MJSIR
Mountain Journal of Science and Interdisciplinary Research
PRINT ISSN: 2619-7855
ONLINE ISSN: 2651-7744
January-June 2026 • 86 (1) : 20-34

Phenotypic Characterization, Pollen Fertility, and PRSV Response of Micropropagated and Seed-Derived Papaya in Cavite, Philippines

Pablito M. Magdalita[1], and Alangelico O. San Pascual[1,2]*

1-College of Agriculture and Food Science, University of the Philippines Los Baños, Philippines
2-Department of Science and Technology - Philippine Science High School Main Campus
**Corresponding author email address: aosanpascual@pshs.edu.ph* DOI: https://doi.org/10.70884/mjsir.v86i1.612

ARTICLE INFO

Date Received: 12-16-2025
Date Last Revised: 06-23-2026
Date Accepted: 06-30-2026

Abstract

The micropropagated-derived and seed-derived papaya trees were characterized based on their performance in Silang, Cavite, Philippines, an area known to be the origin of the deadly PRSV, an area with a long history of papaya ringspot virus (PRSV) occurrence and prevalence. Tree characteristics at first flower showed that plant height, stem diameter and number of nodes of micropropagated and seed-derived trees are not significantly different. On the other hand, the micropropagated trees produced fruits that are significantly heavier, had thicker flesh and had higher titratable acidity than fruits of the seed-derived trees. In contrast, the seed-derived trees produced fruits with firmer flesh. However, no significant differences existed between the micropropagated and seed-derived trees in terms of fruit length, fruit width, peel weight, peel thickness, flesh juice density, extractable juice, TSS and percent edible portion. Both micropropagated and seed-derived trees are moderately tolerant to PRSV. Plants remained vigorous and produced marketable yield. Furthermore, pollen fertility and pollen size of the micropropagated and seed-derived papaya trees are not significantly different. Pollen fertility is higher during June to March, which are the cooler months of the year, and decreased during April to May, which are the hot and dry months of the year in the Philippines.

KEYWORDS

Carica papaya L.
micropropagation
papaya ringspot virus
phenotype
pollen fertility

Introduction

Papaya (*Carica papaya* L.), a monotypic species in the family Caricaceae, is commonly grown for its melon-like fruits which is available throughout the year (TFNet News Compilation, 2016). It is relatively easy to grow and produce fruits within a short period of time, making it as good cash crop (Magdalita et al., 2022). If grown under the right cultural conditions, papaya can give high yield per tree and even on a per hectare basis. In 2024, papaya ranked 4th among the major fruit crops with a total production volume of 149,214.22 MT according to Philippine Statistics Authority (PSA, 2016).

The top four papaya producing regions were SOCCSKSARGEN, Northern Mindanao, Davao Region, and Southern Tagalog (PSA, 2016). Papaya production in these regions are considered small-scale backyard types, but the presence of large commercial plantations exist in the region in two big private companies namely, Del Monte and Dole Philippines contributed to the production

(Francisco, 2020; Sarmiento, 2026). About 92% of the total papaya production is consumed locally as food and the rest are for industrial uses. In 2024, the Philippines ranked eleventh among the top papaya producing countries in the world (Tridge, 2025).

Despite papaya's economic importance, papaya cultivation of the Philippines is being pressed by several problems like diseases and pests. Among these, the most widespread and destructive of which is the papaya ringspot virus (PRSV) (Princi et al., 2026). The PRSV was first detected in Silang, Cavite in 1982 but it spreads rapidly in Luzon including the islands like Marinduque, Panay, Negros, Bohol, Cebu because of the explosive nature of the disease (Magdalita et al., 2022). PRSV was detected in a few areas in 2001 in Davao del Sur and South Cotabato in Mindanao (San Pascual et al., 2023). Papaya ringspot diseases are prevalent globally often causing yield losses of 80 - 100% (Magdalita et al., 2004; Jyotika et al., 2024).

The multiplication of proven papaya genotypes or inbred lines that can be used as parents for hybridization can be achieved by tissue culture specifically through either micropropagation or through production of artificial seeds via somatic embryogenesis (Al-Shara et al., 2020). Micropropagation is a tissue culture technique for rapid mass production of true to type disease free plantlets under aseptic conditions using minute plant organs like nodal cuttings, axillary buds (Thakur et al., 2024). This tissue culture technique is is one of the economic ways of producing uniform planting materials of papaya with proven field performance. Micropropagation will be useful for the maintenance of the phenotype of candidate selections and overcome many of the problems associated with the use of seedlings, overplanting, and thinning (Ryavalad et al., 2019, Magdalita & San Pascual, 2019). Several micropropagation techniques have been developed for papaya using shoot tips and axillary buds (Encina et al., 2023). Although micropropagation technique does not confer resistance to Papaya Ringspot Virus (PRSV), it promotes fast multiplication of papaya lines that possess horticulturally desirable characteristics. Thus, it is important to evaluate these micropropagated plants under natural PRSV disease pressure to determine whether the plants maintain clonal fidelity, reproductive competence, and field performance comparable to conventionally propagated plants. This information is vital to areas with high PRSV disease pressure where deployment of micropropagated plants may be done to sustain papaya production despite persistent disease pressure.

In the early 1990s, Drew (1992) developed an efficient and reliable micropropagation technique via nodal cuttings using De Fossard medium (De Fossard et al., 1974) supplemented with benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). However, this system is limited by the vigor of plant tissues, which are sensitive to the accumulation of gases such as ethylene within the headspace of culture vessels; hence, ethylene protectants like silver thiosulfate have been incorporated into the culture media (Magdalita et al., 1997). Actively growing side shoots from proven trees in the field were induced through the application of BAP and GA_3.

These shoots were then used as cuttings, established in the glasshouse, and maintained as mother stock plants. Small lateral bud explants from side shoots of actively growing cuttings are routinely harvested and used as explants. Elongated shoots are cultured on rooting medium using De Fossard medium supplemented with indole-3-butyric acid (IBA) and subsequently transferred to hormone-free medium to support further development. Fully rooted plantlets are grown in a mixture of peat, perlite, and polystyrene beads and then acclimatized in a high-humidity chamber.

Throughout the process of micropropagation, phenotypic characterization of regenerated plantlets is vital to check for morphological uniformity and growth vigor and its true-to-type fidelity relative to the donor material. Assessment of traits like shoot growth, leaf morphology, and rooting behavior provide a reliable measure of culture success from in vitro to ex vitro conditions and plant's stability. These phenotypic characterizations are widely used as quality control measures for tissue culture systems to determine somaclonal vartiation among the tissue culture-derived plants and ensure clonal fidelity before plants are exposed or established in the field. Such phenotypic assessments are widely recognized as essential quality control measures in plant tissue culture systems to detect possible somaclonal variation and ensure clonal fidelity prior to field establishment (Majumder et al., 2025).

Field performance of micropropagated papayas have been studied by Araya-Valverde et al. (2019)

in Coasta Rica, Gatambia et al. (2016) in Kenya, Pandey and Singh in India (1988) and Drew and Vogler in Australia (1993). In India, the tissue-cultured papayas had bigger stem diameter, larger leaf area, more stomata per unit leaf area, longer petioles, higher chlorophyll content, flowered and fruited earlier by two months and produced higher yields when compared to trees grown from seeds (Pandey & Singh, 1988). In Australia, micropropagated plants have strong root systems that have multiple primary roots, reduced juvenile stage, and short internodes, thus flowering is early (Drew, 1988; Drew & Volger, 1993). This technique has been used in the micropropagation of papaya variety 2001 that have been planted commercially in farmers' field in Queensland, Australia that attained full production in 10-12 months (Drew & Volger, 1993). Further, in Kenya similar performance was observed on seed-derived and micropropagated papaya plants were observed (Gatambia et al., 2016). In the Philippines, no recent studies documented the field performance of tissue cultured papaya plants. Additionally, pollen fertility of micropropagated papaya plants.

