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SHORT COMMUNICATION

STUDIES ON *IN VITRO* MICROPROPAGATION OF SWEET ORANGE AND MANDARIN *VIA* SHOOT TIP CULTURE

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INTRODUCTION

Sweet orange (*Citrus sinensis*) and mandarin (*C. reticulata*) are perennial as well as potential export fruit crops cultivated in Sri Lanka, since ancient times. The main problem in the production of sweet orange and mandarin in Sri Lanka is the lack of elite planting material and the damages caused by pests and diseases. Being cross-pollinated crops, sweet orange and mandarin often fail to produce healthy, high quality planting material from seeds. Therefore, vegetative propagation has become the main technique to produce planting material, and budding and grafting are the most commonly practised methods. Large extents of land, intensive labour and high costs associated with these methods, and their inability to avoid transfer of diseases like *Citrus tristeza* virus to planting material from mother plants are the major problems of these conventional propagation techniques.

Lack of a regular supply of large quantities of homogenous high quality planting material is the main constraint in commercial cultivation of citrus species. Through traditional propagation techniques, it is difficult to obtain large quantities of planting material at a time. Tissue culture offers a quick and efficient technique to mass propagate disease-free planting materials. Through meristem and shoot tip culture clonal propagation is possible during a relatively short period of time (Button and Kochba, 1977). Thus, large amounts of planting material could be made available at any time. The objective of this study was to develop protocols for rapid *in vitro* micropropagation of sweet orange and mandarin *via* shoot tip culture.

MATERIALS AND METHODS

The material for establishment of *in vitro* cultures of sweet orange (*Citrus sinensis*) variety Bibile, and mandarin (*C. reticulata*) variety Madhu, was obtained from the plants raised at the Plant Genetic Resources Centre (PGRC), and sweet orange, variety Valencia, from the Horticultural Research and Development Institute (HORDI), Gannoruwa.

The establishment of *in vitro* cultures of sweet orange and mandarin has frequently been hampered by persistent microbial contaminations. Shoot

tips of actively growing explants of sweet orange and mandarin were taken, leaves removed and 2-3 cm sections of the stem with a bud were excised. These two types of explants were washed in running water for about an hour and kept in Benlate solution (1 g/100 ml) for half an hour. They were then washed thrice in sterilized water and taken into a laminar flow cabinet and further sterilized with 10 % v/v NaOCl (Chlorox) with 2-3 drops of detergent (Tween 20) by shaking for 20 minutes. Then the explants were washed three times in sterilized distilled water. The explants were cut into 1.5 cm pieces with axillary buds and also all browned parts of the stem removed. Thirty explants from each of the two categories of young juvenile shoots and from mature parts of sweet orange variety Bibile were cultured in MS (Murashige and Skoog, 1962) medium with 2 mg/l BAP.

Cultures were kept under a light intensity about 55 $\mu Em^{-2}s^{-1}$ at ± 25 °C and, a photoperiod of 16 hours light and 8 hours darkness. In all other experiments the same culture conditions were maintained. Numbers of explants successfully initiated were counted after 12 weeks and % successful initiation of shoots determined. Results were statistically analyzed by the χ^2 method.

To ascertain the effects of growth regulators on *in vitro* shoot multiplication of shoot tips of sweet orange var. Bibile, the MS medium was modified by adding various levels of BAP in combination of GA_3, IAA or IBA. Four weeks after culturing in the initiation medium of MS with 2 mg/l BAP, shoot tips from young buds where growth has been initiated were sub cultured in hormone-free MS medium for four weeks. Then they were cultured in the proliferating media for a further eight weeks and the number of shoots formed was recorded. The experiment was conducted in a Complete Randomized Design.

To ascertain the effects of growth regulators on *in vitro* multiplication of sweet orange var. Valencia, sterilized young shoot tips were cultured in MS medium with 0.5 mg/l IAA or GA_3 or BAP and also in MS with 1 mg/l and 2 mg/l BAP. Initiation success was determined after eight weeks in culture and growth-initiated shoot tips were subcultured in a media of the same composition. The number of shoots formed in each treatment was recorded after a further time period of eight weeks.

In mandarin var. Madhu, sterilized shoot tips from tender buds were cultured in MS media with BAP levels ranging from 0.5 to 6.0 mg/l. After three weeks in culture shoot initiation was observed and these shoot tips were sub cultured in a media of the same composition. After a further 10 weeks in culture the number of shoots formed was recorded. In another experiment, effects of addition of IAA as an auxin in the medium in addition to cytokinins kinetin or BAP on *in vitro* shoot multiplication of mandarin var. Madhu was

investigated by culturing sterilized tender shoots in MS media with 1mg/l IAA and 2 mg/l kinetin or BAP. After three weeks, the shoot tips with a growth initiation were cultured in media of the same composition and the number of shoots formed was recorded after a further culture period of 10 weeks.

RESULTS AND DISCUSSION

Influence of maturity of explants on shoot tip initiation

Percentage of successful initiation by juvenile and older shoot tips of sweet orange var. Bibile, is given in table 1. The success of growth initiation was 100% with juvenile tissues and 40% with older tissues, indicating the importance of the physiological status of donor plants in culture initiation.

