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COMPARISON OF INFECTION CAUSED BY *PYTHIUM DEBARYANUM*, *PHYTOPHTHORA PALMIVORA* AND *ERWINIA CARICAE* ON TRANSGENIC AND NON-TRANSGENIC PAPAYA RINGSPOT VIRUS RESISTANT PLANTS

Christian Joseph Rili Cumagun[1]*, *Carlos Lamug Padilla*[1]
Malve Sumayao Tolentino[1], *Desiree Menancio Hautea*[2]

[1] Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, Laguna, 4031 Philippines
[2] Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna, 4031 Philippines

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Abstract: The study was conducted to determine the reaction of transgenic papaya ringspot virus (PRSV) – resistant papaya to some major fungal and bacterial diseases of papaya. Four lines of transgenic papaya developed by the Institute of Plant Breeding (IPB) and non-transgenic 'Davao solo' were used in the screening. Plants were artificially inoculated with the most aggressive isolates in a contained greenhouse. Inoculation was done by incorporating *Pythium debaryanum* and *Phytophthora palmivora* – colonized wheat seeds into the base of the plant and by pricking the base of leaf petiole with a needle dipped in suspension of *Erwinia caricae.* Four transgenic papaya lines and 'Davao Solo' were susceptible to *P. debaryanum* and *P. palmivora* under greenhouse conditions. No significant differences in per cent wilted seedlings caused by *P. palmivora* among transgenic four lines and 'Davao Solo' were observed ($p = 0.05$). Similarly, no differences in root rot severity (%) among the same test plants due to *P. palmivora* were noted. The three transgenic papaya lines were initially more susceptible than 'Davao Solo' to *E. caricae* seven days after inoculation but the same degree of infection was attained 14 days after inoculation. The transgenic and the non-transgenic papaya exhibited susceptible reactions to *P. debaryanum* in the greenhouse. No significant difference was observed in one transgenic line and 'Davao Solo' in terms of pre and post germination damping-off incidence in inoculated soil ($p = 0.05$).

Key words: *Erwinia caricae*, *Pythium debaryanum*, *Phytophthora palmivora*, resistance, susceptibility, transgenic papaya ringspot virus (PRSV) resistant plant, damping-off, crown rot, root rot

*Corresponding address:
christian_cumagun@yahoo.com

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important food crops in the tropical and subtropical areas. It is consumed as a fresh dessert fruit, and the green fruits are often used as salad and vegetable. In addition, papaya contains a digestive enzyme, papain, a protease that is useful in tenderizing meat and other protein (Manshardt 1999). The tree is widely planted in backyard because it is relatively easy to grow from seed; the first mature fruits can be harvested nine months after sowing seeds, and fruit is produced continuously year-round. The Food and Agriculture Organization (FAO) estimated that about 5.7 million metric tons of papaya fruit were harvested in 1995, almost double the 1980 harvest (Galinsky 1996). Brazil, India and Mexico are the largest producers of papaya. However, the papaya crop is severely affected by PRSV worldwide.

This study focuses on activities leading to the development of advanced lines of PRSV resistant papaya, evaluation of these lines at the isolated/confined field and regulatory file development. Aside from virus diseases, papaya is a host of several fungal, bacterial and nematode diseases of economic importance. *P. palmivora* is the most important cause of root rot disease on papaya, which also infects the fruit and stem and seedlings, was first reported in the Philippines in 1916 (Ploetz *et al.* 1994). Bacterial crown rot and soft rot of papaya caused by *E. caricae* is widespread and destructive disease posing a major threat to the papaya industry of the Philippines (Obrero 1991). With the development of transgenic PRSV resistant papayas, it is of utmost importance that the resistance or susceptibility of the transgenic papaya to these diseases are tested. The rationale of the experiment is to ensure that varietal reactions to other diseases are not altered or rendered less desirable as a consequence of gene introduction. There is currently no local data available on the resistance of non-transgenic papaya to these diseases.