Assessing pollen fertility in micropropagated papaya are also done to detect clonal fidelity and even somaclonal variation among micropropagated plants and confirm if the regenerated papaya plants have functional male gametes which influences fertilization, fruit setting among others (Thorpe, 2007; Loyola-Vargas & Ochoa-Alejo, 2016). High pollen viability is needed so that the plants can become functional for maintenance of breeding lines and cultivar development.

The objectives of this study were to: a) determine the growth and yield of micropropagated and seed-derived papaya plants; b) pollen fertility was assessed through pollen viability testing using staining techniques and in vitro pollen germination assays; and c) evaluate the micropropagated papaya plants' reaction to PRSV, and yield characteristics. This study aimed to determine whether micropropagation can be used as a propagation strategy for elite papaya lines that retain desirable traits and acceptable field performance in PRSV-endemi c areas.

Materials and Methods

Growth and Yield Data

The experiment was arranged in a Completely Randomized Design (CRD) with two treatments, namely micropropagated and seed-derived papaya. The micropropagated (Figure 1) and seed-derived papaya plants were established in ten alternating rows, with five rows for micropropagated plants and five rows for seed-derived plants. Each row consisted of fifteen plants. The plants were spaced at 2 meters per hill and 3 meters between rows. Selected phenotypic characteristics like plant height, stem diameter, and number of nodes were gathered at flowering stage. Plant height (cm) was measured using a meterstick from ground level up to the first flower. The stem diameter was measured using a vernier caliper (cm) at a point about half of the total stem height, while the number of nodes was counted from the lowest node above the ground up to the first flower.

PRSV field tolerance, yield performance, and fruit quality traits were also assessed for micropropagated and seed-derived papaya. Yield was determined by counting mature fruits per tree at harvest. Fruits at physiological maturity or fruits having a Peel Color Index 2 (PCI 2), defined as fruits having a yellow tinge at the fruit apex, were harvested. Five fruit samples were taken from each tree and allowed to ripen on benches. Fruit characteristics such as fruit weight (g), length (mm), and width (mm); peel and flesh thickness (mm), peel and seed weight (g), and fruit quality traits like total soluble solids (°Brix), and titratable acidity (meq/10mL juice), flesh firmness (kg/cm²) were measured while edible portion (%) was calculated using the formula: (Fruit weight – (Seed weight + Peel Weight) / Fruit weight) X 100. For TSS, 2-3 drops of the juice were squeezed in the calibrated refractometer and readings were recorded. For the titratable acidity, 10mL of the freshly extracted juice were mixed automatically by the machine with a high range titrant and subjected to reading from the automatic titrator (H1I84502, Hanna Instruments, USA).

Figure 1

Micropropagated Papaya Plants Used in the Study

Pollen Fertility Assessment

Hermaphroditic flowers of the seed derived line were used while male flowers of lines derived from the micropropagated plants were collected to determine the pollen germination and viability. The study also aimed to assess the characteristics of pollen grains collected from both micropropagated and seed-derived *Carica papaya* plants. The pollen was gathered from mature flowers at the balloon stage and stained using I2KI solution and was collected for a year. Pollen grains were subjected to staining using I2KI solution and germination using Brewbaker and Kwack's medium. Pollen size was measured using ToupTek viewing software as aided by TrueVision Microscope (Tennesee, USA) at 100x. Pollen size determination is done to assess variation among the pollen size and even shape. Since deformation and small pollen or too large normally are unviable, thus pollen size is determined. The pollen grains of *Carica papaya* have a diameter of approximately 25µm, ranging from 25.18 to 25.72µm depending on flower type (Phuangrat et al., 2013).

Reaction to Papaya Ring Spot Virus

PRSV reaction of papaya plants was assessed at the fruiting stage through visual observation of symptoms like chlorosis in the leaves, ringspots in the fruit and water soaked lesions in the petiole (Figure 5) and confirmed using Enzyme-Linked Immunosorbent Assay (ELISA). Virus titer was quantified based on ELISA absorbance values following standard procedures (Alviar et al., 2012). No rating rubric was used; assessment was based on symptom expression and quantitative ELISA results. A susceptible papaya plant shows severe systemic mosaic symptoms with intense chlorosis, defoliation and shoe stringing in the leaves with prominent water soaked lesions in the petioles, acute plant stunting and production of non-marketable fruit with dense ringspots. While a moderately tolerant phenotype shows mild mosaic or chlorotic patterning without structural leaf degradation or distortion with no plant stunting and maintain high photosynthetic efficiency thus sustaining normal growth with normal flower and production of marketable fruit yield under continuous viral pressure (San Pascual et al., 2023; Magdalita & San Pascual, 2019).

Statistical Analysis

Data were analyzed using an independent-samples t-test at a significance level of $\alpha = 0.05$ to determine differences between micropropagated and seed-derived papaya plants. Statistical analyses were performed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 developed by the International Rice Research Institute (IRRI, 2014).

Results and Discussion

Plant Characteristics

Plant height is an important trait in papaya related to precocity or earliness to flower. Dwarf (<1m) and semi-dwarf (1-1.5m) papaya are wanted in plantations since harvesting of physiologically mature fruits is convenient (International Board for Plant Genetic Resources, 1988). Figure 2 shows no significant difference on the mean plant height of seed and micropropagated plants (p=0.5095). Micropropagated plants have mean height of 1.17m while seed derived plants have mean height of 1.23m. The height of individual plants ranges from 0.55 to 1.9m which indicates that the plants have dwarf to semi-dwarf plant height.

Further, similar mean stem diameter of micropropagated-derived and seed- derived papaya trees established (p=0.7076) were observed (Figure 2). Both micropropagated and seed-derived trees exhibited comparable stem diameter values (2.75–2.80cm), suggesting similar vegetative growth performance under field conditions. The individual stem diameter ranges from 1.1 to 4.0cm. A thick stem is an advantage in papaya because it can support a heavy load of fruits without making the tree fall into the ground especially during typhoons (Magdalita & San Pascual, 2019). In addition, it serves as storage of water that makes the papaya tree adaptable to environments with limiting soil moisture and harsh wind conditions (Encyclopedia Britannica, 2025).

Moreover, no significant mean number of nodes to first flower of micropropagated and seed-derived *C. papaya* plants established (p=0.7148) were also observed (Figure 2). The micropropagated plants have a mean number of nodes to first flower of 19.25 while

Figure 2

Mean Plant Height (m), Stem Diameter (cm), and Number of Nodes of Seed and Micropropagated Plants of C. papaya

