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IN VITRO BUD CULTURE OF KINNOW TREE

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Abstract

Tissues from field grown trees have contamination problems in *In vitro* cultures. Two hundred explants were tried in each sterilizing chemical viz., $HgCl_2$ and NaOCl. Both the chemicals were effective in making clean explants growth as 62 (31%) and 67 (33%) respectively. The dead explants were 25 and 23. The buds sprouted and upon subculturing, shoots were grown in MS medium supplemented with BA (2 mg/l), GA (1 mg/l), proline (5 mg/l). The developed shoots rooted in half strength medium with addition of IBA (2 mg/l). Our objective was to obtain rooted shoots. Although there were callus formation, leaf growth but no true shoot elongation. The rooted shoots were grafted on young rough lemon seedlings. The overall procedure is lengthy but plants can be obtained from *In vitro* bud culture of field grown Kinnow tree.

Introduction

The field grown tree buds are not easy to multiply in culture because they have high rate of contaminations in *In vitro* growth. The harsh tissue sterilization not only damage the growing regions of the buds but also affect their overall growth potential. Objective of this study was to estimate the efficiency of *In vitro* system for multiplication of field grown tree. For clean tissue growth there is culture initiation, subculturing, shoot growth and rooting, acclimatization of rooted shoots by grafting. Such micropropagation process has low probability of somaclonal variations as compared to callusing (Hao & Deng, 2002) or nucellar embryos (Han *et al*., 2002).

In vitro contaminations is one of the most serious problems of commercial tissue culture laboratory, especially of field grown trees and lack of adequate control of contamination levels is the primary reason for failure of commercial production (Niedz & Bausher, 2001). Micropropagation of mature *Citrus* trees has been tried by various workers (Al-Khayri & Abdulaziz, 2002; Baruah, 1996) and in other species like plant regeneration from internodes explants of mature native oil tree (Rahman *et al*., 2004). Contamination and necrosis occurred in shoot apices in *In vitro* conditions of Cherry. Therefore micrografting of shoot apices as alternative method was used (Amiri, 2006). *In vitro Citrus* budwood culture require proper nutritional medium for obtaining shoots (Sas-Sert Kaya & Cinae, 1999), but overall *in vitro* regeneration of mature tree buds is difficult. In *Citrus* most of the studies are on aseptically grown seedling explants (Shailendra *et al*., 2005) and on somatic embryogenesis (Germana, 2003), because multiplication rate is higher by somatic embryogenesis. This paper describes our experience of *In vitro* Kinnow bud culture.

Materials and Methods

The field grown 10 year Kinnow plant was used in this study. Stem buds were taken from around two months old vegetative summer sprouts. The explants stem sections were about 3 cms with axillary bud in the center. Explants were washed with detergent and 200 sections were disinfected with 1% Mercuric chloride for 10 minutes. The other lot of 200 sections were sterilized with 5% NaOCl for 15 minutes. The stem explants were thoroughly washed several times with autoclaved distilled water to remove traces of

sterilizing chemicals. The stem sections were cut from both ends that was expose to sterilizing chemicals leaving l cm stem explants with bud in the center. These explants were placed in the upright position on the surface of MS medium (Murashige & Skoog, 1962). The additions were BA (2 mgs / l) + GA (mg/l) + proline 5 mg/l. Sucrose was 3% and the pH of medium was adjusted as 5.8. It was solidified with 1% agar.

After 45 days of initial culture, the healthy bud cultures were subcultured to fresh medium of the same composition for another 60 days. After first subculture, the growing shoots were separated from the rest of culture vessel growth and were transferred to the same medium for another 60 days and finally to half strength above medium containing of IBA (2 mg/l). All the cultures were incubated at 26 ± 2^{o}C in natural light.

The rooted shoots were removed from the culture vessel, washed with tap water to remove residual agar, dried with tissue paper and side grafted on 1–2 year rootstock stem with the help of an expert budder. After 40 days of shoot graft, the humidity was gradually decreased and polythene cover was finally removed after one month. The graft was kept in shade till hardening of new vegetative growth.

Results and Discussion

Most *Citrus* species are relatively competent to regenerate from *In vitro* culture when *In vitro* grown plantlets are used as explant source or from ovular nucellar masses which has inherent potential of making embryos. A lot of improvement in micropropagation of this woody plant tree has not yet attained the reputation of being model systems. However, the plant regeneration of a single bud is dependent on high potential of bud sprouting. The potential of cell division and bud regeneration in *In vitro* conditions is low in *Citrus* species and cultivars especially in Kinnow mandarin due to browning and poor growth. Bud with differentiating meristem of its own is disturbed with sterilizing chemicals, which had lethal effects on Kinnow buds and 12% of initial cultured explants died and 17% not responded to culture conditions due to $HgCl_2$ and NaOCl exposure (Table 1). Different protocols have been used for cleaning field tree tissues as for *C. junos* 1% NaOCl for 10–15 minutes with 0.01% Tween for 1–30 minutes followed by washing with sterile distilled water, 1 second in 70% ethanol and washing again in distilled water (Oh *et al*, 1991). In cv. Valencia of sweet orange, adventitious bud development was induced in seedling epicotyls segments and the buds originated directly from cambial region on the cut ends of explants (Almeida *et al*., 2006).

