when PRSV-P was still unheard of. Breeding lines were lost due to this disease. The breeding program has been refocused to include resistance/tolerance as a breeding objective. Conventional breeding techniques are supplemented with biotechnology tools like micropropagation of elite lines, embryo rescue of interspecific hybrids and genetic engineering.

This paper will discuss how different approaches are being utilized to develop papaya varieties that are resistant to PRSV-P. Modest success attained by the program is attributed largely to the "Team Approach" in plant breeding, researchers with varied expertise working together on a common research agenda. International collaboration also plays a role in this research.

INTRASPECIFIC HYBRIDIZATION

Germplasm enhancement. Papaya is an introduced crop in the Philippines with its center of origin in Central and South America. Except for introduced varieties that are maintained by self-pollination, our papayas are in the form of strains that are named after the place where they are grown (e.g. 'Cavite') or after traits that describe them (e.g. 'Morado'). We have been collecting papayas since 1981, both from local and foreign sources. The collections represented the available diversity in terms of fruit size, flesh color, plant stature, petiole color and other horticultural traits. We also have other wild relatives of the cultivated papaya (e.g. *C. cauliflora, C. quercifolia, C. pubescens, C. guodotiana, C. parviflora)* which were obtained from papaya researchers from Hawaii, Australia, India, Venezuela and Mexico. Last year, 18 local and 5 foreign accessions were collected.

Screening for PRSV-P resistance. IPB's papaya breeding program started in 1981 through a modest grant from PCARRD. At this time, PRSV was unheard of. Our breeding objectives were focused on fruit quality based on market preference. Natural selection played its role in screening for PRSV-P resistance among our materials. A lot of our breeding lines were lost in the mid 80's due to the virus. The performance of those that survived the natural virus infection was further evaluated. We also screened more than one hundred papaya accessions in the greenhouse through artificial inoculation but found no resistance. Seedlings that showed mild symptoms were field-planted, evaluated and rated tolerant if they produced a good crop for at least one cycle of fruiting. A notable tolerant line came from Florida.

Development of inbred lines. Materials exhibiting good horticultural traits, tolerance to PRSV-P, etc. are continuously purified by self- or sib-pollination for at least five generations. Selection is practiced at each generation of selfing or sib-pollination, losing quite a number each time. We have genetic stocks for small, medium or large fruits, yellow or red flesh, short stature, good shape, etc. Table 1 gives the characteristics of the inbred lines developed at IPB.

Table 1. Characteristic of papaya inbred lines developed at IPB.

InbredLine	**Source**	**Flesh Color**	**Reaction to PRSV-P**
4108	Surigao	Red	Susceptible
4165	Albay	Red	Susceptible
4172	Cavite	Red	Moderately tolerant
4308	Davao	Red	Susceptible
5648	Florida	Yellow	Moderately tolerant
5893	Taiwan	Red	Moderately tolerant
3878	Cagayan de Oro	Yellow	Susceptible

Of these inbred lines, 5648 showed a consistent tolerant reaction to PRSV-P. Other promising lines are being purified to obtain additional genetic stocks for hybridization work.

Hybridization and selection. Diallel crosses were made using our inbred lines. The performance of the hybrids was evaluated in our experimental farm based on fruit quality, precocity, and PRSV-P tolerance for at least three seasons. Three hybrids were selected and placed in on-farm trials for further evaluation. Table 2 gives the characteristics of the three hybrids tested in several farms.

Table 2. Performance of three papaya hybrids with tolerance to PRSV-P

Parameters	5648 x 5893	5648 x 3878	5648 x 4172
Ave # of fruits	13	16	18
Fruit wt (g)	898	929	1166
TSS (°B)	11.9	11.3	11.4
Taste Sweet	Sweet	Sweet	
Flesh color	Yellow	Yellow	Yellow
Flesh texture	Firm	Firm	Firm
Aroma	Mild	Mild	Mild
% Edible portion	62.2	64.0	67.5
Petiole color	Purple	Green	Green

New hybrids have been generated and they are being thoroughly evaluated prior to putting them in on-farm trials.