Non-transgenic papayas are susceptible to *Phytophthora* root rot, *Pythium* damping-off and bacterial crown rot caused by *E. caricae* based on the prevalence of these diseases in the country (Tangonan and Quebral 1992). This study was conducted 1) to isolate aggressive strains of *P. palmivora* and other fungal pathogens including *E. caricae* from the non-transgenic papaya and 2) to determine the susceptibility and/or resistance of transgenic papaya to *P. palmivora*, *P. debaryanum*, and *E. caricae* in comparison with non-transgenic papaya in a contained greenhouse.

MATERIALS AND METHODS

Collection, isolation, identification and maintenance of fungal and bacterial cultures

Diseased plant parts (roots, stems and crowns) and soil samples were collected from several papaya orchards in Laguna. The infected root/stem or crown was placed in a beaker and washed in gently running water for several hours. Small section of advancing root/stem or crown lesions was cut out and surface sterilized, by dipping the tissue in a 1:10 dilution of sodium hypochlorite for 30 seconds and rinsed in sterile water. Tissues were blotted dry, cut in 0.5 cm lengths and plated onto corn meal agar with added antibiotics and potato dextrose agar. Corn meal agar (CMA) amended with antibiotics was used for isolating *Phytophthora* and *Pythium* (Drenth and Sendall 2001). Infected flowers, fruits, and leaves were also used for isolation. Slices of

eggplant fruits were embedded for 4–7 days in partially flooded soil collected from papaya plants showing root rotting symptoms. Infected eggplant tissues were plated onto corn meal agar amended with antibiotics (Lee and Varghese 1974). Identification of *Pythium* and *Phytophthora* species followed the manual of Drenth and Sendall (2001). For bacterial isolation, small pieces of plant tissues were cut out from the diseased and healthy portion, surface sterilized and placed in sterile distilled water for few minutes. A 0.1 ml of bacterial suspension was placed in sterilized petri plates and pre-cooled nutrient agar (NA) was added. The plates were incubated at room temperature for 48–72 h. A single colony with characteristics of *E. caricae* (Gardan *et al.* 2004) was streaked onto NA slants. Fungal isolates were maintained in potato dextrose agar (PDA) slants and bacteria in NA slants.

Pathogenicity test of isolates on non-transgenic papayas

Pathogenicity test of eight fungal and two bacterial isolates was conducted in the greenhouse at the Crop Protection Cluster, UPLB. Three varieties of papaya namely '*Carica cariflora*', 'Cavite Special' and 'Papayang Uwak' were inoculated with two week old test pathogens grown in aforementioned culture media. The test was replicated three times with each replicate containing five seedlings. Each seedling was drenched with 10 ml suspension of concentration 10^4 spores per ml. Disease monitoring was conducted three, five and nine days after inoculation by counting the number of seedlings with damping-off symptoms. For the bacteria, two isolates from Brgy. Labuin and Brgy. Pagsawitan, Laguna were used. Twenty-four hour old isolates grown in NA were added with 9 ml sterile distilled water. Inoculation was done by pricking/stabbing at the base of leaf petiole with a syringe needle dipped in bacterial suspension of concentration 10^6 to 10^7 CFU/ml. Inoculation with sterile distilled water served as control. Inoculated seedlings were covered with plastic for 24 h.

Pathogenicity test of aggressive isolates on transgenic PRSV resistant papaya lines

Pathogenicity test with papaya seedlings was conducted in pots with vermiculite as substrate at the BL greenhouse, IPB. Seeds of four lines of transgenic papaya were soaked in water for three days and sown in beds containing autoclaved soil. The test was replicated three times with each replicate containing five seedlings. After three weeks, papaya seedlings were pricked and transplanted in individual pots. Inocula of *P. debaryanum* and *P. palmivora* were grown on autoclaved wheat seeds in flasks for three to four weeks. Inoculation was done by incorporating 0.2–0.3 g wheat seeds into the vermiculite near the base of the plant. Seven to 14 days after inoculation, the incidence of seedling damping-off was estimated. Four to six weeks after inoculation, plant roots were scored on the following scale: 0 = no root rot; 1 = up to 5% root mass rotted; 2 = up to 10% root mass rotted; 3 = up to 25% of root mass rotted; 4 = 50% of root mass rotted; 5 = all roots rotted up to 20% of seedlings killed; 7= up to 75% of plants killed; 8 = up to 90% of seedlings killed; 9 = all plants killed. Per cent root rot severity was computed as: n(1) + n(2) + n(3)…../n(9) x 100 where n = number of plants corresponding to the rating score in parenthesis. In the field, per cent of wilt incidence on four transgenic and 'Davao Solo' plants was monitored for the first four weeks.

