

Genotype Differences in Seed Germination and Growth, Pollen Fertility and Fruit Traits of Three Promising Papaya (*Carica papaya* L.) F₁ Hybrids

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Papaya F₁ hybrids are generally grown by many growers for their hybrid vigor, superior fruit characteristics and moderate tolerance to the *Papaya ringspot virus* (PRSV). A single-factor experiment conducted in completely randomized design (CRD) with three replications was done to test seed germination, assess seedling growth, and evaluate pollen fertility and horticultural characteristics of three promising papaya F₁ hybrids – 5648 x 336 (Liyag), 097 x 4172 (Hirang), and 4173 x 5648 (Timyas). There were significant differences in percent seed germination and seedling height between the three papaya F₁ hybrids. Hybrid 5648 x 336 (Liyag) had the highest seed germination rate (92.20%) and the tallest seedlings (44.73 mm). In terms of root characteristics, there were significant differences between the hybrids in fresh and dry weights of roots, number and length of secondary roots. Hybrid 5648 x 336 (Liyag) had the heaviest fresh and dry weights of roots and had the highest number of secondary roots while hybrid 097 x 4172 (Hirang) had seedlings with the longest secondary roots. There were significant differences in pollen viability in the three hybrids. F₁ hybrid 4173 x 5648 (Timyas) had the highest percent pollen viability (83.33%), and hybrid 097 x 4172 (Hirang) the lowest (73.33%). Pollen viability and pollen germination were high for the six parental inbred lines of the three F₁ hybrids, but no significant differences were found. In addition, there were significant differences between the three F₁ hybrids in fruit weight, fruit length and flesh thickness. Hybrid 097 x 4172 (Hirang) had red flesh, while hybrid 5648 x 336 (Liyag) and hybrid 4173 x 5648 (Timyas) had yellow orange flesh. The three hybrids are moderately tolerant to PRSV similar to the control cultivar Sinta.

Key Words: *Carica papaya* L., F₁ hybrids, genotypes, germination, roots, seeds

Abbreviations: BCR – bacterial crown rot, I₂KI – potassium iodide, PCI 2 – peel color index 2, PRSV – *Papaya ringspot virus*

INTRODUCTION

The papaya (*Carica papaya* L.), a monotypic species in the family *Caricaceae*, is commonly grown for its melon-like fruits that are available throughout the year. It is relatively easy to grow and produces fruits within a short period, making it a good cash crop. When grown under the right cultural conditions, papaya can give high yield per tree and on a per hectare basis. In the papaya cultivar Sinta, for example, 19,859 kg/ha yield can give the farmer a net income of PhP 99,595 for farmgate and PhP 209,494 for wholesale (PCAARRD 2009). In 2015, papaya ranked 8th in area planted and 4th in production volume among the ten leading fruit crops in the Philippines (PSA-BAS 2016). The top three papaya-producing regions in the Philippines were SOCCSKARGEN in South Central Mindanao (69,008 mt), Northern Mindanao (38,256 mt)

and Davao Region (10,405 mt) (PSA-BAS 2016). Large commercial papaya plantations are found in two big private companies in Mindanao, namely, Del Monte and Dole Philippines. However, most of the papaya plantings in these regions are considered small-scale backyard types. About 92% of the total papaya production is consumed locally as food, while the rest are for industrial use. In 2013, Philippine production of papaya ranked 8th in the world. Export of dried papayas to Australia, Belgium and France, with an initial amount of 320 tons in 1991 paved the way for the Philippine economy to gain US\$ 4 million (PCAARRD-DOST 2013).

Papaya suffers from several diseases and insect pests, the most widespread and destructive of which is the *Papaya ringspot virus* (PRSV). In the Philippines, PRSV was first detected in Silang, Cavite in 1982 (Opina 1986), but it

spread rapidly in Luzon because of the explosive nature of the disease (Magdalita et al. 1989). PRSV was also detected in a few areas in 2001 in Davao del Sur and South Cotabato in Mindanao (Herradura et al. 2001). In the Philippines, PRSV practically decimated commercial papaya in Southern Luzon, leading to a significant decline in production, substantial income losses for farmers, a relative scarcity of the fruit in the market, and higher costs to consumers. To date, PRSV is already endemic in the entire island of Luzon and has spread to other island provinces of Marinduque, Mindoro, and Palawan in Luzon; Leyte, Samar, Panay, Negros, Bohol and Cebu in the Visayas (Magdalita et al. 2012).

Another disease of papaya is bacterial crown rot (BCR), caused by a bacterium, *Erwinia mallotivora* Goto. At its onset, BCR can damage the entire papaya plantation, or at least 50% of the trees (Yap 2017). After infection, the papaya stems start to rot and then collapse (Magdalita et al. 2017).