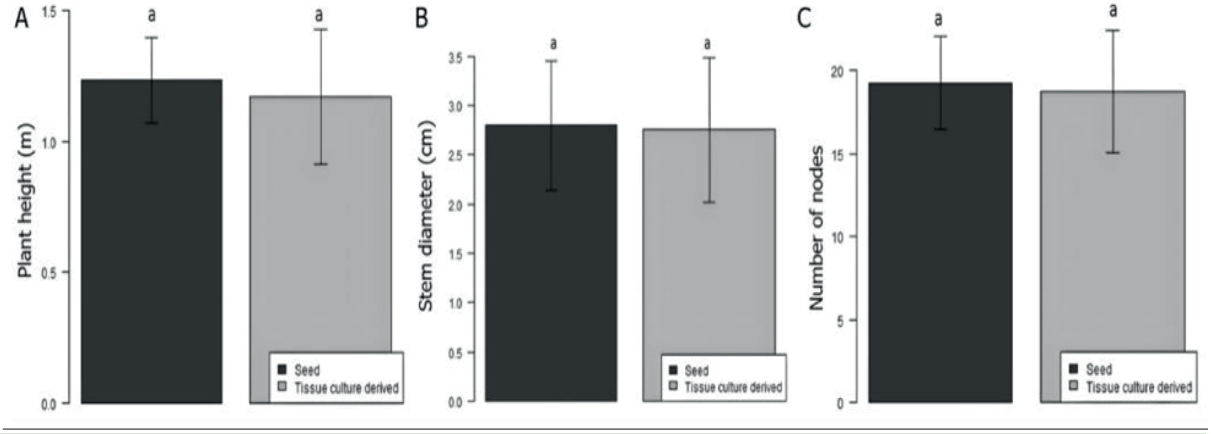
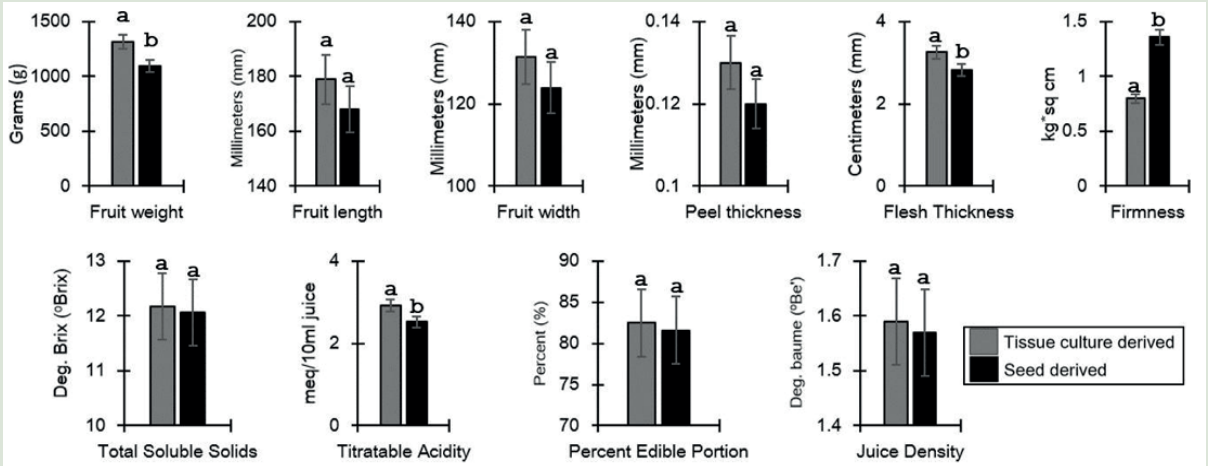


Figure 3

Fruit Characters of Micropropagated-Derived and Seed-Derived Papaya Trees Grown in Silang, Cavite, Philippines



the seed-derived plants have a mean number of nodes of 18.73. The number of nodes to first flowering ranged from 11 to 33 nodes among individual plants. This result suggests that the number of nodes reflects a tree form that can be classified as semi-dwarf which agrees with the plant height. Lesser number of nodes at first flowering is a desirable trait in papaya, since it suggests that the tree can flower at a shorter stature. It has been reported earlier that lesser number of nodes in papaya is an indication that nodes are close to each other, hence giving the plant a short tree stature, leading to precocious or early flowering (Aikpokpodion, 2012). Hence, papaya genotypes with lesser number of nodes are preferred in selection.

Fruit Characteristics

In general, papaya fruits can be classified as large-fruited (>3kg), medium (1-2kg) and small (<1kg). Medium-sized papaya fruits are wanted in the local markets since they are suitable for consumption in one sitting by an average Filipino family, and they are easier to handle and more convenient to carry than large-fruited types.

Figure 3 shows significant differences (p=0.022) on the mean fruit weight of micropropagated and seed-derived papaya trees. Micropropagated papaya trees have significantly bigger fruits (1319.48g) compared to fruits

of seed-derived papaya trees (1072.75g). This finding is corroborated to the Cassava clone 'Señorita' derived from micropropagation via meristem culture that has higher tuber yield compared to vegetatively propagated ones (Garcia et al., 1993). In contrast, Swartz et al. (1981) reported that in strawberries, reduced fruit size was observed in micropropagated plants. In papaya, Talavera et al. (2007) compared field-grown micropropagated and seed-derived hermaphrodite papaya and found comparable vegetative and yield traits like fruit characteristics between the two propagation methods. In addition, two forms of fruit i.e. hermaphrodite form which is elongated and the female form which is rounded and ovate types were borne by the seed-derived trees. The round and the ovate fruit shapes are ideal for packing in cartoon boxes similar to what is being done in apples, pears and peaches. A similar mean fruit length (p=0.17) and width (p=0.10) of the micropropagated-derived and seed-derived papaya trees (Figure 2) was observed. This again suggests that the technique does not induce somaclonal variation. Micropropagated plants have fruits that are 279-129mm long and 170-104mm wide. On the other hand, the seed-derived trees have fruits that are 246-134mm long and 146-100mm wide. Many consumers prefer to buy and medium-sized papaya fruits than larger fruits due to convenience during marketing (Del Carmen et al., 2012).

Figure 3 shows a similar mean peel thickness (p=0.19) of fruits of the micropropagated and the seed-derived papaya trees. Again, this result suggests that the micropropagation technique does not induce somaclonal variation. The micropropagated trees have fruits with peel measuring 1-2cm thick, while the seed-derived plants have fruits with peel measuring 0.5-2cm thick. The thick peel of a papaya fruit is an important postharvest trait that renders the fruit more resistant to bruises, scars and other surface injuries during post-harvest handling and transport. Since papayas are transported to markets, a thick peel is indeed necessary. In addition, a thick peel will prevent the entry of postharvest pathogens readily, thus minimizing fruit rotting and spoilage. For instance, the thick peel of 'Maradol Roja' papaya makes it resistant to bruises during long distant transport from Mexico to Europe (Specialty Produce, nd.).

However, significant differences on the flesh thickness of fruits of the micropropagated and seed-derived papaya trees (p=0.001). Thicker flesh was observed on fruits of micropropagated trees with mean flesh thickness of 3.26cm compared to the fruits of seed-derived trees with mean flesh thickness of 2.78cm. A thick flesh is another important trait in papaya variety since it contributes considerably to the edible portion of the fruit and, also a factor that prevents the fruit from becoming easily soggy when ripe (Budiyanti et al., 2021). The fruit's flesh thickness is like 'Maradol Roja', a Mexican papaya variety with a flesh thickness of >3.0cm (Sanikommu et al., 2021; Fitch, 1995).

Similar total soluble solids of the fruits of microrpopagated and seed-derived papaya trees (p=0.71) was observed. The TSS of papaya fruits obtained from the micropropagated trees was 12.17ºBrix. while for the seed-derived trees it was 12.06ºBrix. This result suggests that the micropropagation technique does not induce somaclonal variation. The sweetness of the flesh indicated by the total soluble solids (TSS) is the most important trait being considered by consumers and buyers of fruits especially for a crop that is being consumed fresh. The TSS of fruits from both the micropropagated and seed-derived papaya trees is similar to the Solo varieties from Hawaii, USA (Manshardt, 1992). This implies that the ripe fruits have sweet-tasting flesh that could be well-liked by consumers. The fleshy fruits like papaya are generally eaten as dessert after meals by most consumers, thus the taste of the flesh must be sweet and pleasant.

