



# Micropropagation of Hermaphrodite *Carica papaya* L. 'Rainbow' Seedlings via Axillary Bud Pathway

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## Introduction

*Carica papaya* L. belongs to the Caricaceae family and is an important tree fruit crop, with production in more than 60 tropical and subtropical countries (Evans & Ballen 2012). Papaya, native to Central America, has three sexual forms: male, female, and hermaphrodite (Storey 1938). Hermaphroditic plants are desired by the industry for their ability to self-pollinate and produce a marketable fruit in terms of size and quality.

Planting seeds is the common means of commercial propagation of papaya, not only in the Hawaiian Islands but around the world (Patel et al. 2013). 'Rainbow', a commercially popular Hawaiian variety, is a transgenic yellow-fleshed *Papaya Ringspot Virus* (PRSV)-resistant  $F_1$  hybrid which was released in 1998 (Manshardt 1998). 'Rainbow' seedlings segregate for sex, producing a 1:1 hermaphrodite/female (Storey 1938). To ensure a uniform stand of hermaphrodite plants in the field, five or more seeds are planted per hole, and then at sexual maturity (4–5 months after germination), surplus hermaphrodites and females are rogued, leaving one hermaphrodite per hole (Estrella et al. 2012). Overplanting adds to the cost of production as it causes early competition for water, nutrients, and



Figure 1. Two-month-old hermaphroditic 'Rainbow' papaya seedlings as source of explants

sunlight among seedlings and requires additional inputs, as well as extra labor to rogue unwanted plantlets. Micropropagation can produce large numbers of elite homogeneous clones, allowing for the planting of a single hermaphrodite in each hole and eliminating the negative aspects of seed propagation.

Wu et al. (2012) reported producing over 100,000 hermaphrodite papaya plants via organogenesis within three years. However, their protocol was developed for cv. 'Meizhonghong'. Since genotype can play a role in *in vitro* success, there was no assurance that their protocol would apply to cv. 'Rainbow'. Therefore, this study was conducted to test cv. 'Rainbow' and its adaptability to the Wu et al. (2012) protocol.

## Materials and methods

Fifty-three 2-month-old hermaphroditic 'Rainbow' seedlings were used as the source of explants for the experiment (Figure 1). Seedlings were grown in a greenhouse at the Magoon Horticulture Facility of the University of Hawai'i at Mānoa. Seeds were sown in Sunshine<sup>®</sup> Mix #4, kept moist, and placed under 50% reflective shade. At two months, 70 potted seedlings that had been identified as hermaphrodites by DNA polymerase chain reaction assay

were taken out of the greenhouse and placed in a room air conditioned to 70°F and given 8 hr office-environment light per day for seven days prior to initiation.

### Stage 1. Initiation of aseptic cultures

Five-centimeter shoot-tips (Figure 2) were excised from 'Rainbow' seedlings and then surface-sterilized as follows:

- They were immersed in 70% isopropyl alcohol for 1 min;
- Sterilized in 10% (v/v) Clorox® bleach for 20 min;
- Rinsed three times in sterile de-ionized water;
- Cut into 1-cm segments and placed in VWR® conical tubes containing 10ml of shoot multiplication medium (SMM). SMM was composed of Murashige and Skoog (MS) basal medium with vitamins (Murashige and Skoog 1962) (Phytotech Labs®, product M519), combined with 4% sucrose, 3 mg/l benzyladenine purine (BAP), and 2.8 g/l Phytigel® (gelling agent). The pH of the medium was adjusted to 5.7.
- Explants were then cultured under 16/8h light/dark photoperiod (cool white 40W fluorescent lamp) providing 45  $\mu\text{mol}/\text{m}^2/\text{s}$  and an average room temperature of 25°C.