Aside from PRSV and BCR, another constraint to production is poor seed germination due to improper storage of seeds saved by farmers who plant open-pollinated varieties (OPVs). While many small-scale papaya producers usually grow the crop from saved seeds, agronomists encourage the use of commercial hybrid seeds for planting to obtain higher yields. However, due to the high costs of commercial hybrid seeds, many farmers still rely on the use of open-pollinated seeds taken from their previous crops. These seeds are usually extracted from ripe papaya fruits of trees grown in farms and backyard gardens in many areas in the Philippines.

To ensure good seed quality, papaya growers must also consider the sexual reproductive type and the genetic variability of the variety. Papaya is heterozygous for many traits and will produce considerable trait differences in the resulting progenies. In addition, OPVs produce progenies with high phenotypic variability due to pollen contamination of neighboring trees. In addition, both dioecious and gynodioecious (bisexual) varieties of papaya are grown commercially in Australia. The dioecious which are outcrossing varieties are generally recommended by the Queensland papaya industry because of the high fruit yields of these varieties (Chay-Prove et al. 2000).

Usually, papaya farmers and growers either sow the seeds directly in the field or germinate them in seedling trays in the nursery. Seed germination may occur within 10–21 d after sowing and may continue intermittently for up to 35–40d (Bhattacharya and Khuspe 2001). In papaya

seeds, the outermost layer containing the gelatinous substance known as sarcotesta contains inhibitors that prevent germination (Ellis et al. 1985; Tseng 1992). Even fresh seeds with unremoved sarcotesta that has not been properly washed usually have very low and variable germination. Soaking the freshly extracted papaya seeds in tap water followed by several washings with tap water over several days can help break the sarcotesta, resulting in leaching of the gelatinous mucilage containing the inhibitors. Breaking the sarcotesta can be done by rubbing the seeds in wire mesh and allowing them to float during several washings with tap water followed by air drying of the seeds in ambient temperature for 1 wk. In addition, floaters can be discarded during washing of the seeds. Soaking papaya seeds in water for 24 h can help increase seed germination (Paz and Vasquez-Yanes 1998).

The ability of papaya as a pollen donor is important to determine if it can be used as a hybrid with other varieties. Three basic sex forms occur in papaya, namely: male, female and hermaphrodite. Due to limitations in temperature, the hermaphrodite is commercially restricted to the tropics. The female morph can be grown successfully in subtropical zones, but commercial success largely depends on the availability and viability of pollen (Allan 1963a, b).

This study was conducted to test the seed germination and assess the seedling characters of three papaya F₁ hybrids (Hirang, hybrid 097 × 4172; Liyag, hybrid 5648 × 336; and Timyas, hybrid 4173 × 5648), assess their flowering characteristics and pollen fertility, and evaluate their fruit characteristics and reaction to PRSV.

MATERIALS AND METHODS

Papaya seeds were procured from the papaya germplasm collection of the Fruit and Ornamental Crops Breeding Section, of the Institute of Plant Breeding (IPB), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños. The experiments were conducted at the same institute from May 2016 to December 2017.

The seeds of the three papaya F₁ hybrids and the control cultivar Sinta were placed in individual seed envelopes and labeled properly. They were stored in tightly sealed glass jars with silica gel as desiccant at the bottom for 6 mo and stored in an air-conditioned room. Seeds of each F₁ hybrid and of the control cultivar Sinta were retrieved and then placed in glass jars containing tap water and soaked overnight. The water was decanted the next day, and the seeds were sown in a sterile potting medium composed of a mixture of garden soil, coir dust and compost (1:1:1, v/v). The medium was placed in well-

drained plastic trays rested on metal benches in the screenhouse and provided with a black net as partial shade. A plastic tray was assigned to each papaya F₁ hybrid. In each plastic tray, three rows with shallow trench were made to serve as the replicates for each genotype. In each row, 20 seeds of each papaya F₁ hybrid were sown for a total of 60 seeds sown for each hybrid. After sowing, the seeds were covered with a thin layer of the same medium and then watered accordingly when the medium started to dry up. The seeds germinated for 7d. After this period, the number of germinated seeds in each replicate for each hybrid was counted, and the percent seed germination was determined as follows:

$$\% \text{ germination} = \left(\frac{\text{no. of germinated seeds}}{\text{total no. of seeds sown}} \right) \times 100$$

To evaluate the seedling traits of the papaya F₁ hybrids and the control Sinta, 25 seedling samples were taken from each replicate for the hybrids. These samples were used to assess seedling height, stem diameter, number of leaves, length and width of fully expanded leaves, petiole length, fresh and dry weights of roots, length of primary root, and the number and length of secondary roots.

