HortScience 57(5):629–633. 2022. https://doi.org/10.21273/HORTSCI16498-22

# Micropropagation of Hermaphrodite Papayas: Increased Root Induction and Plant Survival during Acclimatization

**Maureen M. M. Fitch, Mary Joy Ancheta, Luyen C. Huynh, Xiaoling He, Marjorie A. Ortega, Hideko K. Fields, Carol M. Murakami, Josienellie R. Shaw, and Jeremiah-James Lopez**
*Hawaii Agriculture Research Center (HARC), P.O. Box, Kunia, HI 96759*

*Additional index words. Carica papaya*, virus-resistant papayas

***Abstract.*** **In Hawaii, the commercial papaya industry is based on cultivars that segregate as females or hermaphrodites. Multiple seedlings are planted and then thinned at flowering to single hermaphrodites at each site. The aim of this study was to increase propagation efficiency by improving our procedure for micropropagation of hermaphrodite plants only. Initially, shoots were multiplied in vented jars on M2 medium, a Murashige and Skoog formulation containing 0.25 µM 6-benzyladenine (BA) and 0.1 µM α-naphthalene acetic acid (NAA). At weekly intervals, micropropagated shoots were either incubated for 4 to 7 days in IBA2 medium containing 20 µM indole-3-butyric acid (IBA) or were dipped in autoclaved rooting powder containing 0.8% IBA (DIP); then, they were placed in M2 until root initials or small roots were visible. After root induction in both treatments, plants were transferred to an in vitro medium containing ½ MSO and 30 g·L$^{-1}$ sucrose in vermiculite (VER). The IBA2 treatment produced 467 potted plants compared to 475 produced by the DIP treatment; however, the average number of days that each treatment required from root induction to potting of rooted plants was not significantly different (IBA2: 52.42 ± 5.65 days; DIP: 51.94 ± 3.61 days). Plants from both treatments were grown in either wet potting medium (500 mL water/300 g potting medium) or damp potting medium (120 mL water/300 g potting medium) to test the effect of moisture content on plant survival and growth after potting. Use of damp rather than wet potting medium resulted in significantly higher plant survival and growth. These results could facilitate more efficient commercial practice for papaya growers.**

Papayas (*Carica papaya* L.) are semi-herbaceous trees grown in the tropics and subtropics that fuel a worldwide market of popular cultivars. Their ripe fruit, with high levels of vitamins A and C, are important as breakfast items, ingredients for fruit salads, juices, and jam preserves. Green fruits are cooked with chicken or grated onto salads that include seafood, hot chili spices, and fish sauce (green papaya salad) in Southeast Asian cuisine. The fruits are important fresh exports that provide value-added revenue for many countries.

Papayas from Hawaii became synonymous with transgenic crops. *Papaya ringspot virus* (PRSV) nearly destroyed the flourishing Hawaii papaya industry in 1995; however, the introduction of the world's first genetically engineered, virus-resistant 'Rainbow' papaya (Fitch et al., 1992; Fitch, 2020) saved the industry (Gonsalves, 1998) and has continued to be the most important cultivar grown in Hawaii (Manshardt, 1998, 2014).

Commercial papayas grown from seed are either hermaphrodite or female. The $F_1$ hybrid seedlings segregate 1:1 for each type. Hermaphrodites have perfect flowers that self-pollinate before anthesis. The fruits are pyriform, of uniform size, and are the preferred type grown for the fresh fruit and export markets in many places other than Hawaii (e.g., Belize, Brazil, Malaysia, Mexico, Taiwan) (Chan and Teo, 2002; Lai et al., 1998; Manshardt, 2014; Talavera et al., 2007; Yu et al., 2000). In cooler areas such as Australia, Israel, Japan, and South Africa, female trees are grown (Allan, 1995; Drew, 1992; Hidaka et al., 2008; Reuveni et al., 1990) because they have a more consistent fruit set than hermaphrodites (Storey, 1953). When hermaphrodites are grown, females are removed because their ovate fruit shape is less efficient for packing. Growers plant at least five seedlings at each planting site to ensure a more than 95% chance of having a hermaphrodite plant. Females and extra hermaphrodites are removed; however, this is an expense that could be eliminated if all plants were hermaphrodites. Clonal propagation of papayas, hermaphrodites or females, is a solution that several workers reported in the past 30 years [e.g., rooted cuttings (Allan, 1995; Fitch et al., 2005a; Montas et al., 2016; Reuveni and Shlesinger, 1990) and micropropagation (Chan and Teo, 2002; Drew, 1992; Drew and Vogler, 1993; Fitch et al., 2005a; Hidaka et al., 2008; Litz and Conover, 1978; Reuveni et al., 1990; Talavera et al., 2007; 2009; Teo and Chan, 1994; Yu et al., 2000)].