Figure 2. Papaya shoot-tips (5 cm long) ready for surface sterilization

### Stage 2. Shoot multiplication

After two weeks on SMM, shoot-tips and axillary buds elongated and produced leaves and callus. They were trimmed off and divided into shoot-tips and stem segments. They were then cultured onto fresh SMM in baby food jars. Multiplying shoots were subcultured every four weeks onto fresh medium. By subculture #3, multiplying clumps were large enough to provide a culture mass increase of approximately 400% per month (Figure 3). It was thus confirmed that this medium is suitable for subculture and proliferation of axillary buds of 'Rainbow'.

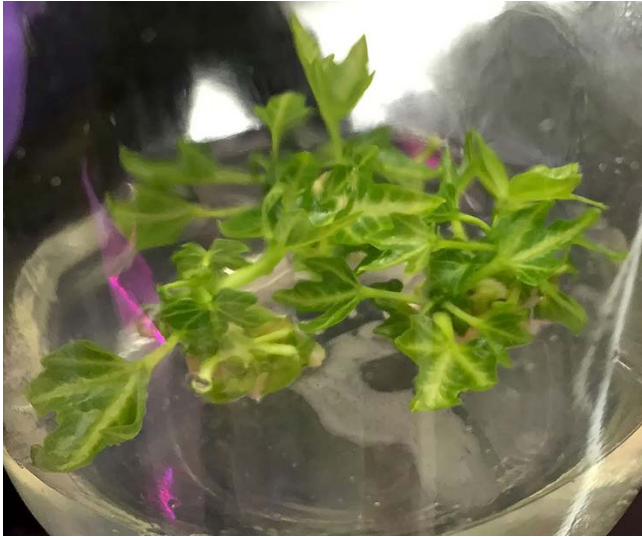
Three months after initiation, an endogenous bacterium developed (Figure 4). Endogenous bacteria have been reported as a major problem in papaya shoot-tip tissue culture (Wu et al. 2012, Thomas & Kumari 2010). Upon detection of the endogenous bacterium, 2 mg/l Plant Contamination Control (PTC<sup>3</sup>, PhytoTechnology Laboratories) was added to the SMM. For long-term production, a bacterial indexing procedure is recommended to eliminate endogenous bacteria.

### Stage 3. Elongation of axillary buds

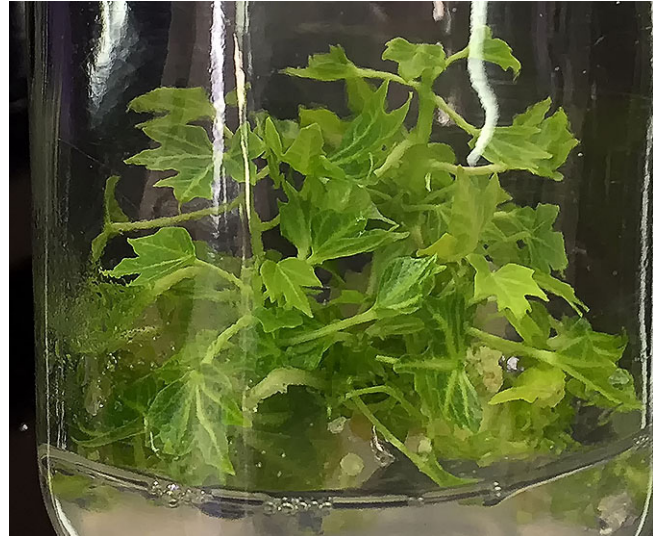
One hundred multiplying clumps were transferred to shoot elongation medium (SEM) composed of 1.5X MS basal medium, 0.25 mg/l BAP, 0.3 mg/l gibberellic acid



Figure 3. Multiplying clump from axillary buds on shoot multiplication medium (SMM).