Variety release. Sinta, a hybrid between 5648 and 4172 showed outstanding performance in both experimental and on-farm trials. It was released in 1995 and has since gained wide acceptance because of its precocity, PRSV-P tolerance and excellent fruit quality. Sinta fruit is medium-sized and has sweet, firm, yellow and smooth flesh.

Growing of Sinta is confined in Luzon only despite the clamor of growers from other islands. This is because of the quarantine restriction issued by the Bureau of Plant Industry that prohibits the movement of any form of papaya planting materials out of Luzon, in an attempt to abate the spread of PRSV-P to other islands. There are other red hybrids under evaluation that are suitable for both fresh and processing.

Seed production. Seed production of Sinta is done at IPB where seeds and seedlings are sold. In 1999, the East-West Seed Co. was licensed to seed produce Sinta on royalty basis, following our established hybrid seed production protocol. We hope to seed produce Sinta in an isolated, disease-free area in Mindanao so that seeds produced there, using indexed micropropagated parental inbreds, can be transported to any island without fear of spreading the disease. A micropropagation protocol from Australia has been adapted to rapidly multiply Sinta and other genotypes. Through this system, the sex of the plant is known prior even to flowering.

Technology transfer. A variety developed at IPB comes with a pocket of technology detailing how to grow the crop properly, covering seed extraction, seedling establishment, transplanting, fertilization, weeding, harvesting, etc. We have a brochure for Sinta in English and Filipino. We also hold trainings on papaya fruit and seed production. Print, radio and television programs also provide good avenues for popularizing our variety and in technology transfer activities.

INTERSPECIFIC HYBRIDIZATION

The genus *Carica* has 21 species but only *C. papaya* bear edible fruits. PRSV-P is not found in the cultivated papaya but is present in *C. cauliflora, C. pubescens* and *C. quercifolia*. We obtained these species and other species like *C. goudotiana, C. monoica and C. parviflora* abroad.

Screening for PRSV-P resistance. The wild *Carica* species were manually inoculated with PRSV-P under greenhouse condition. Their reaction to the virus was verified by ELISA. Our results (Table 3) are similar to the published reports: C. cauliflora, C. quercifolia and C. pubescens are PRSV-P resistant while *C. goudotiana,* and *C. parviflora* are susceptible.

Table 3. Reaction of *Carica* species to PRSV-P Los Baños isolate

Carica species	Reaction to PRSV-P	ELISA test
C. papaya	susceptible	+
C. cauliflora	resistant	–
C. pubescens	resistant	–
C. quercifolia	resistant	–
C. guoudotiana	susceptible	+
C. parviflora	susceptible	+

Interspecific crosses. Papaya is reproductively isolated from its wild relatives, hence it does not cross readily with any of them. Cross incompatibility is due to post-zygotic factors that lead to abortion of embryos. However, earlier work in Hawaii proved that interspecific hybrids can be rescued *in vitro* before they abort.

More than 30 papaya genotypes were crossed with *C. cauliflora, C. quercifolia* and *C. pubescens* to identify the most responsive genotypes. Four genotypes produced embryos that could be rescued by in vitro culture, when crossed with the wild species. These genotypes were used in succeeding pollinations. Ninety to 120 day old embryos were suitable for embryo culture. Fruits harvested before or after this range did not yield good results. We used the de Fossard medium in culturing the interspecific embryos.

Evaluation of F1 hybrids. Interspecific hybrids grew satisfactorily in vitro and acclimatized well in the greenhouse. Their leaf morphology was intermediate between papaya and the male wild relative. The hybrids had the combined isozyme banding pattern of both parents using Shikimate dehydrogenase, confirming their hybrid status. Cytological analysis of the pollen mother cells showed very abnormal meiosis, explaining pollen sterility of the hybrids and the lack of homology between the two genomes.

Manual inoculation with PRSV-P revealed that the interspecific hybrids were resistant to the virus and this was confirmed by ELISA test. A few hybrids of the *C. papaya* x *C. quercifolia* exhibited a small degree of pollen fertility. These are being used in the current backcrossing work. There is more promise with this cross combination since fertile F_1s have been obtained. Backcrossing to papaya is being intensified to effect the transfer of resistance to the cultivated species.