Field tests of cloned papayas exhibited several advantages. Tissue cultures from adult plants had higher yields than seedlings in the spring and reduced juvenile phases (lower height of the first flower and earlier harvest), more fruits per meter of stem, and increased trunk circumferences (Drew and Vogler, 1993). Cloned plants helped to reduce field establishment costs during the first year (Talavera et al., 2007). Cloned plants produced earlier and higher yields than single hermaphrodite seedlings and multiple planted seedlings thinned to a single hermaphrodite (Fitch et al., 2005b).

## Materials and Methods

### Plant material

All experiments were conducted at the Hawaii Agriculture Research Center (HARC) using the most important hermaphrodites, PRSV-resistant commercial papaya hybrids 'Rainbow' (Manshardt, 1998) and 'Laie Gold' (Fitch, 2002). 'Laie Gold' was micropropagated in 90% or more of the in vitro experiments because 'Rainbow' was being propagated in another project as rooted cuttings of greenhouse-grown hermaphrodite plants destined for Hawaii island (M. Fitch, unpublished results). 'Laie Gold' was readily sold to growers on Oahu where HARC is located. Previous experience comparing micropropagation of both cultivars showed no obvious differences between them. Data comparing treatments were averaged without considering the effects of a small percentage of one cultivar.

### In vitro culture

Media used in all experiments are listed in Table 1. In vitro cultures were initiated as needed. Approximately 2 to 3 cm of apical shoots of germinated hermaphrodite seedlings (Deputy et al., 2002) and shoots from selected hermaphrodite field plants (Fig. 1) were rinsed overnight in running water and agitated in 1.11% sodium hypochlorite with a drop of Tween 20 for 30 min. Explants were trimmed of bleached tissues, cut into shoot

Received for publication 13 Jan. 2022. Accepted for publication 18 Feb. 2022.
Published online 20 April 2022.
This work is supported by Development of Genetic Resources for Select Tropical Crops grant no. 58-2040-8-002 from the USDA Agricultural Research Service. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.
We thank the U.S. Department of Agriculture, Agricultural Research Service (ARS) and the Hawaii Department of Agriculture, Agribusiness Development Corporation (ADC) for funding; Michael Kohn, Pa'ina, LLC, for project support; and volunteers for assistance with laboratory and growth room tasks.
M.M.M.F. is the corresponding author. E-mail: mfitch@harc-hspa.com.

Table 1. Papaya micropropagation media.

| Type of culture | Medium | Components |
|---|---|---|
| Shoot stock maintenance in gel in 10-cm petri dishes, ≈25 mL | MBN in petri dishes | 1 L MBN:<br>MS (Murashige and Skoog, 1962) salts + MS organic components<br>+<br>BA 1 μM = 0.2 mg·L$^{-1}$<br>NAA 1 μM = 0.2 mg·L$^{-1}$<br>+<br>30 g·L$^{-1}$ sucrose<br>2.8 g·L$^{-1}$ gellan gum<br>pH 5.7 |
| Shoot proliferation and elongation in 30 mL of M2 gel medium in 7-cm-tall jars | M2 in jars with vented caps, two vented bags | 1 L M2:<br>MS salts + MS organic components<br>+<br>BA 0.25 μM = 0.05 mg·L$^{-1}$<br>NAA 0.1 μM = 0.02 mg·L$^{-1}$<br>+<br>30 g·L$^{-1}$ sucrose<br>2.8 g·L$^{-1}$ gellan gum<br>pH 5.7 |
| Root induction and initiation on ≈3-cm-tall shoots trimmed of calli and inserted in IBA2 medium | IBA2 in jars with vented caps, two vented bags | 1 L MSO:<br>MS salts + MS organic components<br>+<br>IBA 20 μM = 4.0 mg·L$^{-1}$<br>+<br>30 g·L$^{-1}$ sucrose<br>2.8 g·L$^{-1}$ gellan gum<br>pH 5.7 |
| Root induction and initiation on ≈3-cm-tall shoots trimmed of calli, dipped in autoclaved rooting powder (0.8% IBA in a commercial product), and inserted in M2 gel medium | M2 in jars with vented caps, two vented bags | 1 L M2:<br>MS salts + MS organic components<br>+<br>BA 0.25 μM = 0.05 mg·L$^{-1}$<br>NAA 0.1 μM = 0.02 mg·L$^{-1}$<br>+<br>30 g·L$^{-1}$ sucrose<br>2.8 g·L$^{-1}$ gellan gum<br>pH 5.7 |
| Induced (IBA2) and rooted shoots (DIP) subcultured to root and grow in 40 mL VER medium in 10-cm-tall jars until planting in potting media | VER in jars, solid caps, two bags, vented caps and bags or no bags when dense roots and well-developed canopies form | 1 L ½ MSO:<br>½ (MS salts + MS organic components)<br>+<br>30 g·L$^{-1}$ sucrose<br>pH 5.7, 40 mL liquid medium in 40 mL vermiculite |