No significant difference on the mean percent edible portion of fruits harvested micropropagated and seed-derived papaya plants (p=0.61) was observed (Figure 2). Fruits from the micropropagated papaya plants have a mean percent edible portion of 82.49%, while the seed-derived papaya trees have a mean edible portion of 81.53%. Another important trait being considered by papaya consumers is the percent edible portion. This suggests that the amount of edible flesh is higher than the amount of non-edible parts like the peel and the seeds. In general, papaya fruits with high edible portion, aside from being sweet, juicy, and firm are preferred by consumers. In addition, the cavity size of the papaya fruit affects the edible portion, hence papayas with small cavities have higher edible portion than those with large cavities.

Figure 3 shows significant differences on the titratable acidity (TA) (meq/10mL juice) of fruits from micropropagated and seed-dervied papaya trees. Slightly higher TA was obtained in fruits of micropropagated papaya plants (2.92 mEq/10mL juice) than in seed-derived papaya trees (2.58mEq/10mL juice). The significantly higher titratable acidity observed in micropropagated papaya fruits, although slight, may be advantageous for commercial production due to its postharvest and shelf-life extension implications. Argañosa et al. (2008) indicated that higher TA values are preferred for storage of papayas because this is correlated with low pH values that inhibits growth of microorganisms on fresh fruits.

On the other hand, no significant difference on the mean firmness (kg/cm²) of fruits of the micropropagated and seed-derived papaya plants (p=0.0006). This result suggests that the micropropagation technique does not inducesomaclonal variation. Fruits from the micropropagated papaya plants have mean firmness of 0.8kg/cm² while the seed-derived papaya trees have mean firmness of 1.36kg/cm². A firm flesh of the fruit is another important trait that most consumers and processors like in papaya, but soggy flesh is unwanted (Del Carmen et al., 2012). Furthermore, no significant difference was observed on the mean juice density (ºBe') of fruits of micropropagated-derived and seed-derived papaya trees (p=0.61) (Figure 3). This suggests that somaclonal variation is not being induced by the micropropagation technique. Fruits from micropropagated papaya plants have mean juice density of 1.59 ºBe', while seed-derived papaya plants have mean juice density of 1.57 ºBe'. Juice density is important for health-conscious consumers since high density implies that more ions are present in the juice which is needed by the body especially during hot weather to replenish lost ions.

Among the different parameters measured, differences only in fruit weight, flesh thickness, firmness and titratable acidity were observed between the micropropagated and seed-derived plants. Larger fruits and thicker flesh were observed in micropropagated plants. Furthermore, higher titratable acidity was observed in micropropagated papaya plants, while firmer flesh was observed in seed-derived trees. These findings are similar to Lopez-Terros (2018), who reported higher titratable acidity across different ripening stages of papaya. This implies that the observed values in both plant sources fall within the expected physiological range despite being significantly different. The titratable acidity levels recorded in this study were relatively lower compared to those reported by Lopez-Terros (2018), this indicates that even though micropropagation has influenced this specific characteristic, it remains within the acceptable limit for papaya fruit characteristics. Moreover, this finding agreed with the result of Yapo et al. (2011) in pineapple who also found significant differences among TSS and titratable acidity of micropropagated pineapple plants as compared to plants derived from classical propagation techniques. The same authors further indicated though that even if significant differences were observed, in both pineapples from micropropagation and classical techniques, the values still fall within the range of TA for pineapples as reported previously by Py et al. (1987) and Cano et al. (1994). Similarly, in Hawaii, Fitch et al. (2005) found no variation on the performance of micropropagated and seed-derived 'Solo' papaya trees.

Further in Kenya, Gatambia et al. (2016) also indicated very low variation between micropropagated and seed-derived papaya plants, suggesting that in vitro propagation does not alter key morphological or fruit quality traits under field conditions. This supports the concept that papaya is relatively stable under clonal propagation when micropropagated using stable regeneration protocols as noted in earlier micropropagation studies (Drew, 1992). Similarly, in vitro plant tissue culture conditions and protocols when properly optimized, are found to preserve and maintain high genetic fidelity across the plants regenerants that resulted to low to no variation among regenerants (George et al., 2008; Veera et al., 2020).

Pollen Size and Fertility

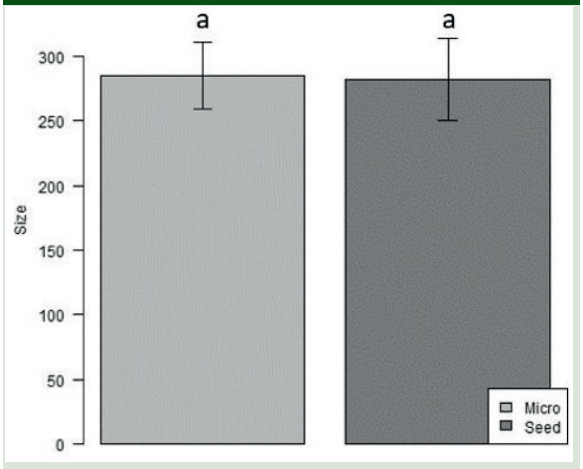
The micropropagated papaya trees have bigger pollen grains of 28.96µm but no significant differences were observed with the pollen grains (p=0.6559, CV=9.81) of seed-derived papaya trees (Figure 4). Pollen of papaya is generally 30 µm in diameter and this agreed with the previous report of Science and Plants for Students (2017). More et al. (2010) indicated that *C. papaya* pollen, are very similar to those plants under the families Myrtaceae and Arecaceae which have pollen grains ranging from 26-28µm in size. Very small and large pollen grains fail to germinate (Srikantha

& Jacob 2001). However, pollen size is not a good determinant of pollen germinability. In fact, Srikantha and Jacob (2001) found that too large and too small pollen, and even those that are darkly stained fail to germinate or have a poor germination. Moreover, McCallum and Chang (2016) also indicated that pollen size influences the delivery of sperm to the egg.

Structural pollen staining was also conducted on the pollen grains of the micropropagated and seed-derived papaya trees using I2KI stain to detect the presence of starch serving as carbohydrate source of the germinating pollen. If the pollen is unstained or lightly stained, it does not have enough food reserves for it to grow and develop their pollen tube for germination and eventual fertilization. High pollen viability was observed among micropropagated and seed-derived papaya trees having a range of 87.5–100% (Figure 8). No significant difference was observed among the pollen viability of micropropagated and seed-derived plants (p=0.7846, cv=4.32) (Figure 5), indicating that the micropropagation technique did not induce somaclonal variation. Similarly, Siar et al. (1998) detected high pollen fertility in papaya at 96% by staining using 2% acetocarmine. In addition, Magdalita et al. (1997) found 98% pollen viability on *C. papaya* and 94% on *C. cauliflora*. Magdalita and San Pascual (2019) indicated that Papaya hybrids have high pollen fertility ranging from 80-95%.

Figure 4

Size of Pollen Of Papaya Trees Derived from Micropropagation (a) and Seed-Derived Papaya Trees (b) (in Micrometers)



In structural pollen staining using I2KI, the presence of starch is an indication of food reserves, but assessment of livability can be determined by pollen tube growth during pollen germination. High percentage of stained pollen in micropropagated and seed-derived papaya trees were detected during the wet season but not significantly different. This result is shown by the darkly stained pollen of both the micropropagated (Figure 6A) and the seed-derived (Figure 6B) papaya trees.