A ruler was used to measure seedling height (mm) from the base of the seedling or at ground level up to the tip of the tallest leaf. A digital Vernier caliper was used to measure the stem diameter (mm) at the middle of the stem of the seedling. The number of leaves was obtained by counting the five most fully expanded leaves of each sample. A digital Vernier caliper was also used to measure the length (mm) of the most fully expanded leaves from the leaf base to the tip of the leaf of five samples. The width (mm) of the five most fully expanded leaves of the seedling samples was obtained by measuring from one end of the widest portion of the leaf to the other end. A ruler was used to measure the length (mm) of the petiole of the same leaf samples by measuring from the base of the petiole to the point of leaf attachment. Fresh and dry weights (mg) of the roots were obtained by using an analytical balance. The root samples were wrapped in aluminum foil and dried in an oven for 1 wk. Subsequently, the samples were weighed. The length (mm) of the primary root was measured from the junction of the root and the stem up to the tip of the primary root. A ruler was used to measure the length (mm) of five secondary root samples of each plant sample from the point of attachment at the primary root up to the tip of the secondary root. The number of secondary roots was obtained by counting the secondary roots from each seedling sample, and the average was taken.

Pollen Fertility Test

The pollen fertility of the papaya F₁ hybrids and the parental inbred lines should be known and characterized to find out if the papaya F₁ hybrids and the parental inbred lines can be good pollen donors. Flowers of the F₁ hybrids and the parental inbred lines were observed until the flower reached full balloon stage. The flowers were collected from 7:00 to 10:00 am. Afterwards, the flowers were brought to the laboratory and used for the pollen fertility test.

The collected pollen grains were put in Petri dishes and dusted onto microscope slides. A few drops of I₂KI solution (1 g KI and 0.5 g I) dissolved in 100 mL distilled water (Cavusoglu and Sulugsoglu 2013) were applied on the pollen grains by using a medicine dropper. A cover slip was placed on top of the slide. The excess stain was removed by blotting with tissue paper at the side of the cover slip. Pollen grains are considered viable if they are fully stained. Forty pollen grains were counted for each of the three replicates. In each replicate, a flower at the full balloon stage was used as source of pollen for staining. Pollen fertility testing by structural staining was computed as follows:

$$\left(\frac{\text{No. of darkly stained pollen grains}}{\text{Total no. of pollen}} \right) \times 100$$

A pollen fertility test by germination was also conducted by dusting pollen grains onto a depressed slide with semi-solid Brewbaker and Kwack's medium (Brewbaker and Kwack 1963) added with 5% sucrose. After 4 h incubation at 25°C, the germinated pollen grains were counted. Pollen fertility was computed as follows:

$$\left(\frac{\text{No. of geminated pollen}}{\text{total no. of pollen}} \right) \times 100$$

Horticultural Characteristics and PRSV Reaction of Promising Papaya F₁ Hybrids

The horticultural characteristics and PRSV reaction of the three promising papaya F₁ hybrids were evaluated at the Central Experiment Station in Tranca, Bay, Laguna. The PRSV-moderately tolerant variety Sinta was the positive control, while the PRSV-susceptible variety Cavite Special served as the negative control. The experiment was conducted in RCBD with three replications and 25 plant samples per plot for a total of 375 experimental plants in a 2,250 m² lot (Fig. 1). Each replication was bordered with the Sinta hybrid. The experimental plants were spaced 3m between rows and 2m between plants. Seedlings were raised in the screenhouse at 25–30°C and placed on steel benches. Seeds were sown in a light soil medium

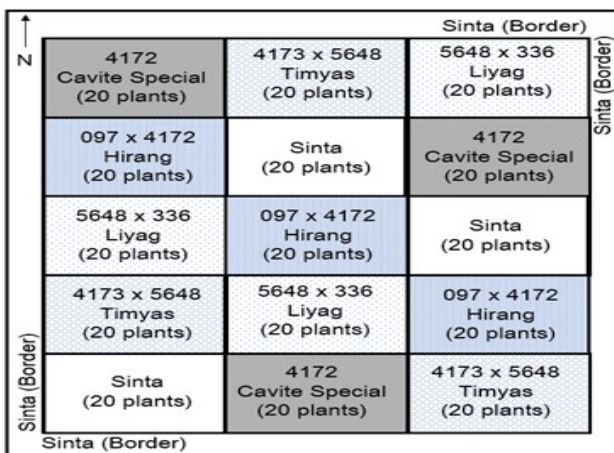


Fig. 1. Replicated field trial of candidate papaya hybrids.

consisting of a mixture of garden soil, coir dust and vermi-compost (1:1:1 v/v) and watered regularly. Seedlings (7d old) were fertigated with complete water-soluble fertilizer to hasten their growth. After 2 mo, they were hardened for 1wk by gradual exposure to sunlight and minimal watering. The plants were transplanted in holes just enough to accommodate the bole of soil with the plant in it. The bottom of the hole was provided with 50 g basal fertilizer (mixture of urea and complete fertilizer, 2:1 v/v).