An isolate of *E. caricae* that had been found highly aggressive to non-transgenic papaya was used in the screening. Inoculum was produced on NA coming from a freshly restreaked, isolated colony in slants. Plates were incubated for 46–95 h until

colonies were formed on the medium. The bacteria from colonies were suspended in water and adjusted to approximately 10^6 to 10^7 CFU/ml. Two-month old seedlings of transgenic and non-transgenic papaya were inoculated with the bacteria using a syringe needle. Plants were pricked at the base of leaf petiole with a needle previously dipped into bacterial suspension, and sterile distilled water served as control. Inoculated plants were placed in a contained greenhouse. Crown rotting incidence was observed seven and 14 days after inoculation.

Pythium sp. isolated from transgenic plants was used in the screening to determine the reaction of transgenic and the non-transgenic papaya plants for on pre- and post germination damping-off. Inoculum of *P. debaryanum* was grown in autoclaved wheat seeds in flasks for two weeks. Inoculation was done by incorporating wheat seeds into the sterilized soil at the rate of 1.0 g: 1.0 l of inoculum with a concentration of 10^6 spores per ml. and sterilized soil respectively, and mixed thoroughly. Seeds of papaya soaked in water for three days were sown in plastic trays containing inoculated soil. Sterilized soil served as control. Each treatment was replicated three times. Pre- and post- germination damping-off were monitored seven days and 14 days after sowing.

Analysis of data

Differences in the degree of infection between transgenic and non-transgenic papaya were determined using Fisher's least significant difference using PLABSTAT software (Utz 2000).

RESULTS AND DISCUSSION

Screening for aggressiveness in non-transgenic varieties showed that two out of eight fungal isolates were consistently pathogenic against the three local varieties of papaya (Fig. 1, 2 and 3). These isolates are Pya1 and Pya2. Pya1 and Pya2 were identified as *P. debaryanum* and *P. palmivora*, respectively (Drenth and Sendall 2001). The most aggressive bacterial isolate (Ec1) was isolated from Brgy. Labuin Sta Cruz, Laguna. Isolates Pya1, Pya2 and Ec1 were used to determine their aggressiveness on transgenic PRSV-resistant papaya lines.

The four transgenic papaya lines and 'Davao Solo' were all susceptible to isolate Pya1 (*P. debaryanum*) and Pya2 (*P. palmivora*) under contained greenhouse conditions (Table 1). No significant difference in per cent root rot severity caused by Pya2 among transgenic lines and 'Davao Solo' was observed ($p = 0.05$) (Fig. 4). In a field trial, 'Davao Solo' reacted similarly to 132–2.17–12.37 in terms of wilt incidence. The pathogen causing wilt was isolated as pure culture and identified as *P. debaryanum*. The same isolate was identified to be causing wilt and root rot in the BL2 greenhouse.

The three transgenic papaya lines were more susceptible compared with 'Davao Solo' to *E. caricae* in 7 days after inoculation (Fig. 5). But after 14 days from inoculation, the incidence of crown rotting in 'Davao Solo' was increased. This indicated that crown rot expression in 'Davao Solo' was delayed. Inoculated plant showed visible symptoms three to four days after inoculation and severe crown rotting was observed beyond seven days.

Susceptible reaction was exhibited by transgenic and the non-transgenic papaya plants at pre- and post-germination damping-off using *Pythium debaryanum* sp.

