

# Acclimatization, Rooting and Field Establishment of Micropropagated Papaya Plants

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## Abstract

Export markets for papaya Maradol require elongated fruits that come from hermaphrodite flowers rather than rounded fruits from female flowers. That forces growers to try to have plantations with 100% hermaphrodite plants. Currently commercial growers use seeds that show only 75% hermaphroditism or lower. For this reason, they transfer 3 plants/pot to the field, water and fertilize them for further 2 months, only do then eliminate 2 of the 3 plants after flower sex examination. By comparison our micropropagated plants have 100% hermaphrodite flowers and 100% of their fruits are elongated in shape, and therefore the grower can directly transfer 1 plant/pot, reducing the need for plants from 6000/ha to only 2000 plants/ha. Our protocol includes rooting medium with efficient root production in vitro. However, the survival rates can be improve by inducing rooting in vitro and root development ex vitro, using commercial rooting products. The ventilation of the vessel has shown to be important to increase plant survival ex vitro. The best rooting, ex vitro performance and plant survival were achieve using ventilated vessels in vitro and rooting ex vitro with Radix 1500. In a commercial-scale field-trial, for 4 consecutive years, micropropagated plants conserved their 100% hermaphroditism in their flowers and consistently 100% of their fruits were elongated in shape while the control plants derived from seeds showed only 75% hermaphrodite flowers and therefore 25% of the less commercial rounded fruits. In addition, no differences were found in plant performance between the micropropagated plants and plants originated by seeds. As far as the physiology, photosynthetic rates and chlorophyll fluorescence they were very similar in both types of plants. In terms of plant height, micropropagated plants were slightly shorter but this is advantageous for harvesting. Fruit size and yields were also comparable and in terms of fruit appearance and quality, fruits from both plant types were equivalent.

## INTRODUCTION

Mexico occupies second place after Brazil in the world production of papaya (FAOSTAT, 2006). Mexico is the main exporter of papaya fruits to the USA with important income for the country. The cultivation of papaya Maradol in Mexico has increased considerably in the last years. All the plantations of papaya in Mexico are established using seedlings and therefore there is currently a large demand of quality seeds. The plants of papaya Maradol originating from seed produce two types of flowers: female or hermaphrodite. Growers prefer hermaphrodites since they produce fruits of elongate shape that are in greater demand in the international market.

To obtain hermaphrodite plants in their plantations, growers transplant up to three seedlings per pot in the field. This increases the production costs, labor, irrigation and diseases control in the first 3 months prior to flowering when 2 out of 3 plants are eliminated from the field to eliminate females.

Micropropagating plants bearing hermaphrodite flowers seemed a good alternative. The in vitro culture has shown to be an efficient way to get from a single

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individual, thousands of cloned plants conserving the same characteristics of the donating plant including the floral type.

CICY developed an efficient protocol for micropropagation of papaya Maradol. In previous experiments, it was demonstrated that once the plants survive the glasshouse acclimatization phase, and are transplanted to a plantation they showed a physiological behavior similar to those derived from seeds (Talavera et al., 2004). Nevertheless, the protocol efficiency can be increased by improving the rooting percentage as it is an important step in the glasshouse phase and later for good anchorage in the field.

The objective of the present work was to improve the protocol for micropropagation developed by PROPLANTA-CICY using strategies for rooting to increase the efficiency and survival of papaya micropropagated plants when they are transferred to the field; and to study the performance of the micropropagated plants in commercial plantations.

## **MATERIALS AND METHODS**

Micropropagated papaya hermaphrodite Maradol plants originating from a single clone were grown under 2 vessel-ventilation-systems: intact commercial (Magenta) vessels (without filter) and vessels bearing a hole in the lid containing a filter (with filter). For rooting under ex-vitro conditions, 2 commercial root inducers containing indol-3-butyric acid (IBA) were tested: Raizone plus (IBA 3%) and Radix 1500 (IBA 1500 ppm) (Fig. 1). In the glasshouse, using commercial substrate (sunshine) plantlets were transferred to plastic trays (Fig. 2). The percentages of rooting and survival, initial and final size of plants, fresh and dry weight were evaluated for both treatments.

A second field trial was established in Regadio in Sucilá Yucatán, México using commercial conditions. Plant height, number of leaves, noon photosynthetic rates from the youngest fully expanded leaves using an IRGA (LICOR 6200, USA), and the chlorophyll fluorescence (efficiency of the PSII) with fluorometer PEA (Hansatech MK2, UK) were measured. Fruit yields were evaluated in micropropagated plants and compared to those obtained from plants that originated from commercial seed (CARISEM) used as controls (Fig. 6).