Figure 5

Percent Pollen Viability (A) by Pollen Staining and Germination (B) from Micropropagated-Derived and Seed-Derived Papaya Trees

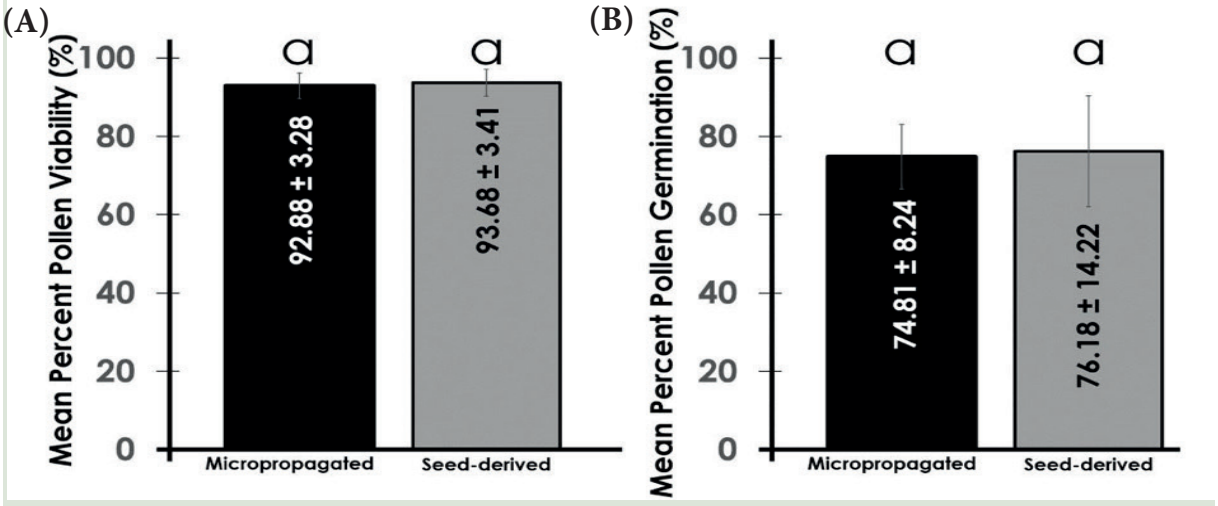
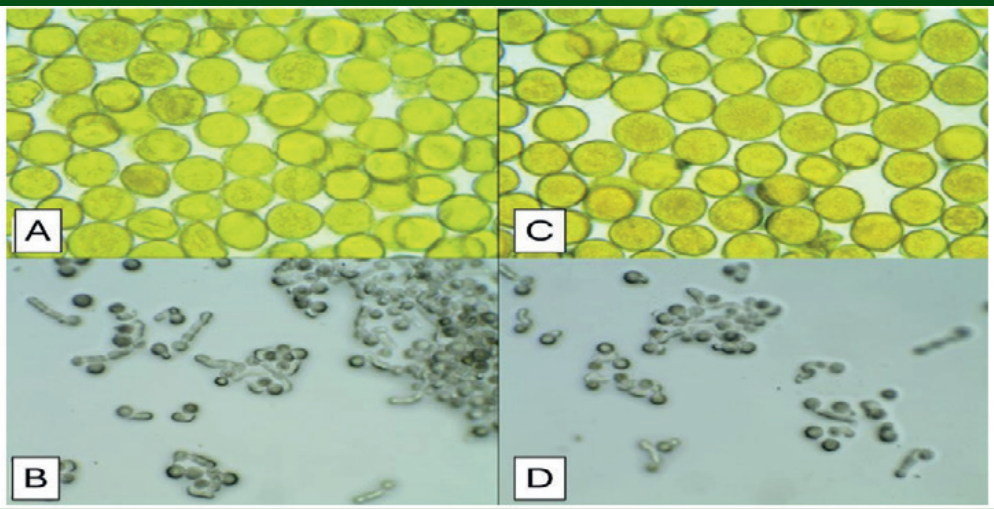


Figure 6

Pollen Grains Structurally Stained with I2KI and Germinating Pollen of Micropropagated-Derived (A & B) and Seed-Derived Trees (C & D) of C. papaya After 5hrs of Incubation in Brewbaker's and Kwack's Medium



As a morphological marker for growth and life, pollen tube growth is essential to test using either in-vitro germination in a germination medium or in vivo germination. In the present study, pollen germination test was done in vitro wherein the pollen grains were sown in a semi-solid medium and then incubated at ambient room temperature 25-28°C for 5 hrs. Pollen of the micropropagated and seed-derived papaya trees had high percent germination on the Brewbaker's and Kwack medium (1963) with 10% sucrose under ambient room temperature after 5 hrs incubation. No significant difference (p=0.88) on pollen germination of micropropagated and seed-derived papaya trees was detected (Figure 6). This result is shown by the germinated pollens with their elongating pollen tube of both the micropropagated and seed-derived papaya trees (Figure 6B). After 5 hrs incubation at ambient room temperature. The rate of pollen germination ranges from 73.84-76.18%. This observation corroborated with the study of Tamaki et al. (2011) indicating that the highest pollen germination in papaya was observed at 20 and 25°C with pollen germination ranging from 70 – 80%. According to Tamaki et al. (2011), under Japan conditions, high pollen germination was observed from April to June and October to November when the temperature ranges from 19.4–25.8°C. In the Philippines, similar pollen viability was also observed either in the hot or cool months of the year.

One of the indications that papaya will be a productive bearer of fruits is the production of seeds which is influenced by pollen fertility, suggesting that as more seeds are produced, more pollen grains have fertilized the ovules. In the present study, no significant differences were observed among pollen fertility of micropropagated and seed-derived papaya trees. Therefore, micropropagated and seed-derived trees are similar in terms of pollen fertility. In micropropagation, clones or duplicate copies of one plant were developed and they do not differ from the original plant. This further implies that there was no occurrence of somaclonal variation as far as pollen fertility on micropropagated trees is concerned which is the same as seed-derived papaya trees. This proved that micropropagation is an effective procedure in mass production of papaya since there is no change in several tree, fruit pollen characteristics and PRSV reaction when compared to seed-derived papaya trees. This shows that micropropagation of papaya does not alter the genetic make-up of the plants.

Furthermore, a one-year monitoring period of pollen fertility of the micropropagated and seed-derived papaya trees was conducted to ascertain pollen behavior. Figure 7 shows the monthly pollen germination rate of micropropagated and seed-derived papaya trees. Checking of the percent pollen germination was done monthly for a period of 12 months. No significant difference among

the percent pollen germination was observed between micropropagated and seed-derived papaya trees (Figure 7). The same trend was observed in pollen fertility by staining where highest pollen germination rate was observed in hot and dry season when the temperature is 28ºC. However, pollen germination was lowest the following month of April 2018 when the temperature is higher at 29ºC and atmospheric moisture is lower. But as the temperature decrease and atmospheric moisture is higher, pollen germination slowly increases. This result is similar with the previous report of Tamaki et al. (2011) who observed that the highest pollen germination in papaya at 70-80% was observed at lower temperature. High pollen germination rates were observed during months wherein the temperature ranged from 19.4 to 25.8ºC. In Japan, high pollen germination was observed from April to June and October to November wherein the temperature range is conducive for papaya pollen germination (Tamaki et al., 2011). In the Philippines, during April, very high temperatures exceeding 25ºC were observed which are not conducive to pollen germination. However, during the months of December and January wherein temperature is lower, pollen germination is high.

Fruiting Behavior and Reaction to PRSV

Figure 8 shows the fruiting habit of the micropropagated and seed-derived trees. The plants show high vigor and heavy fruiting with an average of 22 fruits on the tree. The fruits are dark green in color and very plump. No lumpiness was observed on the fruits, and there are no misshapen or cat-faced and pentandria-type fruits. The papayas are moderately tolerant to PRSV showing mild symptoms of the disease including mottling, chlorosis, small water-soaked lesions on petioles and some ringspots on the fruits.