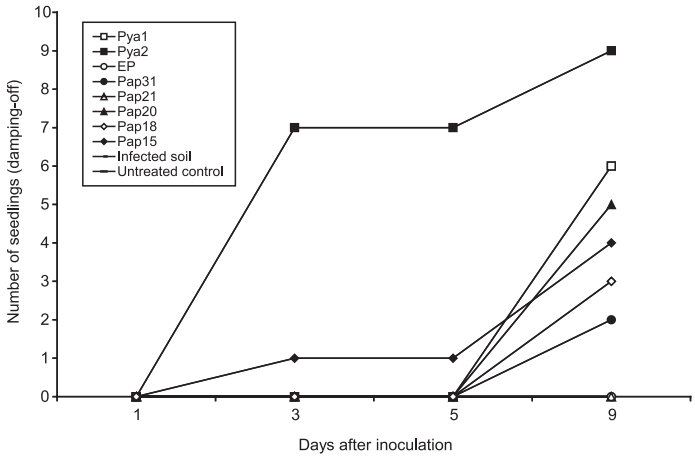

Fig. 1. Virulence test of eight fungal isolates on papaya variety '*Carica casiflora*'

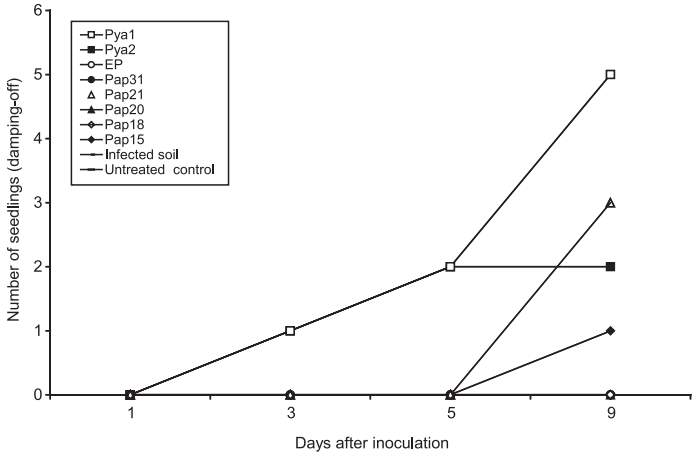

Fig. 2. Virulence test of eight fungal isolates on papaya variety 'Papayang Uwak'

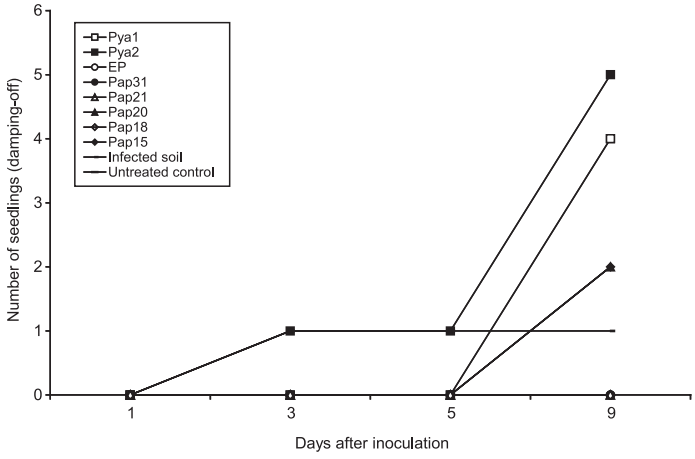

Fig. 3. Virulence test of eight fungal isolates on papaya variety 'Cavite Special'

Table 1. Reaction of four transgenic lines and 'Davao Solo' to *Pythium debaryanum* (Pya1) and *Phytophthora palmivora* (Pya2) expressed as per cent of infected seedlings (wilting).

Papaya line	Mean [%] of infected seedlings		
	P. debaryanum	*P. palmivora*	Control
132–2.9–13.27	46.7a	46.7a	0
132–2.19–9.19	66.7a	60.0a	0
124–3.9–21.17	60.0a	60.0a	0
132–2.17–12.37	46.7a	73.3a	0
Davao Solo	60.0a	46.7a	0

[1] Numbers in column, followed by the same letter do not differ at 5% significance