The plants were watered twice a week at field capacity if there was no rain and sprayed with the appropriate insecticides and fungicides as needed.

At flowering stage, data on horticultural characteristics of the three promising F₁ hybrids and the controls were evaluated. Plant height at first flower (m) was measured from the base of the plant to the first flower by using a meter stick. Stem girth or diameter (cm) was measured halfway of the stem by using a Vernier caliper, while the number of nodes at first flower was counted from the lowest node to the node bearing the first flower. Assessment of PRSV tolerance was done based on a rating scale used by Alviar et al. (2012).

At maturity stage, the number of mature fruits per tree was counted to determine the yield. Marketable and non-marketable fruits were counted. Physiologically mature fruits with a peel color index of 2 (PCI 2), i.e., with a tinge of yellow at the apex of the fruit, were harvested. Five fruit samples were taken from each tree and allowed to ripen on the steel benches. At full ripe stage, they were evaluated for fruit qualities. Fruit weight (kg) was measured by using a top loading balance. A Vernier caliper was used to measure fruit length (cm), fruit width (cm), and flesh thickness (cm). A digital refractometer was used to measure the total soluble solids (TSS °Brix), a relative measure of sweetness of the flesh. The percent

edible portion was computed by dividing the edible portion by the fruit weight and the quotient was multiplied by 100. Flesh firmness (kg cm⁻²) was measured by using a hand-held penetrometer while titratable acidity (meq*10 mL juice⁻¹) was measured by using a titration machine. Qualitative traits, e. g., flesh color and peel color, were evaluated. Each tree was assessed for tolerance to PRSV.

Statistical Design and Analysis

The screenhouse experiments to test seed germination and assess seedling growth were conducted in CRD with three replications. In the germination test, 60 seeds were used for each papaya F₁ hybrid and the control Sinta. Twenty-five seedlings in each of the 3 replications for each hybrid and the control Sinta were used to assess the seedling growth characteristics. Pollen fertility testing by using LKI structural staining and pollen germination were also conducted in CRD with three replications. Five flower samples were used in each replicate giving a total of 15 samples for pollen viability. The pollen fertility data were subjected to one-way analysis of variance (ANOVA) for CRD using F-test and treatment means were compared using LSD at 0.05 level of significance. The field evaluation of horticultural characteristics was conducted in RCBD with three replications. All data on seed germination, seedling growth, pollen fertility tests and horticultural characteristics were subjected to ANOVA using the F-test. At 0.05 LSD, significant differences were found between treatment means using the Statistical Tool for Agricultural Research (STAR) Program (IRRI 2014).

RESULTS AND DISCUSSION

Seed Germination and Seedling Characteristics

The papaya seed belongs to the intermediate type according to storability (Zulhisyam et al. 2013). Seeds are still the most practical method of raising seedlings due to ease of handling. While germination is the most efficient method of seed propagation, there are constraints to the successful germination of the seeds. For example, papaya seeds do not germinate readily when inhibitors found in the sarcotesta that adhere to the seed coat are not completely removed during seed processing (Okeyo and Ouma 2008). Seed germination of the three papaya F₁ hybrids and the control Sinta occurred 1 wk after sowing.

Significant differences were found in percent seed germination and seedling height of the three papaya F₁ hybrids and the control Sinta (Fig. 2a and b). Hybrid 4173 x 5648 (Timyas) (91.06%) and hybrid 5648 x 336 (Liyag) (92.2%) had similarly high percent germination (Fig. 2a). Similarly, high seed germination rate was also obtained in the commercial F₁ hybrids including Cariñosa and Red

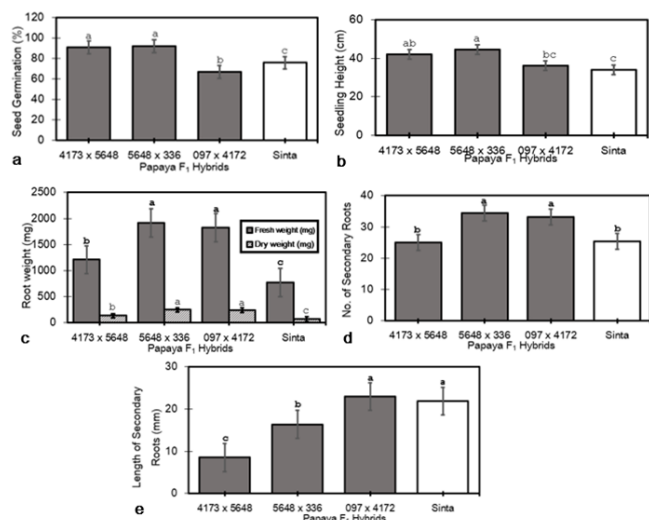


Fig. 2. Seed germination (%) (a), seedling height (cm) (b), root fresh and dry weight (mg) (c), number (d), and length (e) of secondary roots of 3 papaya F₁ hybrids including the control Sinta.