## **RESULT AND DISCUSSION**

### **Growth and Survival during Ex Vitro Stage**

The results showed that the best biomass production in terms of fresh and dry weight was obtained in plantlets treated with Radix 1500 but the best plants were cultured in ventilated vessels and then treated with Radix 500. Plantlets cultured in vitro under sealed conditions (without filter) and treated with Raizone plus did not survive (Table 1). Similarly, plants cultured in vitro with ventilation showed both higher rooting and survival percentages than their sealed counterparts independently of the rooting treatment used, although the best treatment was growing plants in ventilated vessels followed by rooting induction ex vitro with Radix 500 (Fig. 3).

### **Field Trial Evaluation**

The results of the field validation showed that the micropropagated plants behaved similar to plants from commercial seeds. In terms of growth, plant height and number of leaves, micropropagated plants showed equivalent leaf numbers to those of plants derived from commercial seeds but micropropagated plants were slightly shorter than commercial plants (Figs. 4A and B). Similar results have been reported in other micropropagated varieties of papaya (Fitch et al., 2005) and this may prove to be advantageous at harvesting time since the fruits may be more accessible to the harvester. In physiological terms, no significant differences were found in the photosynthetic rate and chlorophyll fluorescence (Figs. 4C and D) for both plant types.

The main advantage of micropropagated plants is that they produced 100% hermaphrodite flowers while the plants from commercial seeds produced 75% of her-

maphrodites flowers and 25% of female flowers. As a result, micropropagated plants produced 100% of elongated shaped fruits while the plants from commercial seeds produced at least 25% of rounded shaped fruits.

In terms of fruit production and yields, the time for first fruit harvest was similar for both plant types. Furthermore, fruit yields were similar for micropropagated plants and plants obtained from commercial seeds (Figs. 5 and 6).

## CONCLUSIONS

The use of ventilation in culture vessels produced ex vitro rooting and survival during the acclimatization stage of papaya Maradol plants. From our results, it is important to test different commercial rooting inducers for ex vitro rooting of papaya Maradol as important differences were found in terms of rooting and survival among the products tested.

In the field under commercial conditions, the performance of the micropropagated plants was similar to that of plants obtained from commercial seeds. Both in terms of growth as well as their physiological performance, with the advantages that the micropropagated plants maintained, for 4 consecutive years, the trait of 100% hermaphroditism in the floral type and all their fruits were elongated. These features make micropropagation a feasible alternative for papaya Maradol as they satisfy the international market demand for elongated shape fruits, and they produce fruit yields that are comparable to those of plants from commercial seeds, while using only a third of the plants required per hectare (i.e. 2000 vs 6000 plants/ha).

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## Tables

Table 1. Fresh and dry weights of total plant, leaves, stems and roots of papaya Maradol micropropagated plantlets cultured in vitro under two types of ventilation and two ex vitro rooting treatments, taken after 21 days of treatment ( $n=5$ ).

	Radix 1500				Raizone plus			
	Fresh weight (g)		Dry weight (g)		Fresh weight (g)		Dry weight (g)	
	With filter	Without filter	With filter	Without filter	With filter	Without filter	With filter	Without filter
Roots	1.04	0.44	0.07	0.02	0.76	*	0.05	*
Stems	0.44	0.18	0.08	0.08	0.27	*	0.02	*
Leaves	0.77	0.40	0.10	0.10	0.60	*	0.07	*
Total	2.25±0.08	1.02±0.16	0.25±0.08	0.20±0.08	1.63±0.60	*	0.14±0.09	*

\*Plants did not survive.