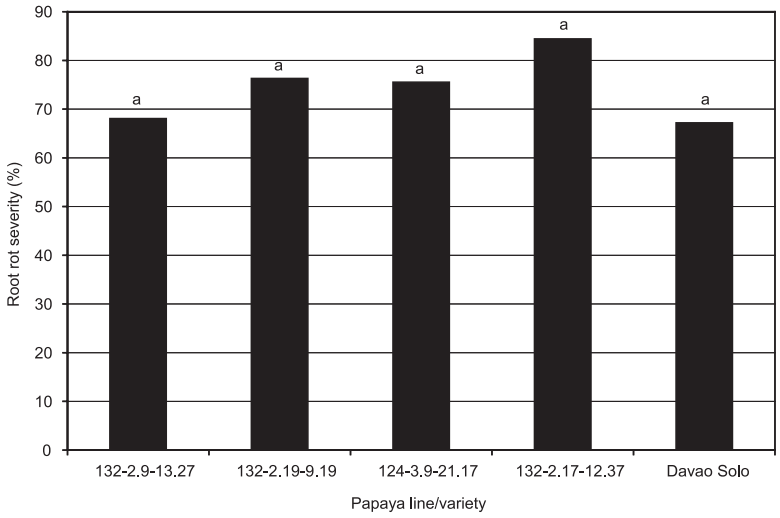

Fig. 4. Reaction of four transgenic lines and 'Davao Solo' to *Phytophthora palmivora* expressed as root rot severity

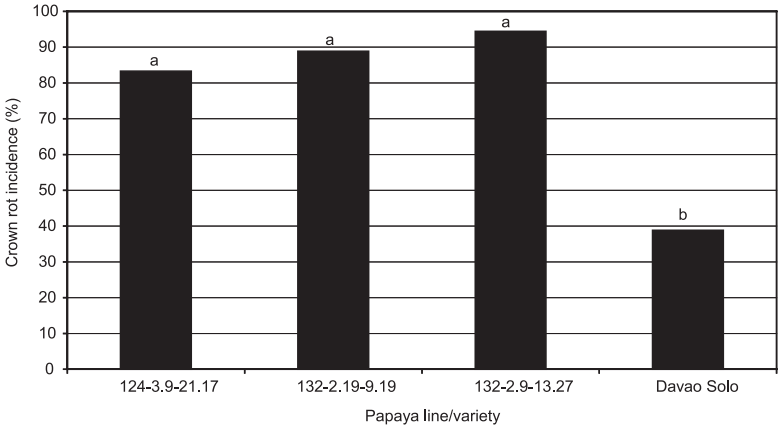

Fig. 5. Reaction of three transgenic lines and 'Davao Solo' to *Erwinia caricae* expressed as crown rot incidence

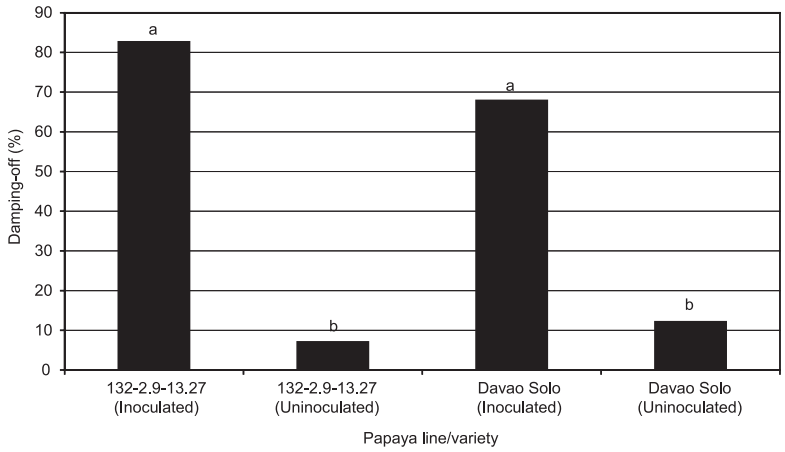

Fig. 6. Reaction of transgenic line 132–2.9–13.27 and 'Davao Solo' to *Pythium debaryanum* expressed as pre- and post-germination damping-off (%)

isolated from transgenic plants (Fig. 6). High pre- and post-germination damping-off incidence 82.7% was observed in the transgenic line, and 67.9% in the non-transgenic line, in inoculated soil compared to sterilized soil.