Royale (EWSC no date).

Drying the seeds, storing them properly and pre-soaking them in tap water overnight prior to sowing activated the metabolic processes and promoted enzyme and hormone metabolism, resulting in good germination (Ali and Elozeiri 2017). Similarly, Riley (1981) and Yahiro (1979) reported that seeds of *Carica* species pre-soaked in water for 24 h, germinated and then dried resulted in higher percent germination compared with seeds that were not pre-soaked in water. In terms of seedling traits, hybrid 5648 x 336 (Liyag) had significantly the tallest stature at 44.73 cm (Fig. 2b), while the control Sinta had the shortest stature at 34.13 cm (Fig. 2b). Hybrid 5648 x 336 and hybrid 4173 x 5648 had similar seedling height, while hybrid 097 x 4172 and the control Sinta, a semi-dwarf variety, had similar seedling height (PCARRD 2009). This result indicates that the three promising papaya F₁ hybrids may have potential semi-dwarf characteristics similar to those of the control Sinta. There are similar observations for the seedlings of other F₁ hybrids such as 'Red Royale' and 'Cariñosa' (EWSC no date). In the face of climate change, semi-dwarf papaya varieties are a valuable commodity in most typhoon-stricken areas as they could withstand strong winds. Harvesting is also convenient and easy with semi-dwarf varieties. In addition, integrated pest management is easier to apply on small trees. However, there were no significant differences between the three papaya F₁ hybrids in stem diameter, number of leaves, leaf length and width, and petiole length.

There were significant differences in terms of root characteristics including fresh and dry weights of roots,

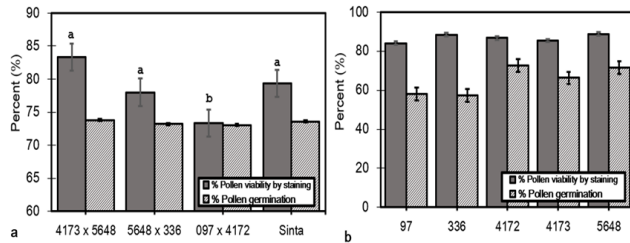
number and length of secondary roots. However, no significant differences were observed in the length of the primary roots among the three papaya F₁ hybrids and the control Sinta. Hybrids 5648 x 336 and 097 x 4172 had significantly the heaviest fresh weight of roots at 1,915.62 g and 1,826.52 g, respectively (Fig. 2c). The same trend was observed in the dry weight of the roots (Fig. 2b). This result suggests that these hybrids had more root mass for anchorage and for absorption of water and nutrients from the soil that could render the seedlings vigorous. However, the control Sinta had the lightest weight of fresh roots at 774.70 mg.

The three new papaya F₁ hybrids and the control Sinta had no significant differences in the length of their primary root. This similarity in length of the primary root of the hybrids could be due to the general natural morphology of the papaya root being relatively short, non-axial and non-fibrous (Carniero et al. 2009). Two of the hybrids (5648 x 336 and 097 x 4172) had significantly more secondary roots at 34.40 (35) and 33.20 (34), respectively, compared with hybrid 4173 x 5648 and the control Sinta (Fig. 2d) which had 25.00 (25) and 25.40 (26), respectively. This result suggests that these hybrids developed more secondary roots. There was a similar observation in Sorghum-Sundangrass hybrids which have more secondary roots. The same hybrid has an aggressive root system and is a high biomass producer (Clark 2007). These roots developed from the upper sections of the primary root and then branched profusely (Carniero et al. 2009). In papaya 'Eksotika', Masri (1993) found that higher density of root tips would mean higher root activity and higher concentration of root tips 30 cm from the soil surface would mean higher nutrient uptake.

In terms of the length of secondary roots, significant differences were observed among the hybrids and the control Sinta (Fig. 2e). The F₁ hybrid 097 x 4172 and the control Sinta had significantly longer secondary roots at 22.96 mm and 21.92 mm, respectively, compared with hybrid 4173 x 5648 at 8.56 mm (Fig. 2e). However, hybrids 5648 x 336 and 4173 x 5648 had significantly the shortest secondary roots.