GENETIC ENGINEERING

Coat protein-mediated resistance has been shown to be effective against PRSV-P. In 1998, Hawaii released 'Rainbow', a transgenic papaya that contains the coat protein gene of the Hawaiian isolate. This genetically modified (GM) papaya is resistant only to the Hawaiian isolate, it is not effective against the isolates from Taiwan, Thailand and Mexico, indicating that resistance is very specific. Similar strategy is in progress in Taiwan, Mexico, Thailand and some other countries.

Our genetic engineering project on the development of PRSV-P resistant papaya is funded by PCARRD/DOST. We accessed the technology through the International Service for the Acquisition of Agri-biotech Applications (ISAAA) and in collaboration with the Plant Genetic Engineering Unit of Kasetsart University (PGEU-KU). We adhere to the very strict guidelines of the National Biosafety Committee of the Philippines (NCBP). Our BL2 Greenhouse has been commissioned by the NCBP for use in greenhouse testing of our transgenic materials.

Cloning and characterization of PRSV-P coat protein. A gene construct (pCP-LBP) of the Philippine isolate was made in collaboration with PGEU. The presence of a 900 bp fragment in the construct, representing the PRSV-P coat protein (cp) was isolated using polymerase chain reaction (PCR) and restriction enzyme digestion. This is being used in the transformation work. We are also cloning other cp genes from other isolates. Through blast analysis, the cp gene sequence was found to be 92% homologous to the Thai isolate, 89 % to Vietnamese isolate, 88% to the Australian and the Hawaiian isolates and 86% to the Puerto Rican and Sri Lankan isolates.

Transformation and regeneration of transgenic plants. The optimized bombardment conditions were established using a pressure level of 1200 kPa and at a distance of 12.5 cm. At this level the impact of the microprojectiles were highly seen as fine dark marks. These marks were impacted exactly on the center of the target and radiated to the sides.

More than 17,000 zygotic embryos of Davao Solo were explanted and more than 6,000 targets were bombarded with the cp gene. Bombarded tissues were placed in the selection medium and those that survived were transferred to the regeneration medium. Putative trangenics containing the cp gene are now regenerating.

DISCUSSION

Plant breeders today are better equipped with research tools compared to our counterparts decades ago. Overcoming incompatibility barriers between species and fixing superior genotypes can now be done routinely, in collaboration with researchers with varied expertise. The case of the development of papaya varieties resistant o PRSV-P is a good example. Screening for resistance in the cultivated papaya did not yield positive results. The best effort in our conventional breeding work led to the development of genotypes with tolerance to the virus. Local accessions, complemented by foreign introductions, enabled us to identify tolerant genotypes which give a good yield despite the disease pressure. These materials are being used to breed for toelant varieties with good fruit quality. Sinta is a medium-sized hybrid papaya with yellow, sweet and firm flesh that is moderately tolerant to PRSV-P. Other genotypes that are, sweet, red or yellow, small, medium or large-sized, depending on the market requirements, are currently being developed.

Wide hybridization, involving *Carica* species that have resistance to PRSV-P is rather difficult due to post-zygotic incompatibility, hybrid breakdown and sterility. These limitations can be overcome by embryo rescue and increasing the number of interspecific hybrids generated to find vigorous hybrids with a certain degree of fertility for use in backcrossing work. Initial work using *C. cauliflora* as male parent did not yield fertile hybrids but techniques for wide hybridization were developed using this species. The *C. papaya x C. quercifolia* cross gave several fertile hybrids and these are being used for modified backcrossing work.

Modern biotechnology enables us to transfer genes across species in a more precise manner. It is a powerful tool that can complement the conventional breeding techniques. Coat protein mediate-resistance has been shown to work in Hawaii and Taiwan and we are adapting the same strategy to develop PRSV-P resistant papaya. Our modest progress in genetic engineering is attributed to the collective contribution of our team members, support from PCARRD/DOST and the collaboration/ networking with a laboratory in Thailand and the experience shared by the American researchers. The International Service for the Acquisition of Agri-biotech Applications (ISAAA) also assists in technology access and other form of support. In all these, effective team work networking and collaboration are crucial.

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