## Figures

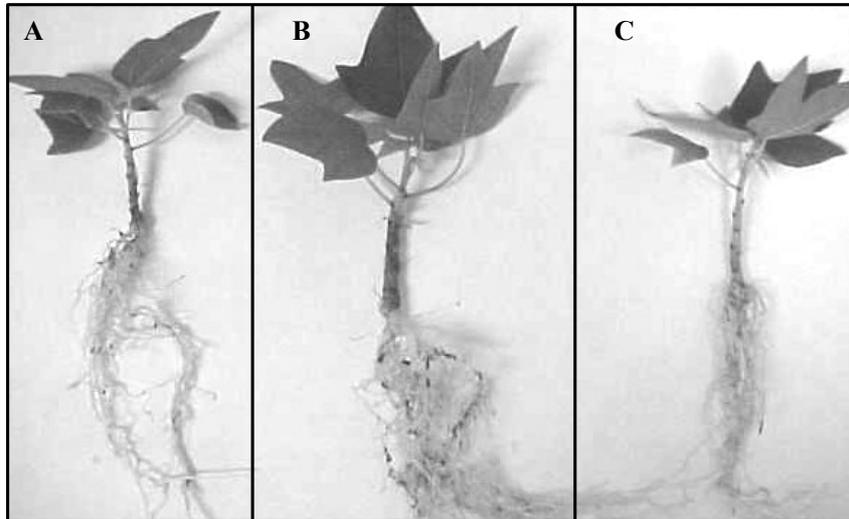


Fig. 1. Rooting with 2 different vessel ventilation and 2 commercial rooting inducers. (A) Plants grown in vitro in sealed vessels followed by treatment ex vitro with Radix 1500; (B) Plants grown in vitro in ventilated vessels followed by treatment ex vitro with Radix 1500; and (C) Plants grown in vitro in sealed vessels followed by treatment ex vitro with Raizone plus.



Fig. 2. Detail of a papaya Maradol micropropagated plantlet during the ex vitro rooting process under glasshouse conditions.

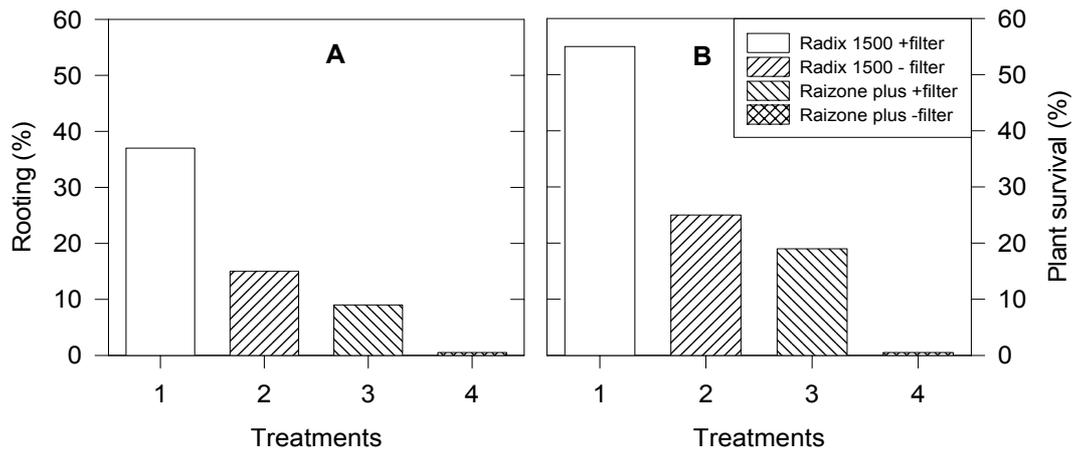


Fig. 3. Rooting (A) and plant survival (B) percentages of micropropagated papaya Maradol plantlets with different root inducers and types of ventilation, 21 days after being transferred ex vitro under glasshouse conditions ( $n=5$ ).

