In Vitro Propagation of Mango (*Mangifera indica*)©

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INTRODUCTION

A Japanese leading and monoembryonic mango (*Mangifera indica* L.) cultivar, 'Irwin', is propagated by grafting onto seedling of polyembryonic mango. The grafted nursery stocks of tropical fruit trees sold in Japan are very expensive mainly because of heating cost in winter. Hence, in vitro propagation of mango will provide a stable supply of nursery stocks and a shortening of raising period possible. However, establishment of tissue culture of mango has been very difficult because of browning of medium and contamination. Therefore, reports on tissue culture of mango are few. Thomus and Ravindra (1997) reported that the solidified medium caused better survival of explants than a liquid medium and the longer explants established better. We made preliminary experiments by using explants from seedlings and succeeded in proliferation of shoots without browning of medium. The objective of this study was to explore the best basal medium for propagation of in vitro shoots from mango seedlings. In addition, we tried to minimize contamination of in vitro culture establishment of explants from mature trees.

MATERIALS AND METHODS

Effect of Basal Medium on Growth of In Vitro Shoots. The in vitro shoots used in this experiment were proliferated from explants obtained from mango seedlings, and were planted in 5 basal media: Murashige and Skoog (MS) (Murashige and Skoog, 1962), ½ MS, wood plant medium (WP) (Lloyd and McCown, 1981), ½ WP, and Ganborg (B5) (Gamborg et al., 1986). All media were supplemented with 10 µM zeatin, 0.2% Plant Preservative Mixture (PPM™, Plant Cell Technology, Washington D.C., U.S.A.), 3% sucrose, and 0.8% agar (pH 5.8). The shoots were planted singly in a 100-ml conical beaker containing 20 ml of culture medium autoclaved at 121 °C for 15 min. All cultures were maintained at 28 °C under 16-h photoperiod. The shoot length and number of leaves were measured every 5 days. Data were subjected to ANOVA.

Prevention of Contamination in In Vitro Culture Establishment. Growing shoots of potted 'Irwin' plants in a greenhouse were collected, and the leaves were removed. The explants were surface-sterilized for 30 min in 1%, 2%, and 3% sodium hypochlorite solutions, containing 0.1% Tween 20 each, and washed three times with sterile water. Before the chlorine treatments, half of the explants were immersed in 70% ethanol for 1 min and the rest were not. The explants, 2–3 cm long, were placed on WP medium with 10 µM zeatin. The culture conditions were the same as those of the basal medium test. The contamination rate of the explant was investigated every day.

RESULTS AND DISCUSSION

The basal medium influenced the in vitro shoot growth. It was on the MS medium that the shoots elongated most. The average of shoot elongation on the MS medium for 2 months was 1.6 cm. However, because some shoots withered and died on the MS medium, the WP and ½ WP media seemed to be the most suitable ones for the in vitro propagation (Fig. 1). The shoots grew the poorest on the ½ MS medium. Browning was not observed in any media. Thin and long explants, producing less phenol, were reported to be better ones for in vitro establishment (Thomus and Ravindra, 1997). We used thin and long explants and shoots, and these materials probably brought the success of shoot proliferation in this study.

Figure 1. Mango shoots derived from ' Irwin' seedlings, developing after 30 days in culture in ½ WP medium with 10 μM zeatin.

Contamination occurred in most explants in in vitro culture establishment regardless of the concentration of sodium hypochlorite solutions. The 70% ethanol treatment delayed the occurrence of contamination, but it did not completely prevent the occurrence. Contamination did not occur in 33% of the explants sterilized by 70% ethanol + 1% sodium hypochlorite.

CONCLUSION

Both WP and ½ WP media were effective basal ones in in vitro propagation of mango. The relationships between the size and age of explants and the browning of medium and withering of shoots should be investigated. As for prevention of contamination, a further improvement using ethanol treatments is necessary. In a preliminary experiment, we also succeeded in rooting of mango shoots (Fig. 2) and acclimatization of rooted shoots (Fig. 3). However, because the roots developed

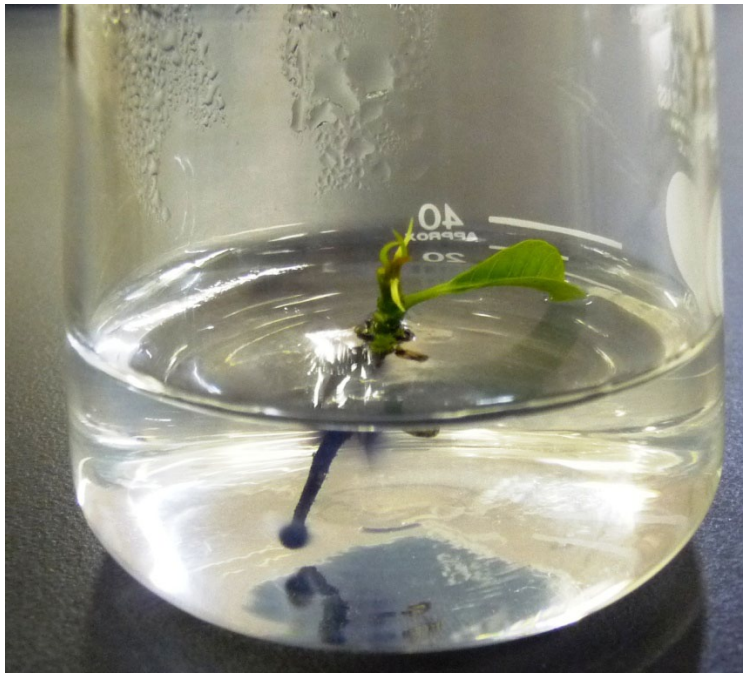

Figure 2. A mango shoot derived from ' Irwin' seedlings, rooting after 20 days in culture in 1/4 WP medium.

Figure 3. Mango micropropagules derived from 'Irwin' seedlings, being acclimatized after 60 days in culture in Jiffy pots filled with fine vermiculite.

poorly, the rooting treatment must be improved. We now use the root development medium, which improved rooting of Japanese chestnut (*Castanea crenata* Sieb. et Zucc) (Tetsumura and Yamashita, 2004).

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