All micropropagated and seed-derived papaya plants showed moderate tolerant reaction to PRSV from the vegetative to the fruiting stage. It is in this place where PRSV was first detected in 1982 and up to now, the virus inoculum is still very high in the area. All the micropropagated and seed-derived trees showed moderate tolerance to PRSV. The trees bear fruits heavily, despite the presence of the virus (Figure 8). Very mild symptoms of PRSV like the appearance of some ringspots of the fruit, chlorosis and mottling on the leaves and few water-soaked lesions on the petioles were observed

Figure 7

Pollen Germination and Pollen Viability of Micropropagated and Seed-Derived Papaya Grown in Silang, Cavite, Philippines.

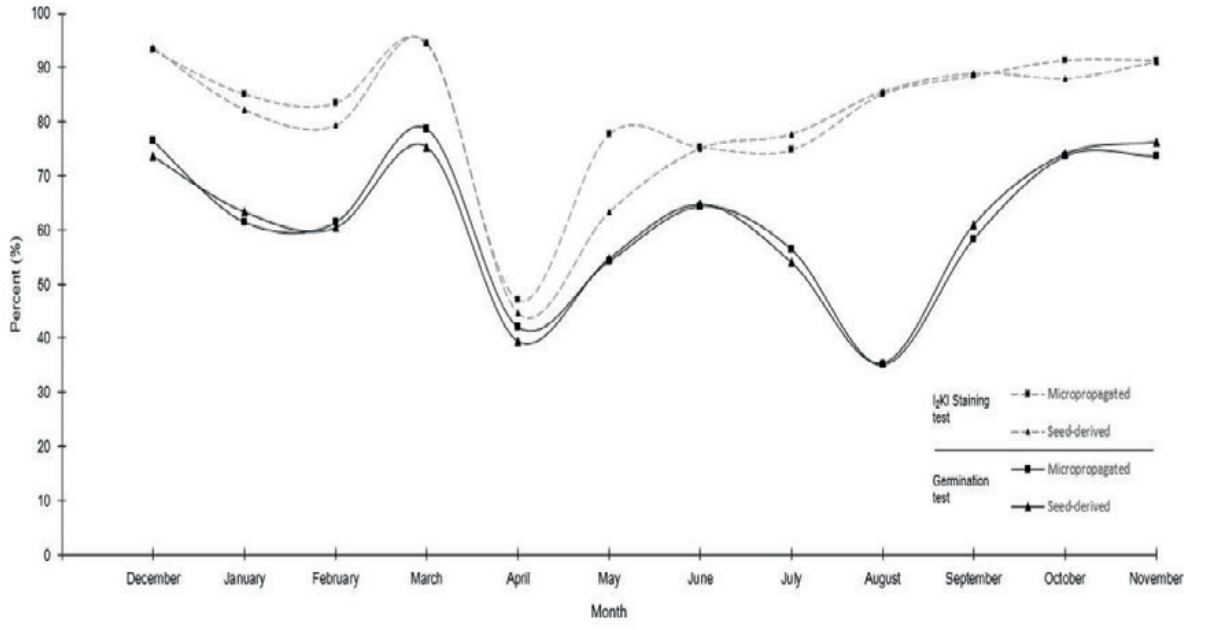
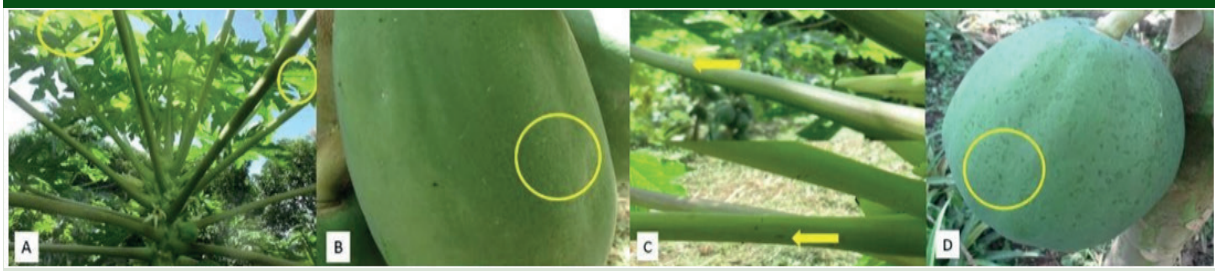


Figure 8

Fruiting Habit and Moderately Tolerant Reaction to PRSV of Micropropagated (D-F) and Seed-Derived Papaya Trees (A-C)

Figure 9

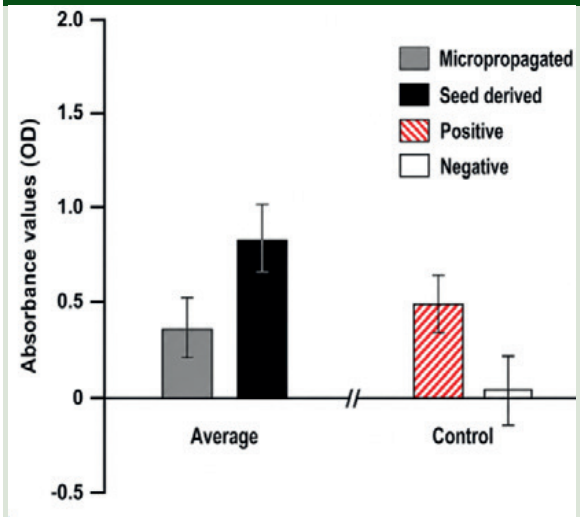
Moderate Tolerance Reaction to PRSV of the Micropropagated and Seed-Derived Papaya Trees in a Field Trial in Silang, Cavite, Philippines, Where the Inoculum of the Virus was Very High. Very Mild to Mild Symptoms of PRSV Including Chlorosis and Mottling on the Leaves (A), Ringspots of the Fruit (B), Water-Soaked Lesions on the Petioles (C) were Exhibited by the Trees. Severe Ringspots on the Fruit were Exhibited by the Control Which is Susceptible to PRSV (D)



on both micropropagated and seed-derived plants (Figure 9). In addition, the enzyme-linked immunosorbent assay (ELISA) test indicated that the micropropagated had high mean virus titer at A405nm absorbance of 0.3306 while the seed-derived trees had absorbance at A405nm of 0.8642 which is similar to the positive control at 0.601, but the negative control had only 0.018 (Figure 10). This shows that both the micropropagated and the seed-derived trees are infected with PRSV like the positive control, indicating that they are indeed moderately tolerant to the virus, since they still produce marketable yield despite the presence of the virus. In tomato, similar response was observed where some varieties have shown high infection level in ELISA but not compromising yield indicating their tolerance (Ellouze et al., 2020).