BA = 6-benzyladenine; NAA = α-naphthalene acetic acid; IBA = indole-3-butyric acid.

apex and single node pieces, plated on MBN, and subcultured monthly or bimonthly as stocks. Contamination was controlled by subjecting all stocks to the hypochlorite treatment every 3 to 4 months without an overnight rinse. One to three shoots or clusters of shoots were transferred to M2 jars with vented lids and grown in the laboratory under cool white fluorescent [photosynthetically active radiation (*PAR*) of 35 μmol·m$^{-2}$·s$^{-1}$] or light-emitting diode (LED) lights (*PAR* of 100–125 μmol·m$^{-2}$·s$^{-1}$). Room temperature was 23 °C, and lights remained on for 24 h every day.

Fig. 1. A shoot like this 'Rainbow' hermaphrodite in bud is typical of materials used to initiate cultures.

*Data collection.* Data regarding cultivar names and dates of subculture from MBN to M2 or from M2 to M2 were obtained.

**Root induction**

Experiments were initiated by transferring shoots or clusters either from MBN stock plates to M2 jars or from M2 jars to M2 jars. Cultures were subcultured monthly until individual shoots were ≈3 cm tall (Fig. 2A). Tall shoots were used singly or cut from clusters, trimmed of subtending calli, and induced to root using either of two treatments (IBA2 or DIP). The workers put 20 shoots into each of the two treatments per transfer time. They repeated both treatments numerous times because there were growers who would use all the 'Laie Gold' plants that could be produced.

IBA2 involved placing one to three trimmed shoots (Fig. 2B) in each container of IBA2 gel medium for 4 to 7 d. After induction, shoots were inserted in VER medium for root initiation and growth. Jars were capped with solid lids, sealed in double bags, and placed under lights in the laboratory.

In the DIP method, shoots were dipped in autoclaved rooting powder (0.8% IBA in a commercial rooting product) (Fig. 2C), and one to three shoots were inserted in each jar of fresh M2 medium where they formed root initials. With both methods, shoots from one stock jar were treated together and not mixed with shoots from other stock jars to minimize cross-contamination. Jars were capped with vented lids and placed in two vented bags.

*Data collection.* Data of cultivar names and dates of IBA2 or DIP treatments were obtained.

**Root emergence, growth, and elongation**

*IBA2.* Induced shoots in VER jars were left to form roots and develop leaf canopies.

*DIP.* One to three shoots that had formed root initials or short roots in M2 (Fig. 2D) were transferred to VER for continued root


Fig. 2. **(A)** The micropropagated plant (MP) with a callus to be removed. **(B)** The MP with the callus removed and ready for insertions into IBA2 medium or for dipping into rooting powder. **(C)** The MP dipped in rooting powder before being placed into M2. **(D)** The MP in M2 for 1 month showing many roots. **(E)** The MP in VER after 2 months. **(F)** Two freshly potted MPs from VER in damp potting medium and dry bags. **(G)** Plants photographed 7.5 weeks after potting. The bag is still sealed and the MP appeared healthy. **(H)** One of the bags from **(G)** showing a pool of water that accumulated in a corner of the initially dry bag. Transpiration and condensation presumably occurred as the plant continued to thrive and enlarge.

and shoot development (Fig. 2E). Solid lids and bags were used until plants formed dense root systems and full canopies. Then, vented lids and bags were replaced to acclimatize the plants to growth conditions outside of the laboratory.