**Figure 4. Endogenous bacteria (milky, yellowish) around plant tissue and on the medium surface.**



**Figure 5. Papaya shoots in shoot elongation medium.**

(GA3), 2.8 g/l Phytigel<sup>®</sup>, and 4% sucrose. Cultures were then placed under light-emitting diode (LED) lights (GreenPower LED, Philips<sup>®</sup>) with a blue:red ratio of 3:8, providing an average of 49  $\mu\text{mol}/\text{m}^2/\text{s}$ . Red LED lighting has been shown to increase shoot elongation (Miyashita et al. 1997). Multiplying clumps were on SEM for 1 month (Figure 5) before exposure to indole-3-butyric acid (IBA) for rooting.

#### **Stage 4. Root initiation**

Elongated shoots (20 1-cm and 20 2-cm) were excised from clumps and transferred to root initiation medium (RIM) composed of 1 mg/l IBA, full-strength MS basal medium, and 2.8 g/l Phytigel<sup>®</sup>. Elongated shoots were cultured on the RIM for one or three days. After exposure to RIM, all shoots were transferred to root elongation medium (REM) composed of 1.5X MS basal medium, 0.5 g/l activated charcoal, 5 g/l sucrose, and 2.8 g/l Phytigel<sup>®</sup>.

Root initials were not observed on shoots placed on RIM for 1 day regardless of shoot size. Among shoots that had been placed on RIM for three days, root initials were first observed after they had been on REM for one month. After seven weeks on REM, shoots were scored for rooting. A rooting scale from 0 to 3 was used: 0=no initials, 1=initials only, 2=weak root, 3=multiple roots.

One-centimeter shoots that had been on RIM for



**Figure 6. Healthy papaya root initials after 5 weeks in REM.**

three days resulted in 65% rooting, with a mean rooting score of 0.9. Two-centimeter shoots that had been on RIM for three days resulted in 95% rooting, with a mean rooting score of 1.77 (Table 1).

Shoots and leaves appeared healthy, with 5–6 root initials (Figure 6).

### Stage 5. Plantlet establishment

Thirty-seven shoots with root initials were transferred to Magenta™ plant-culture boxes with vented lids, with 10 g of autoclaved fine vermiculite (Hoffman horticultural grade) containing plantlet development medium (PLM) composed of 30 ml of 0.5X MS medium (Figure 7a). Two months later, well-developed root systems were observed in 54% of the plantlets (Figure 7b).

### Future Research

This research has demonstrated that the Wu et al. (2012) protocol can be repeated with slight modifications for mass propagation of hermaphrodite 'Rainbow' papaya.

This paper improves the explant excision method and increases the speed of multiplication. From 100

explants (apical and axillary buds) we were able to produce 1,600 multiplying cultures (average of 3 shoots/clump) in 30 weeks.

Rooting success appears to be greatly influenced by shoot size and exposure to IBA. Induction of root initials achieved 95% based on 20 shoots of 2 cm length. However, only 54% of plantlets developed good root systems in vermiculite.

**Table 1. Rooting after seven weeks on REM (three-day RIM pretreatment).**

	1-cm shoots	2-cm shoots
Rooting Success	65%	95%
Mean Score	0.9	1.77



**Figure 7a. Rooting in vermiculite; 7b. Well-developed root system.**



Further research to improve root development can be based on previous work done in China (Wu et al. 2012), Bangladesh (Roy, Roy, & Hakim 2013), Taiwan (Yang & Ye 1992), India (Seal, Mishra, Chandra, & Srivastava 2010; Patel, Patel, Shah, & Shinde 2010), and Egypt (Rady 2004). The main research goal would be to improve thickness of stem of elongated shoots and their overall health condition by evaluating the following:

- Addition of activated charcoal and removal of GA and BA during shoot elongation stage.
- Extension of the duration of auxin treatment (IBA or NAA) after root initiation in REM medium.
- Use of IBA or NAA at various concentrations to determine which hormone is better for root initiation and development (optimization of root development).
- Testing of other basal media such as WPM, 0.5X MS, and 0.25X MS.
- Reduction of sucrose concentration in rooting medium.

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