Table 1. Percentage of successful initiation of 'Bibile' sweet orange with juvenile and older shoot tips treated with MS+BAP 2 mg/l, at 12 weeks after culture.

Treatment No.	Explant type	Initiation %
1	Juvenile shoot tips	100
2	Older shoot tips	40

In vitro rapid multiplication *via* shoot tip culture of sweet orange, variety Bibile

The treatment, BAP 2.0 mg/l (treatment No. 3), significantly increased the number of *in vitro* shoots formed in sweet orange var. Bibile (table 2). The lowest number of shoots was observed from the treatment No. 4, BAP 4 mg /l. Treatment No. 5 and 6 gave higher levels of shoot multiplication and they were not significantly different. The results clearly indicated that BAP alone (treatment No. 3) is sufficient to increase the rate of multiplication of shoots.

Table 2. *In vitro* shoot multiplication of 'Bibile' sweet orange (mean number of shoots formed in different treatments after 16 weeks in culture).

Treatment No.	Treatments	Mean Number of Shoots
1	MS + BAP 0.5 mg/l	2.22
2	MS + BAP 1.0 mg/l	2.36
3	MS+ BAP 2.0 mg/l	4.11
4	MS + BAP 4.0 mg/l	1.00
5	MS + BAP1.0 mg/l+ GA_3 0.1 mg/l +0.5 IBA	3.35
6	MS + BAP0.5 mg/l+ GA_3 0.1 mg/l	3.11
7	MS + BAP1.0 mg/l+ GA_3 0.1 mg/l +0.1 IAA	1.63

LSD (p=0.05) =1.23

In-vitro rapid multiplication via shoot tip culture of sweet orange, variety Valencia

Media used for *in vitro* shoot initiation and multiplication of sweet orange var. Valencia and, the number of shoots formed in different treatment levels is given in table 3. Shoot initiation was 100% in all media. Of the three hormones tested BAP has responded better than the other two, IAA and GA_3, at tested levels. Increasing BAP levels of 0.5 and 1.0 mg/l did not show any significant increase in the multiplication rate, but BAP 2 mg/l level had significantly increased the rate of multiplication of shoots *in vitro* (table 3).

Table 3. Mean number of shoots formed by of sweet orange 'Valencia' in different Treatment levels after 16 weeks in culture.

Treatment No.	Treatment	Mean Number of Shoots
1	MS + IAA 0.5 mg/l	1.75
2	MS + GA_3 0.5 mg/l	1.1
3	MS + BAP 0.5 mg/l	2.3
4	MS + BAP 1.0 mg/l	2.3
5	MS + BAP 2.0 mg/l	3.1

LSD (p=0.05) =1.113

In-vitro shoot multiplication at 0.5 mg/l BAP level for mandarin variety Madhu

The results showed that the best treatment for shoot proliferation of mandarin var. Madhu was 0.5 mg/l BAP or 2 mg/l BAP, as the average number of shoots formed was 3.77 and 3.55 per shoot, respectively (table 4). There was no significant difference between those two treatments. Sim *et al.* (1989) obtained an average 3.7 *in vitro* shoot production per explant with *Citrus mitis* with a BAP level of 0.9 mg/l in MS medium. The lowest number of shoots was observed for the treatment with 6 mg/l of BAP.

Table 4. Mean number of shoots formed in different BAP levels after a total culture period of 13 weeks.

Treatment No.	Treatment Level (BAP mg/l)	Mean Number of Shoots
1	0.5	3.7778*
3	2.0	3.5556*
2	1.0	2.4444
4	4.0	2.4444
5	6.0	1.0000

LSD (p= 0.05) =1.3313

In the experiment where *in vitro* shoot multiplication rate of mandarin was further investigated by adding IAA as an auxin, the treatment had a significant ($p<0.05$) effect on the number of *in vitro* shoots formed. The number of shoots formed was 3.6 and 1.0 for the treatments of IAA 1 mg/l +

Kinetin 2 mg/l and IAA 1 mg/l + BAP 2 mg/l, respectively. Thus, for mandarin var. Madhu, IAA 1 mg/l + Kinetin 2 mg/l responded better than IAA 1 mg/l + BAP 2 mg/l.

Increasing BAP levels from 1.0 to 6.0 mg/l suppressed the shoot multiplication rate (table 4). Grinblat (1972) reported that BAP promoted *in vitro* bud formation of *Citrus* spp. Altman and Goren (1974) found that sprouting was retarded by IAA but slightly increased by GA_3, while GA_3 increased the internode length.

CONCLUSIONS

Tender shoot tips of sweet orange responded better *in vitro* shoot tip initiation than older tissues. *In vitro* shoot tip initiation, as well as *in vitro* shoot multiplication of sweet orange varieties Bibile and Valencia could be achieved efficiently in MS medium with 2 mg/l BAP. For mandarin variety Madhu, the best response of *in vitro* shoot multiplication was in MS with 0.5 or 2 mg/l BAP.

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