*Data collection.* Cultivar names, subculture dates to VER, and the number of subcultured plants were recorded.

### Acclimatization and transplantation to potting medium

After 1 to 2 months or more in VER (Fig. 2E), plants were rinsed to remove vermiculite. They were potted in 9-cm-wide pots in a commercial peat/perlite potting medium (67% Canadian sphagnum peatmoss, 33% perlite) containing a small quantity of nutrients (Fig. 2F). Potted plants in sealed bags were grown under LED lights (*PAR* of 100–125 $\mu mol \cdot m^{-2} \cdot s^{-1}$) with a 16-h light/8-h dark photoperiod. Approximately 300 g of potting medium from newly opened bags was moistened with 120 or 500 mL of water for the damp potting medium experiments and for the wet potting medium experiments, respectively.

After 2 weeks in the wet potting medium treatment, plants were fertilized with ≈20 14N–4.2P–11.6K timed-release granules and bags were re-sealed except for 5-cm vents. After another 2 to 4 weeks, the bags were opened fully and plants were checked weekly and watered as needed. Damp potting medium plants remained in the sealed bags for 2 to 3 months before bags were opened; plants were watered with 40 mL of dilute all-purpose indoor plant fertilizer (24N–3.5P–13.3K) and bags were re-sealed. Plants were grown for another 3 to 4 weeks before bags were fully opened; 20 to 30 mL of water was added together with ≈20 timed-release nutrient granules. Plants were watered weekly with 80 mL of water or as needed. Plants in fully opened bags in the growth room or greenhouse were ready for distribution.

*Data collection.* Cultivar names, subculture dates, potting dates, fertilization and watering dates, distribution dates, and number of potted plants that survived were recorded.

## Results

### Culture initiation, frequency of subcultures, and the number of plants potted in the two root induction treatments

The time from culture initiation to stocks ready for micropropagation on M2 ranged from 1 to 3 months. An additional 3 months of monthly (32.8 ± 2.7 d) subculture on M2 was necessary before shoots grew to 3 cm for root initiation experiments.

*IBA2.* After 4 to 7 d in IBA2 medium, shoots occasionally lost a few older leaves. Shoots that lost leaves and failed to root in VER after 2 to 4 months were returned to M2 to grow new canopies. Losses caused by contamination or failure to develop after subculturing from M2 to VER were estimated to range from less than 10% to more than 30% per week. A total of 467 IBA2 plants were potted after 52.42 ± 5.65 d in VER.

*DIP.* Approximately 10% of rooting powder-dipped shoots (Fig. 2C) formed root initials in M2 after 31.0 ± 0.5 d; they were transferred to VER (1 to 3 per jar) and then planted after 20 to 70 d. Shoots that failed to root within 3 months were re-dipped after subtending calli were removed and placed in M2 to form fuller leaf canopies. All clean, healthy shoots were processed weekly, and all rooted. A total of 475 DIP plants were potted after 51.94 ± 3.61 d in VER. Interestingly, even plants contaminated with bacteria or fungi rooted well enough to grow after being potted. The contaminants were not identified and their pathogenicity was not determined.

### Acclimatization of rooted plants in potting soil

Data regarding a random sample of rooted plants that were sold were analyzed for the average time interval in VER before potting. For 67 plants that were sold, the average number of days in VER medium was 59.36 ± 2.85 d, which was close to the average of all potted DIP plants (51.94 ± 3.61 d).

Most plants grew in the terrarium-like sealed bags (Fig. 2F). Damp potting medium plants that were potted, bagged, and sealed remained sealed longer than those in the wet potting medium group (up to 3 months) (Fig. 2G), with no apparent adverse effects. Plants grew taller, stem diameters thickened, and roots appeared in the moisture that collected at the bottom of bags (Fig. 2H).

The difference between the percentage of plants that survived and grew in wet potting medium (20.7 ± 3.4%) compared with that in damp potting medium (75.8 ± 3.9%) was highly significant ($P \leq 0.0002$) according to *t* test statistics (Fig. 3).