Figure 10

Virus Titre (Mean Absorbance Value at A405nm) of the Micropropagated and Seed-Derived Papaya Trees Grown in the Field Trial in Silang, Cavite, Philippines



Conclusions

At first flowering, plant height, stem diameter, and number of nodes showed no significant differences between micropropagated and seed-derived plants, showing comparable vegetative growth. However, some differences were observed in fruit quality traits, where fruits from micropropagated trees were significantly heavier, had thicker flesh, and exhibited higher titratable acidity compared to those from seed-derived trees, while seed-derived trees produced fruits had firmer flesh. Regarding PRSV response, both micropropagated and seed-derived plants exhibited moderate tolerance, with ELISA confirming high viral titers but only mild symptoms such as chlorosis and occasional ringspots and water-soaked lesions on fruits and petioles, while still maintaining vigor and productivity under field conditions. In terms of pollen fertility, both plant types showed similarly high viability during the wet season as indicated by I_2KI staining, with 93.3% for micropropagated and 93.7% for seed-derived plants, and comparable pollen germination using Brewbaker and Kwack's medium, recorded at 77.5% and 78.4%, respectively. Seasonal monitoring further revealed that pollen fertility remained high from June to March but declined during April to May in both plant types. Overall, the findings demonstrate that micropropagated papaya performs comparably to seed-derived plants in growth, pollen fertility, and PRSV tolerance, with some advantages in fruit quality traits, supporting micropropagation as a reliable method for maintaining desirable horticultural/phenotypic characteristics in papaya.

Recommendations

The researchers recommend the utilization of micropropagation for papaya lines to enable the mass production of different lines, owing to the comparable phenotypic traits, pollen fertility, and moderate tolerance to papaya ringspot virus (PRSV) observed between seed-derived and micropropagated papaya plants. Additionally, micropropagation may be considered a viable method for producing horticulturally desirable papaya lines and generating uniform planting materials, particularly in areas with a long history of papaya ringspot virus (PRSV) occurrence and prevalence. Future studies on papaya micropropagation should focus on multi-location field evaluations and the assessment of its economic feasibility for large-scale production.

Acknowledgment

The authors acknowledge the financial support provided by the Department of Agriculture-Biotechnology Program Unit (DA-BIOTECH) to the project on "Micropropagation and Artificial Seed Production of Candidate PRSV-Tolerant and Table-Type Papaya Inbreds and Hybrids for Field Trials and Variety Release" from which this article was based. They also acknowledge the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna for the facilities provided to the project. The contributions of Ms. Besseluz DLC. Abayon, Mr. Jessie V. Silverio and Mr. Marcelino T. Gregorio and the cooperators for the field trial in Silang, Cavite. Ambassador Meynardo LB Monteleagre, Mr. Joseph Montealegre and Ms. Elsa Montealegre) are also highly appreciated.

References

Al-Shara, B., Taha, R., Mohamad, J., Elias, H., & Khan, A. (2020). Somatic embryogenesis and plantlet regeneration in the *Carica papaya* L. cv. Eksotika. *Plants,* 9, 360.

Aikpokpodion, P. (2012). Assessment of genetic diversity in horticultural and morphological traits among papaya (*Carica papaya*) accessions in Nigeria. *Fruits,* 67(3), 173–187.

Araya-Valverde, E., Bogantes, A., Holst, A., Vargas-Mora, C., Gomez-Alpizar, L., Brenes, A., Sánchez-Barrantes, E., Chavarría, M., & Barboza, L. (2019). Field performance of hermaphrodite papaya plants obtained through molecular selection and micropropagation. *Crop Breeding and Applied Biotechnology*, 19, 420–427.

Argañosa, A.C.S., Raposo, M.F.J., Teixeira, P.C., & Morais, A.M. (2008), Effect of cut-type on quality of minimally processed papaya. *J. Sci. Food Agric.,* 88: 2050-2060. https://doi.org/10.1002/jsfa.3309

Brewbaker, J.L., & Kwack, B.H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany,* 50, 859–865.

Budiyanti, T., Noflindawati, Prihatini, R., Fatria, D., Sunyoto. (2021). The papaya selection based on fruit characters: a way to assembly new preferred variety. *IOP Conf. Ser.: Earth Environ. Sci.*. 653. 10.1088/1755-1315/653/1/012012

Cano, M.P., Torija, E., Marín, M.A., & Cámara, M. (1994). A simple ion-exchange chromatographic determination of non-volatile organic acids in some Spanish exotic fruits. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 199, 214–218.

De Fossard, R., Myint, A., & Lee, E. (1974). A broad spectrum tissue culture experiment with tobacco (*Nicotiana tabacum* L.) pith callus. *Physiologia Plantarum,* 31, 125–130.

Del Carmen, D., Esguerra, E.B., Absulio, W.L., Maunahan, M.V., Masilungan, G.D. (2012). Understanding consumer's preference for fresh table-ripe papaya. *Philippine Journal of Crop Science,* 37, 75-80.

Drew, R. (1992). Improved techniques for in vitro propagation and germplasm storage of papaya. *HortScience*, 27, 1122–1124.

Drew, R. (1998). Rapid clonal propagation of papaya in vitro from mature field-grown trees. *HortScience,* 23, 609–611.

Drew, R., & Vogler, J. (1993). Field evaluation of tissue-cultured papaya clones in Queensland. *Australian Journal of Experimental Agriculture,* 33, 475–479.

Ellouze, W., Mishra, V., Howard, R.J., Ling, K.-S., & Zhang, W. (2020). Preliminary Study on the Control of Cucumber Green Mottle Mosaic Virus in Commercial Greenhouses Using Agricultural Disinfectants and Resistant Cucumber Varieties. *Agronomy,* 10(12). https://doi.org/10.3390/agronomy10121879

Encina, C.L., Granero, M.L., & Regalado, J.J. (2023). In vitro long-term cultures of papaya (*Carica papaya* L. cv. Solo). *Horticulturae*, 9(6), 671.

Francisco, G.V. (2020). Papaya: The fruit with anti-cancer properties. *Cebu Daily News*. https://cebudailynews.inquirer.net/281804/papaya-the-fruit-with-anti-cancer-properties

Fitch, M.M., Moore, P.H., Leong, T.C., Akashi, L.A.Y., Yeh, A.K., White, S.A., Cruz, A.S.D., Santo, L.T., Ferreira, S.A., & Poland, L.J. (2005). Clonally propagated and seed-derived papaya orchards: II. Yield comparison. *HortScience*, 40(5): 1291–1297.

Fitch, M. (1995). *Somatic embryogenesis in papaya* (*Carica papaya* L.). In Y.P.S. Bajaj (Ed.), *Biotechnology and Agriculture and Forestry*, 30. Springer-Verlag.

Gatambia, E.K., Kihurani, A.W., Rimberia, F.K., & Waiganjo, M.M. (2016). Evaluation of growth rate and phenotypic traits of meristem-cultured papaya plants. *Journal of Experimental Agriculture International,* 14(4): 1–8.

Garcia, M.G., Vega, V.M., & Morales, S.R. (1993). Effect of meristem culture micropropagation on the vigor and yield of the cassava clone Senorita. In Roca W.M. & Thro A.M. (Eds.), *Proceedings of the 1st International Scientific Meeting of the Cassava Biotechnology Network* (pp. 149–153). CIAT.

George, E.F., Hall, M.A., & De Klerk, G.-J. (2008). *Plant propagation by tissue culture (3rd ed.)*. Springer. https://doi.org/10.1007/978-1-4020-5005-3

International Board for Plant Genetic Resources. (1988). Descriptors for papaya. https://www.bioversityinternational.org/e-library/publications/detail/descriptors-for-papaya/

Jyotika, R.K., Harish, S., Karthikeyan, G., Kumar, K.K., Murugan, M., Jayakanthan, M., & Chen, T.C. (2024). Molecular approaches for the management of papaya ringspot virus infecting papaya: a comprehensive review. *Molecular biology reports,* 51(1), 981. https://doi.org/10.1007/s11033-024-09920-9

Loyola-Vargas, V.M., & Ochoa-Alejo, N. (2018). *An Introduction to Plant Tissue Culture: Advances and Perspectives*. In: Loyola-Vargas, V., Ochoa-Alejo, N. (eds) *Plant Cell Culture Protocols. Methods in Molecular Biology,* 1815. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-8594-4_1

Magdalita, P., Godwin, I., Drew, R., & Adkins, S. (1997). Effect of ethylene and culture environment on papaya nodal culture development. *Plant Cell, Tissue and Organ Culture,* 49, 93–100.