Generally no resistance reaction was established in the PRSV resistant transgenic as well as the non-transgenic papaya tested against *P. palmivora* and *P. debaryanum* and *E. caricae* under contained greenhouse conditions. Wilt incidence was moderate to severe in the plants inoculated with *P. palmivora* and *P. debaryanum* isolates. A high degree of root rot severity was also observed in the transgenic and non-transgenic plants inoculated with *P. palmivora*. The same reaction as that of plants inoculated with the bacterial isolates, a high incidence of crown rot was stated. Pre- and post-germination damping-off incidence observed was 82.7% in transgenic line and 67.9% in the non-transgenic line sown in inoculated soil. In comparison with other related study, Colyer *et al.* (1999) evaluated several transgenic cotton genotypes and their non-transgenic parents for two years in a naturally infested field for resistance to the root-knot nematode (*Meloidogyne incognita*)/Fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum*) disease complex. They found that in transgenic and non-transgenic cotton, root gall ratings were high and wilt ratings were moderate. There were significant differences in wilt or root-knot gall ratings between some parental cultivars and their transgenic progeny. These results indicate that some transgenic cultivars respond differently to the root-knot nematode/Fusarium wilt complex than their parental cultivar. The potential causes and importance of these differences were discussed. Eighteen transgenic and non-transgenic cotton varieties were evaluated for resistance to the reniform nematode (*Rotylenchulus reniformis*) and yield. The test was conducted in a field located in Huxford, Alabama which was naturally infested with the reniform nematode. Similarly, Mclean *et al.* (2001) also observed that in their test none of the cotton varieties showed resistance to the reniform nematode.

The initial finding indicated that gene resistance in transgenic papaya was found to be specific to papaya ring spot virus (PRSV). Therefore our findings support the view that susceptibility and/or resistance of the transgenic papaya to the aforementioned diseases were the same as in non-transgenic plants.

However, Wei *et al.* (2006) studied the effect of transgenic PRSV resistant plant in microbial communties. They concluded that transgenic papaya could indeed alter chemical properties, enzyme activities, and microbial communities in soil. This aspect has not been studied in this project and therefore could be explored.

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POLISH SUMMARY

PORÓWNANIE INFEKCJI WYWOŁANEJ PRZEZ *PYTHIUM DEBARYANUM, PHYTOPHTHORA PALMIVORA* I *ERWINIA CARICAE* NA ROŚLINACH PAPAJI TRANSGENICZNYCH I NIETRANSGENICZNEGO NA WIRUSA PIERŚCIENIOWEJ PLAMISTOSCI PAPAJI

Celem badań było określenie reakcji roślin Carica papaja odpornych transgenicznych na wirusa plamistości pierścieniowej na niektóre ważne, patogeniczne grzyby oraz bakterie. W przeprowadzonej pracy wykorzystano 4 transgeniczne linie Carica papaja wyhodowane w Instytucie Hodowli Roślin (Filipiny) oraz nie transgeniczną odmianę C. papaja „Davao Solo", odporne na wyżej wymienionego wirusa. Rośliny rosnące w szklarni były sztucznie zakażane wysoce agresywnymi izolatami grzybów *Pythium debaryanum* i *Phytophthora palmivora*, oraz bakterią *Ervinia caracie*. Zakażenie Roślin przeprowadzano umieszczając u podstawy pędów nasiona pszenicy zasiedlone przez grzyby patogeniczne, natomiast zakażenie szczepem bakterii *E. caracie* wykonano nakłuwając podstawę ogonka liściowego igłą umoczoną w zawiesinie komórek bakteryjnych. Zarówno 4 transgeniczne linie C. papaja jak i odmiana „Davao Solo" okazały się wrażliwe na *P. debaryanum* i *P. palmivora* w warunkach szklarniowych. Nie stwierdzono istotnych różnic pomiędzy liniami transgenicznymi C. papaja i odmianą „Davao Solo" w procencie siewek wykazujących objawy więdnięcia wywoływanego przez *P. palmivora* ($p = 0,05$). Trzy linie transgeniczne C. papaja wykazywały w okresie pierwszych 7 dni po inokulacji wyższą wrażliwość na *E. caracie* niż odmiana „Davao Solo", ale po 14 dniach stopień ich porażenia był jednakowy. Zarówno transgeniczne i nie transgeniczne linie C. papaja były wrażliwe na *P. debaryanum*. Nie stwierdzono istotnej różnicy w wystąpieniu zgorzeli przedwschodowej i powschodowej u jednej z badanych linii transgenicznych C. papaja i odmiany „Davao Solo" ($p = 0,05$) w zakażonej ziemi.