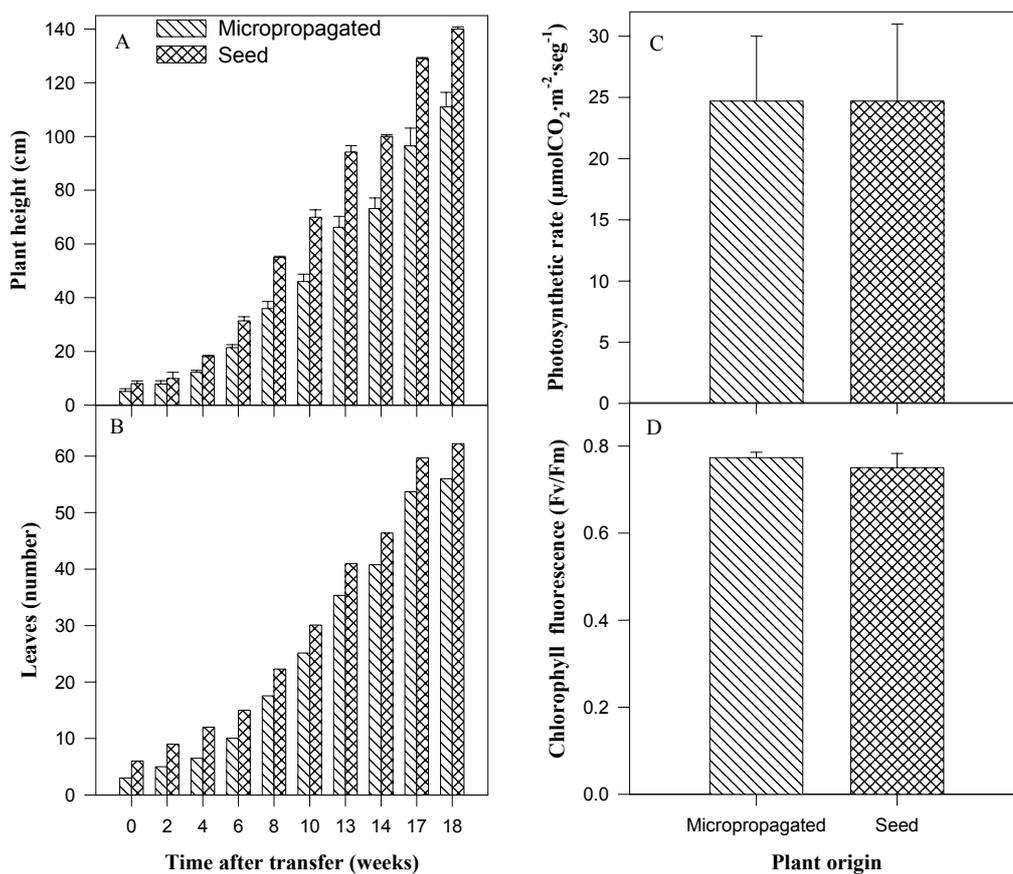


Fig. 4. (A) Plant height, (B) number of leaves measured during 18 weeks following transplant to the field trial, (C) noon net photosynthetic rate, and (D) chlorophyll fluorescence in micropropagated papaya Maradol plants vs. plants obtained from seeds taken 18 weeks after being transplanted. Data are means $\pm$ SD from 10 plants in each point.

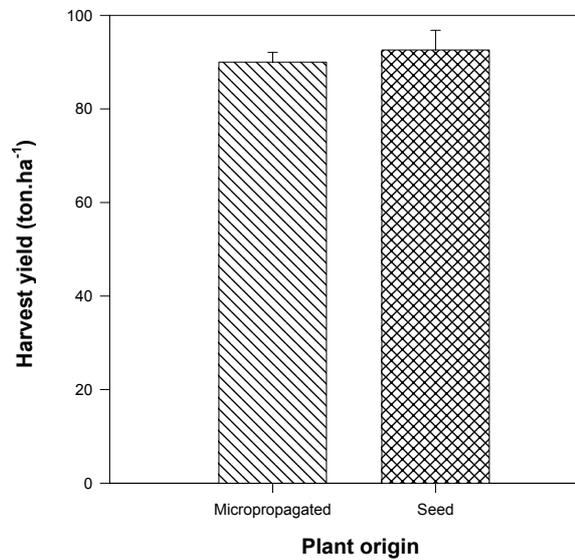


Fig. 5. Fruit yield, from both micropropagated papaya Maradol plants and from plants obtained from commercial seeds. Data are means $\pm$ SD from 60 plants for each plant type.

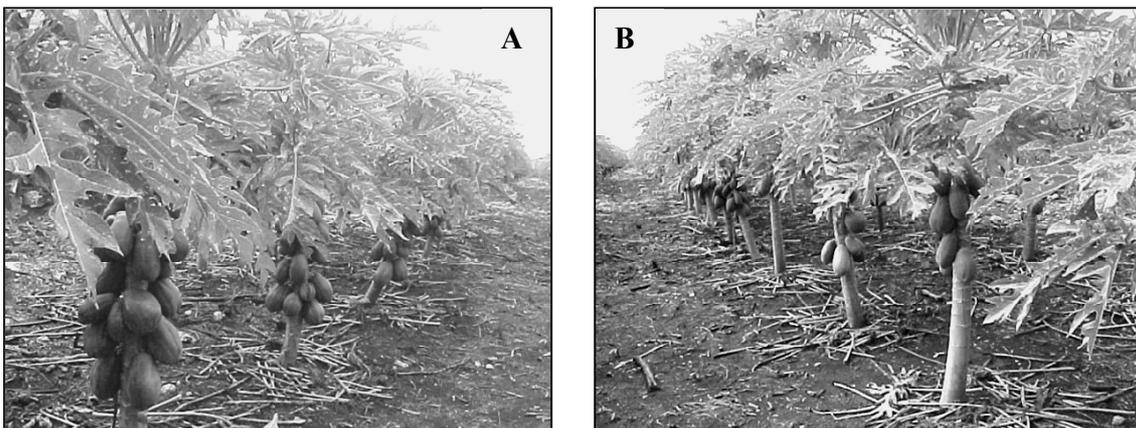


Fig. 6. View of the field trial handled under grower commercial conditions at Sucilá, Yucatán, México, showing (A) fruits from micropropagated plants, and (B) from plants of seeds, 35 weeks after transplant.