## Discussion and Conclusions

'Rainbow' and 'Laie Gold' were micropropagated because they are the most important commercial cultivars in Hawaii and could be distributed to growers after data were collected. More than 90% of the papayas micropropagated were 'Laie Gold' because it is the most important cultivar grown in Oahu, and the HARC is convenient for pickup. 'Rainbow' papaya on Hawaii island represents 95% of Hawaii's industry; however, it was only micropropagated in small quantities because shipping costs make the price prohibitive for growers. The use of important local cultivars is consistent with studies performed in other locations such as Australia (Drew, 1992; Drew and Vogler, 1993), Israel (Reuveni et al., 1990), Japan (Hidaka et al., 2008), Malaysia (Chan and Teo, 2002; Teo and Chan, 1994), Mexico (Talavera et al., 2007, 2009), and Taiwan (Yu et al., 2000). The Australian, Israeli, and Japanese clones were selected females important to local grower groups. The Australian cultivar needed to be pollinated and required one male plant per 10 females, whereas the seedless female Japanese cultivar did not require pollination.

The numbers of shoots that initiated roots were similar for both IBA2 and DIP protocols and were consistent with those researched in Malaysia and Mexico. The Malaysian group (Chan and Teo, 2002; Teo and Chan, 1994) used either IBA2-like medium containing 2.2 μM BA and 29.5 to 49.2 μM IBA or DIP-like treatment with a highly concentrated medium containing 12.3 mM IBA to induce roots on shoot cuttings before insertion in water agar; however, rooting was dependent on the genotype.

The difference between the survival of plants in the wet and damp potting medium treatments was highly significant. Rooted plants in VER before potting were sometimes contaminated but still grew and were distributed to growers. Higher moisture could have resulted in heavy losses attributed to rot, whereas the damp potting medium may not have had sufficient moisture to support contaminant growth but was sufficient for root and shoot growth. Peat potting medium is known to suppress microbial growth.

In Mexico (Talavera et al., 2009), in vitro shoots were induced to root using a presumably proprietary in vitro protocol developed at

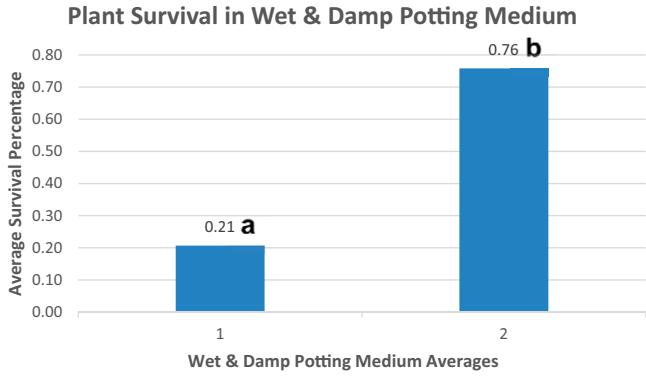


Fig. 3. Comparison using an analysis of variance. The average percentage of plants that grew after potting in wet potting medium was significantly different from that after potting in damp potting medium ($P \leq 0.0002$).

Centro de Investigación Cientifica de Yucatán (CICY) by PROPLANTA CICY and were grown using three methods: method 1, ventilated in vitro vessels for root induction followed by ex vitro treatment with IBA and Radix 1500; method 2, nonventilated in vitro vessels followed by ex vitro treatment with the same Radix product; or method 3, ventilated in vitro vessels followed by ex vitro treatment with Raizone plus (IBA 3%). Shoots cultured in vitro in sealed vessels and treated with Raizone plus did not survive. The best practice for achieving the highest rooting, survival, and fresh and dry weight gains was method 1. The Mexican group produced 5000 plants for field tests that had 100% hermaphrodites compared with 75% hermaphrodite seedlings. During a field test that compared micropropagated plants with seedlings, micropropagated papaya plants were smaller than seedlings (Talavera et al., 2007). Comparative harvest data regarding the clones and seedlings showed higher yields for seedlings. Apparently, all fruits were harvested, and seedling fruits including females were larger and developed faster. The Hawaii field tests (Fitch et al., 2005a, 2005b) involved fewer (332) micropropagated 'Rainbow' hermaphrodites; unlike the Mexican group who harvested both females and hermaphrodites, the yields of cloned hermaphrodites were significantly higher for both micropropagated plants and rooted cuttings compared with hermaphrodite seedlings during the first year of growth. Other attributes of the micropropagated plants, such as labor savings and decreased time to harvest, were reported by the two groups.