Pollen Fertility

Pollen viability obtained using I₂KI structural stain significantly differed among the three papaya F₁ hybrids and the control Sinta (Fig. 3a). Two of the papaya F₁ hybrids (4173 x 5648 and 5648 x 336) including the control Sinta had significantly more viable pollen at 83.33%, 78.00%, and 79.33%, respectively, compared with hybrid 097 x 4172 (73.33%) during the summer months (Fig. 3a). In terms of pollen germination, similar rates among the hybrids and Sinta were observed ranging from 73.06% to



*Letters with similar letters are not significantly different with each other at $\alpha=5\%$ LSD.

Fig. 3. Pollen viability and germination (%) of three papaya F₁ hybrids and the control Sinta (a) and their parental inbred lines (b).

73.57% (Fig. 3a). This result indicates that the three papaya F₁ hybrids and the control Sinta could develop viable pollen needed for self-fertilization during hot weather, hence, good fruit production under natural conditions. Similar results were observed in a previous report in Australia where pollen viability of the maintainer line of papaya cultivar 2001 known as Supermale is high during summer when the average temperature is approximately 29°C (Magdalita et al. 1998). Conversely, minimum temperatures below 10 °C in Israel and South Africa significantly affect pollen viability, possibly because of the degeneration of the pollen mother cells (Allan 1963a, b; Cohen et al. 1989; Garrett 1995).

On the other hand, seed production of the three papaya F₁ hybrids is an important aspect of hybrid technology. For this reason, pollen fertility of the parental lines should be known before their use as pollen source for the male parent and as ovule source for the female parent prior to mass seed production. While the pollen viability of five parental inbred lines of the three F₁ hybrids was not significantly different, it remained high, ranging from 84% to 88.67% (Fig. 3b).

All the five parental lines had numerous dark-stained pollen grains since the proteins in them reacted with the stain and have normal shape as indicated by the I₂KI

structural stain (Fig. 4). Conversely, the lightly and partially stained pollen grains with deformed shape can be interpreted as non-viable and incapable of germination (Fig. 4). It indicates that the dark-stained pollen grains of the parental lines have normal nuclei and that they are in good condition, and therefore capable of fertilization, giving rise to the male gametophyte of a seed plant. Hence, all these parental lines are amenable for use in large-scale seed production under local conditions. This result concurs with the previous observation that the parental lines of the Sinta papaya hybrid had high pollen viability (EWSC pers. communication).

While pollen viability is high in the five parental inbred lines of the three F₁ hybrids as indicated by I₂KI staining, it is equally important to check the germination of the pollen. *In vitro* pollen germination allows the measurement of the intrinsic aptitudes of the pollen to germinate outside and become a guide to simulate the interaction between the pollen and the stigma (Zaid and De Wet 2002). This intrinsic aptitude can be estimated by the ability of the pollen to fertilize the ovule and finally develop into a fruit (Boughediri et al. 1987). Pollen germination percentage of the five parental inbred lines ranged from 47.53% to 72.68% (Fig. 3b). While the parental line 4172 had the highest percentage pollen germination, it is not significantly different from that of the other parental lines. This result indicates that the five parental lines are all capable of germinating their pollen which is essential in the production of the F₁ hybrid progenies via fertilization of the female parent during cross-pollination.

The elongating pollen tubes can reach the embryo sac and enable the transfer of the male gamete to the female to effect successful fertilization (Fig. 5), which is possible via the union of the sperm and the egg nuclei that will become the diploid zygote and in turn develop into an embryo of the F₁ hybrid, and the union of the second gamete to the polar nuclei that will give rise to the

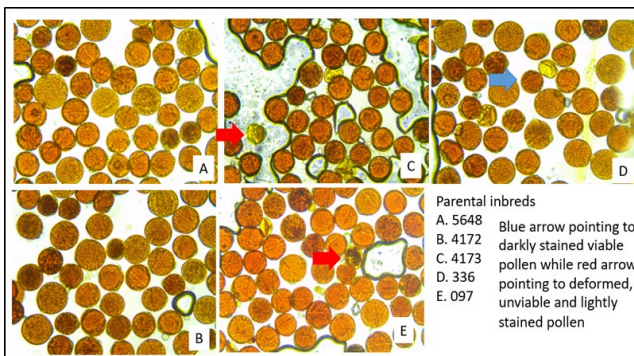


Fig. 4. Pollen viability of different parental lines of three F₁ hybrids subjected to I₂KI structural staining and magnified 400X using ToupTek (USA) microscope viewing software.