Magdalita, P., Drew, R., Adkins, S., & Godwin, I. (1997). Morphological, molecular and cytological analysis of *Carica papaya* × *C. cauliflora* interspecific hybrids. *Theoretical and Applied Genetics*, 95, 224–229.

Magdalita, P.M., Rivarez, M.P.S., Dela Cueva, F.M., San Pascual, A.O., & Trinidad, L.M. (2022). Ultrastructural characterization and field performance of papaya regrowth selections recovered from bacterial crown rot infection for disease tolerance breeding. *Philippine Journal of Crop Science*, 46(3): 18–26.

Magdalita, P.M., & San Pascual, A.O. (2019). Somatic embryogenesis, regeneration, phenotypic and cytological evaluation of selected Philippine papaya (*Carica papaya* L.) genotypes. *Philippine Journal of Crop Science*, 44(3): 20–30.

Magdalita, P.M., Valencia, L.D., Ocampo, A.T.I.D., Tabay, R.T., & Villegas, V.N. (2004). Towards development of PRSV resistant papaya by genetic engineering. In *Proceedings of the 4th International Crop Science Congress*. Brisbane, Australia. https://www.agronomyaustraliaproceedings.org/images/sampledata/2004/poster/3/7/4/749_magdalita.pdf

Majumder, S., Igamberdiev, A.U., & Debnath, S.C. (2025). Somaclonal Variation and Clonal Fidelity in Commercial Micropropagation: Challenges and Perspectives. *Agronomy,* 15(6). https://doi.org/10.3390/agronomy15061489

Manshardt, R.M. (1992). *Papaya*. In F.A. Hammerschlag & R.E. Litz (Eds.), *Biotechnology of Perennial Fruit Crops* (pp. 489–511). Cambridge University Press.

McCallum, B., & Chang, S.M. (2016). Pollen competition in style: Effects of pollen size on siring success in the hermaphroditic common morning glory, Ipomoea purpurea. *American Journal of Botany,* 103(3): 460–470.

More, S., Ghorai, N., & Bera, S. (2010). Study on the selection of some local pollen plants in West Bengal, India as pollen brood feed in *Apis cerana* larvae by worker bees. *Proceedings of the Zoological Society,* 63(1): 39–44.

Pandey, R., & Singh, S. (1988). Field performance of in vitro raised papaya plants. *Journal of Horticulture*, 45, 1–7.

Philippine Statistics Authority. (2016). Crop Statistics. Quezon City, Philippines.

Phuangrat, B., Phironrit, N., Son-ong, A., Puangchon, P., Meechai, A., Wasee, S., Kositratana, W., & Burns, P. (2013). Histological and Morphological Studies of Pollen Grains from Elongata, Reduced Elongata and Staminate Flowers in *Carica papaya* L.*Tropical Plant Biol*. 10.1007/s12042-013-9118-0

Princi, S.K., Umar, S., & Noorani, M.S. (2026). Papaya ringspot virus: from pathogenesis to genetic resistance—a global perspective. *Plant Biotechnol Rep.,* 20, 25. https://doi.org/10.1007/s11816-025-01038-z

Py, C., Lacoeuilhe, J.J., & Teisson, C. (1987). *The pineapple: Cultivation and uses*. G.-P. Maisonneuve & Larose.

Ryavalad, S., Malabasari, T.A., Shantappa, T., Uppar, D.S., Biradar, B.D., & Mantur, S.M. (2019). Micropropagation studies in papaya (*Carica papaya L.*) cv. Surya. *International Journal of Current Microbiology and Applied Sciences*, 8(4): 2362–2367.

San Pascual, A.O., Magdalita, P.M., Sotto, R.C., & Lalusin, A.G. (2023). Performance evaluation of new PRSV-tolerant papaya (*Carica papaya* L.) F1 hybrids in three selected locations. *Science Diliman*, 35(1): 3–25.

Sanikommu, V.R.R., Aj, S.C.K., & Kalal, P. (2021). *Papaya* (*Carica papaya* L.). In *Tropical Fruit Crops: Theory to Practical* (pp. 426–468). Jaya Publishing House.

Sarmiento, B.S. (2026). "Lowly" no more: Papaya outranks canned tuna in Soccsksargen 2025 export list. *MindaNews*. https://mindanews.com/business/2026/04/lowly-no-more-papaya-outranks-canned-tuna-in-soccsksargen-2025-export-list/

Science and Plants for Students. (2017). *Pollen image library*. http://www-saps.plantsci.cam.ac.uk/pollen/index2.htm

Siar, S., Geronimo, S., Sierra, Z., & Villegas, V. (1998). Cytology of *Carica papaya* and *Carica cauliflora* and their F1 interspecific hybrid. *Philippine Journal of Crop Science,* 23(2): 90–96.

Specialty Produce. (n.d.). Maradol papayas information and facts.https://specialtyproduce.com/produce/Maradol_Papayas_17069.php

Srikantha, A., & Jacob, V. (2001). Pollen studies on *Carica papaya* L. http://agrilearning.goviya.lk/papaya/Research_papow/papowpdf/cu/12.pdf

Swartz, H.J., Galletta, G.J., & Zimmerman, R.H. (1981). Field performance and phenotypic stability of tissue culture-propagated strawberries. *Journal of the American Society for Horticultural Science,* 106(5): 667–673.

Talavera M.C., Gil y Espadas F., Contreras, M., Santamaria, J.M. (2007). Field Performance of 100% Hermaphrodite Micropropagated Papaya Plants. *Acta Horticulturae.* 10.17660/ActaHortic.2007.748.29

Tamaki, M., Urasaki, N., Sunakawa, Y., Motomura, K., & Adaniya, S. (2011). Seasonal variations in pollen germination ability, reproductive function of pistils, and seed and fruit yield in papaya (*Carica papaya* L.) in Okinawa. *Journal of the Japanese Society for Horticultural Science,* 80(2): 156–163.

TFNet News Compilation. (2016). Papaya: Name, taxonomy, botany. International Tropical Fruit Network. https://www..itfnet.org/v1/2016/05/papaya-name-taxonomy-botany/.

Thakur S., Shruti, Hashmi S., Mishra, S., Ekka S.K., Kushwaha, A., & Kujur R. (2024). A Review on Plant Tissue Culture. *Asian Journal of Biology,* 20(2): 14-18.

The Editors of Encyclopaedia Britannica. (2025). Papaya. *Encyclopaedia Britannica*.

Thorpe, T.A. (2007). History of plant tissue culture. *Mol Biotechnol,* 37, 169–180. https://doi.org/10.1007/s12033-007-0031-3

Tridge. (2025). Fresh papaya. https://www.tridge.com/intelligences/papaya

Veera, S., Chirumamilla, P., & Taduri, S. (2020). High Efficiency In vitro Regeneration and Genetic Stability of Corallocarpus epigaeus - An Endangered Medicinal Plant. *Plant Tissue Culture and Biotechnology*, 30(2): 219–229. https://doi.org/10.3329/ptcb.v30i2.50692

Yapo, E.S., Tanoh, H.K., Mongomaké, K., Justin, Y.K., Patrice, K., & Jean-Michel, M. (2011). Regeneration of pineapple (*Ananas comosus* L.) plant through somatic embryogenesis. *Journal of Plant Biochemistry & Biotechnology*, 20(2): 196–204.