The combined in vitro induction protocol (Talavera et al., 2009) that included an ex vitro treatment with a commercial product was similar to the IBA2/DIP treatment reported here and is worthy of further study because the group produced 5000 hermaphrodites, which is a number that would satisfy many growers in Hawaii. Their commercial field comparisons between seedlings and micropropagated plants differed from those in Hawaii because both females and hermaphrodites were grown in the seedling field compared with the micropropagated plant field with hermaphrodites only. They planted three seedlings per hole, waited 2 months until flowering, and left one seedling per planting hole. They had 75% hermaphrodites in the seedling field; however, the yields of hermaphrodites were not higher because, commercially, only hermaphrodites were sold. The Hawaii field tests (Fitch et al., 2005a, 2005b) involved fewer (332) micropropagated 'Rainbow' hermaphrodites, but all plants were hermaphrodites.

In the Hawaii example, there was a significantly higher yield in the cloned papayas vs. thinned hermaphrodite seedlings over an 8-month period in the first year of harvest. This was consistent with research in Australia (Drew and Vogler, 1993).

The micropropagation method may help increase the number of plants that can be provided to commercial growers of both 'Rainbow' and 'Laie Gold.' The much larger market for the few large growers of 'Laie Gold' on Oahu who sell 'Laie Gold' as a specialty crop rather than as a commodity and the major growers of papaya on Hawaii island who produce 95% of the crop (almost exclusively 'Rainbow') are still challenges. Within ≈1 year, ≈1000 plants were distributed to growers by HARC. Approximately half were from micropropagation, the other half were from rooted cuttings. A method of producing a portion of the large numbers of plants required by growers with low loss rates (e.g., <20% to 30%) after potting could help support micropropagation laboratories in the state and elsewhere.

## Literature Cited


Allan, P. 1995. Propagation of 'Honey Gold' papaya by cuttings. In: J. Gomes (ed.). International symposium on tropical fruits. Acta Hort. 370:99–101. https://doi.org/10.17660/ActaHortic.1995.370.15.

Chan, L.K. and C.K.H. Teo. 2002. Micropropagation of Eksotika, a Malaysian papaya cultivar, and the field performance of the tissue culture derived clones. In: R. Drew (ed.). Proceedings of the international symposium on tropical & subtropical fruits. Acta Hort. 575:99–105, https://doi.org/10.17660/ActaHortic.2002.575.80.

Deputy, J.C., R. Ming, H. Ma, Z. Liu, M.M.M. Fitch, M. Wang, R. Manshardt, and J. Stiles. 2002. Molecular markers for sex determination in papaya (*Carica papaya* L.). Theor. Appl. Genet. 106:107–111, https://doi.org/10.1007/s00122-002-0995-0.

Drew, R.A. 1992. Improved techniques for in vitro propagation and germplasm storage of papaya. HortScience 27:1122–1124, https://doi.org/10.21273/HORTSCI.27.10.1122.

Drew, R.A. and J.N. Vogler. 1993. Field evaluation of tissue cultured papaw clones in Queensland. Aust. J. Exp. Agric. 33:475–479, https://doi.org/10.1071/EA9930475.

Fitch, M.M.M. 2002. *Papaya ringspot virus* resistance genes as a stimulus for developing new cultivars and new production systems. In: R. Drew (ed.). Proc. IS on Trop. & Subtrop. Fruits. Acta Hort. 575:85–91, https://doi.org/10.17660/ActaHortic.2002.575.6.

Fitch, M.M.M. 2020. 6.1 *Carica papaya* Papaya. In: R.E. Litz, F. Pliego-Alfaro, and J.I. Hormaza (eds.). Biotechnology of fruit and nut crops. 2nd ed. CAB International. https://doi.org/10.1079/9781780648279.0131.

Fitch, M.M.M., R.M. Manshardt, D. Gonsalves, J.L. Slightom, and J.C. Sanford. 1992. Virus resistant papaya plants derived from tissues bombarded with the coat protein gene of *Papaya ringspot virus*. Bio/Technology 10:1466–1472, https://doi.org/10.1038/nbt192-1466.