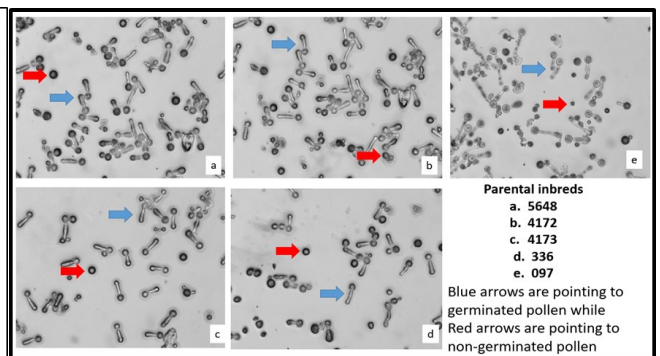


Fig. 5. Germinated pollen of the parents of three papaya F₁ hybrids on Brewbaker and Kwack's germination medium magnified 100X using ToupTek (USA) microscope viewing software.

endosperm of the seed. Hence, pollen germination of the male parent is necessary for successful cross-pollination to produce F₁ hybrid seeds.

Fruit Characteristics and PRSV Reaction of Promising Papaya F₁ Hybrids

The horticultural characteristics of the three papaya F₁ hybrids grown in the Central Experiment Station in Tranca, Bay, Laguna are presented in Table 1. There were significant differences in the fruit weight of the papaya F₁ hybrids (Table 1). The individual fruit weight ranged from 393 to 3196 g. Liyag (5648 x 336) had the highest fruit weight of 1338.74 g, which is significantly different from those of Hiram (097 x 4172) and Timyas (4173 x 5648) and the control cultivars Sinta and Cavite Special. Magdalita and Signabon (2017) also found in a preliminary study that Liyag (5648 x 336) produced the heaviest fruits among the three hybrids. This fruit size is characteristic of medium-sized papayas (1–2 kg) which are the most preferred in the local market due to their suitability for consumption in one sitting by an average Filipino family.

The fruit length of the hybrids and the controls ranged from 315 to 116 mm. F₁ hybrid Liyag (5648 x 336) produced the longest fruit at 209.16 mm that is significantly different from that of the other hybrids. This result suggests that the elongated fruits of this hybrid were produced by the hermaphrodite sex form. This fruit form is preferred by consumers because of convenience in handling. Conversely, the control Sinta produced the shortest fruit at 174.21 mm (Table 1). No significant differences were found in the fruit length of 097 x 4172 (Hiram), 4173 x 5648 (Timyas), and the controls Sinta and Cavite Special.

Flesh thickness of the hybrids and the controls were found to be significantly different. Hybrid 5648 x 336 (Liyag) and the controls Sinta and Cavite Special had the thickest flesh which is significantly different from those

of 097 x 4172 (Hiram) and 4173 x 5648 (Timyas). This result implies that hybrid 5648 x 336, being thick-fleshed, had more edible flesh. Its flesh thickness is similar to that of Maradol Roja, a Mexican papaya variety with a flesh thickness of >3.0 cm (Fitch 2005). Thick flesh is an important varietal trait since it contributes considerably to the edible portion of the fruit and is also a factor that prevents the fruit from becoming easily soggy when ripe.

Flesh color is an important trait to consider in papaya because it is usually eaten fresh and fruit colors are appealing to consumers. The three F₁ hybrids significantly differed in their flesh color. Hybrid 097 x 4172 (Hiram) has vermilion red (RHCC 41B) flesh in particular (Fig. 6). This hybrid also expressed the red



Fig. 6. Whole and cross-section of the fruits of three papaya F₁ hybrids.

flesh color in a previous evaluation in the field (Magdalita and Signabon 2017). F₁ hybrid 5648 x 336 (Liyag) has saffron yellow (RHCC 21A) flesh, while 4173 x 5648 (Timyas) has maize yellow (RHCC 21D) flesh. Red-fleshed papayas are preferred in the Bicol Region in the Philippines, and in other places where there are more

Table 1. Fruit characteristics of different papaya F₁ hybrids in the Laguna field trial.

F ₁ Hybrids	Fruit Wt. (g)	Fruit Length (mm)	Fruit Width (mm)	Flesh Thickness (mm)	Edible Portion (%)	Total Soluble Solids (°Brix)	Mean No. of Harvestable Fruits	Titrateable Acidity (meq 10 juice ⁻¹)	Firmness (kg cm ⁻²)	Tolerance to PRSV	Flesh Color
Hiram (097x4172)	1051.82b	180.86b	108.80a	24.53b	83.01a	10.7a	19.33a	2.12a	2.91a	Moderately tolerant	Red
Liyag (5648x336)	1338.74a	209.16a	118.14a	27.32a	84.35a	11.04a	14.46ab	1.79a	2.99a	Moderately tolerant	Yellow orange
Timyas (4173x5648)	936.48b	181.88b	108.46a	24.93b	82.46a	12.08a	19.15a	1.95a	4.06a	Moderately tolerant	Yellow orange
Sinta	1030.62b	174.21b	111.18a	27.94a	27.94a	11.84a	17.82a	2.20a	2.84a	Moderately tolerant	Yellow orange
Cavite Special (4172)	968.06b	190.80ab	102.56a	27.94a	27.94a	11.67a	9.92a	2.07a	3.08a	Susceptible	Red orange

*Values with similar letters are not significantly different at $\alpha = 5\%$ LSD.