Fitch, M.M.M., P.H. Moore, T.C.W. Leong, L.A.Y. Akashi, A.K.F. Yeh, S.A. White, A.S. Dela Cruz, L.T. Santo, S.A. Ferreira, and L.J. Poland. 2005a. Clonally propagated and seed-derived papaya orchards: I. Plant production and field growth. HortScience 40:1283–1290, https://doi.org/10.21273/HORTSCI.40.5.1283.

Fitch, M.M.M., P.H. Moore, T.C.W. Leong, L.A.Y. Akashi, A.K.F. Yeh, S.A. White, A.S. Dela Cruz, L.T. Santo, S.A. Ferreira, and L.J. Poland. 2005b. Clonally propagated and seed-derived papaya orchards: II. Yield comparison. HortScience 40:1291–1297, https://doi.org/10.21273/HORTSCI.40.5.1291.

Gonsalves, D. 1998. Control of *Papaya ringspot virus* in papaya: A case study. Annu. Rev. Phytopathol. 36:415–437, https://doi.org/10.1146/annurev.phyto.36.1.415.

Hidaka, T., S. Komori, M. Yamada, and H. Fukumachi. 2008. Mass production of papaya (*Carica papaya*) saplings using shoot tip culture for commercial use. So. Pac. Stu. 28:87–95, https://id.ndl.go.jp/bib/9524847.

Lai, C.-C., T.-A. Yu, S.-D. Yeh, and J.-S. Yang. 1998. Enhancement of *in vitro* growth of papaya multishoots by aeration. Plant Cell Tissue Organ Cult. 53:221–225, https://doi.org/10.1023/A:1006004216935.

Litz, R.E. and R.A. Conover. 1978. *In vitro* propagation of papaya. HortScience 13:241–242. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL7910053862.

Manshardt, R. 1998. "UH Rainbow" papaya. University of Hawaii Germplasm G-1. https://gms.ctahr.hawaii.edu/gs/handler/getmedia.ahsx?moid=3128&dt=3&g=12.

Manshardt, R. 2014. Chapter 6 history and future of the solo papaya. In: R. Ming and P.H. Moore (eds.). Genetics and genomics of papaya. Plant Genetics and Genomics: Crops and Models-Springer Science+Business Media, New York. https://doi.org/10.1007/p78-1-4614-8087-7_6

Montas, W., P. Moon, and J.H. Crane. 2016. Propagation of papaya (*Carica papaya* L.) with large-sized cuttings. Proc. Annu. Meet. Fla. State Hort. Soc. 129:22–24. https://journals.flvc.org/fshs/article/view/106019/101671.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497, https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Reuveni, O. and D.R. Shlesinger. 1990. Rapid vegetative propagation of papaya plants by cuttings. Acta Hort. 275:301–306, https://doi.org/10.17660/ActaHortic.1990.275.36.

Reuveni, O., D.R. Shlesinger, and U. Lavi. 1990. In vitro clonal propagation of dioecious *Carica papaya*. Plant Cell Tissue Organ Cult. 20:41–46, https://doi.org/10.1007/BF00034755.

Storey, W.B. 1953. Genetics of papaya. J. Hered. 44:70–78, https://doi.org/10.1093/oxfordjournal.jhered.a106358.

Talavera, M.C., F. Espadas y Gil, M. Contreras, G. Fuentes, and J.M. Santamaria. 2007. Field performance of 100% hermaphrodite micropropagated papaya plants. In: J.M. Santamaria and Y. Desjardins (eds.). Proceedings of the 2nd international symposium on acclimatization and establishment of micropropagated plants. Acta Hort. 748:219–222. https://doi.org/17660/ActaHortic.2007.748.29.

Talavera, C., F. Espadas y Gil, M. Contreras, G. Fuentes, and J.M. Santamaria. 2009. Acclimatization, rooting and field establishment of micropropagated papaya plants. Acta Hort. 812:373–378, https://doi.org/17660/ActaHortic.2009.812.52.

Teo, C.K.H. and L.K. Chan. 1994. The effects of agar content, nutrient concentration, genotype, and light intensity on the in vitro rooting of papaya microcuttings. J. Hort. Sci. 69:267–273, https://doi.org/10.1080/14620316.1994.11516454.

Yu, T.-A., S.-D. Yeh, Y.-H. Cheng, and J.-S. Yang. 2000. Efficient rooting for establishment of papaya plantlets by micropropagation. Plant Cell Tissue Organ Cult. 61:29–35, https://doi.org/10.1023/A:1006475901439.