Chinese costumers. In addition, red-fleshed papayas are preferred for canning as component of fruit cocktail.

On the other hand, yellow-fleshed papayas are generally preferred by consumers in Southern Tagalog and Central Luzon including the Visayas and Mindanao where the yellow-fleshed Solo papayas are very popular and where consumers have become used to yellow-fleshed papayas.

In addition, the three F₁ hybrids had a large edible portion (82.46–84.35%), firm flesh (2.91–4.06 kg cm⁻²), low titratable acidity (1.79–2.12 meq 10 juice⁻¹), and high total



Fig. 7. Titration extracts after (A) and before (B) titration of fruit juice of three papaya F₁ hybrids.

soluble solids (10.7–12.08 oBrix) (Table 1). Titratable acidity (TA) is a measure of the concentration or the presence of acids in the fruit extract showing differences in juice color after titration (Fig. 7), while TSS is indicative of the relative presence of sugars.

While the three F₁ hybrids had sweet flesh, they still have small amount of acids which could be attributed to the presence of vitamins and minerals in the fruit extract which are nutritious and beneficial to papaya consumers.

The three F₁ hybrids were moderately tolerant to PRSV. Disease onset was observed 32wk from transplanting. In general, the crown leaves had very mild chlorosis and mottling while very few water-soaked lesions appeared on the trunk and petioles. However, the inbred parent Cavite Special (4172) is susceptible to PRSV, showing typical symptoms of the virus such as mottling and chlorosis, leaf distortion, shoe stringing and severe lesions on the stem and petioles. PRSV tolerance can be reflected in the ability of the tree to produce yield despite the presence of the disease. The mean number of fruits produced by the three F₁ hybrids differed significantly (Table 1). Hybrid 097 x 4172 (Hirang) had significantly the highest number of fruits at 19.33 (20), while the control inbred parent Cavite Special, which is susceptible to PRSV, had the lowest number of fruits at 9.92 (10). The three F₁ hybrids had a prolific fruit-bearing habit as the column is loaded with fruits (Fig. 8).

SUMMARY AND CONCLUSION

Papaya production is constrained by diseases, insects, and low seed germination (Magdalita and Signabon 2017). Therefore, development of tolerant varieties is necessary to continuously secure production even in the presence of diseases. The newly developed PRSV-tolerant hybrids and candidate varieties should be characterized and their performance assessed. Their seed germinability, tree and fruit characteristics, and PRSV tolerance should be determined. The seed germination test was done to generate baseline data on the behavior and viability of the three papaya F₁ hybrid seeds prior to planting in the field. The experiment was conducted in CRD with three replications to assess seed germination, seedling growth, pollen fertility and fruit characteristics including reaction to PRSV.

Significant differences for percent seed germination and seedling height were found between the three new papaya F₁ hybrids. Hybrid 5648 x 336 (Liyag) had the highest percent seed germination and the tallest seedling height. However, no significant differences were found for seedling stem diameter, number of leaves, length and width of expanded leaves, and petiole length. In terms of root characteristics, hybrid 5648 x 336 had the heaviest fresh and dry weights of roots, the longest primary root, and the highest number of secondary roots. Furthermore, 097 x 4172 (Hirang) had the longest secondary roots. While in terms of pollen viability, hybrid 4173 x 5648 (Timyas) had the highest percent pollen viability. The six parental inbred lines of the three F₁ hybrids had similarly high pollen viability and germination. Significant differences between the three promising F₁ hybrids were observed for fruit weight, fruit length and flesh thickness. Hybrid 5648 x 336 (Liyag) had significantly the heaviest, longest fruit and the thickest fruit flesh. All hybrids were moderately tolerant to PRSV and their fruits had a large edible portion. The fruit flesh of hybrid 097 x 4172



Fig. 8. Prolific fruit-bearing habit of papaya F₁ hybrids growing in the Central Experiment Station in Tranca, Bay, Laguna, Philippines.

(Hirang) was red, while that of 5648 x 336 (Liyag) and 4173 x 5648 (Timyas) was yellow orange. The three F₁ hybrids were moderately tolerant to PRSV.

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