Table 1. *In vitro* stem bud culture of Kinnow tree.

Surface sterilization of explant	1% $HgCl_2$ for 10 minutes	5% NaOCl for 15 minutes
Explants cultured	200.0	200.0
Contaminated explants after 45 days	85.0	79.0
Dead Explants	25.0	23.0
Buds not responded	38.0	31.0
Buds started growth	62.0	67.0
Buds produced shoots	35.0	43.0
Shoots rooted in IBA 2 mgs/l	46.0	45.0
Successful graft of rooted shoots	39.0	37.0
Success % of initial cultured explant	19.5	18.5
Callussing with leaf growth upon subculturing	5.0	17.0

Fig. 1. Bud sprouting

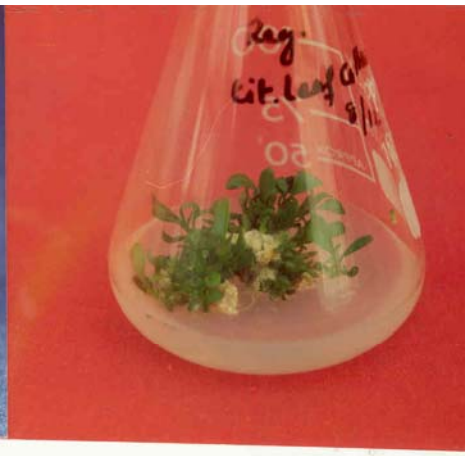

Fig. 2. Callus and shoot in sub-culture

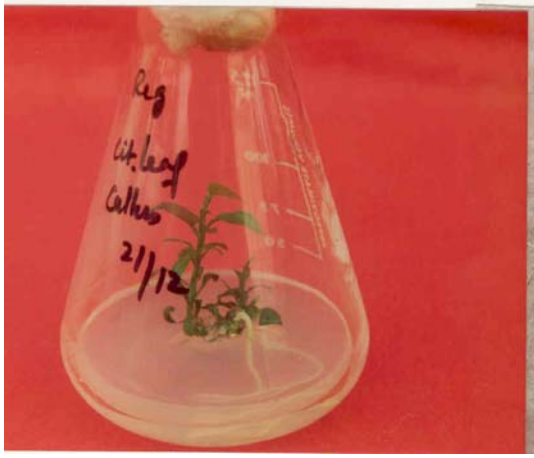

Fig. 3. Shoot and root growth

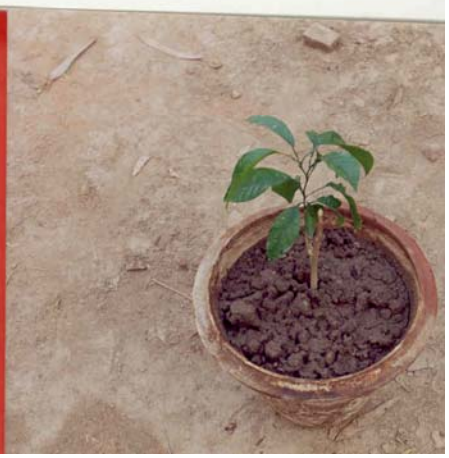

Fig. 4. Grafted shoot

Kinnow buds responded to BA and GA medium. Nodal segments needed longer culture period for shoot regeneration. The subculture period was important. The growth was comparatively faster in the second passage as compared to initial culture. The bud sprouting and shoot development and proliferation were normal and stable (Fig. 1) indicating that hormonal balance present was optimal and addition of GA in the medium was necessary for shoot growth.

In some bud subcultures, there was callusing (Fig. 2) with shoot primordia and leaves. Shoot initiation period was reduced in callusing cultures. However, in most cases, there was no callus production at all and this fact should guarantee the genetic stability and the homogeneity of the plants derived from *In vitro* cultures. Continuous subculturing of shoot propagules from one nodal explant are difficult. The number of shoots increased in the foot half of *in vitro* derived shoots then top half of the shoot.

Rooting was induced by IBA in *In vitro* grown shoots derived from Kinnow stem buds (Fig. 3) some of the unrooted shoots, when transferred to fresh IBA medium were able to produce root. The initial lack of rooting in IBA might be creating an unsuitable hormonal balance which prevented root induction. Grafting of rooted shoots (Fig. 4) was superior to shoots with calluses at the base without rooting. The improved phytosanitary status of *In vitro* plants makes the planting material of choice with reduced risk of diseases in new planting area. The growth regulators GA and IBA were suitable for initiation and growth of shoot tip explants of 11 *Citrus* cultivars and IBA resulted in good rooting of shoots (Omura & Hidaka, 1992). BA was superior to K for shoot proliferation in all *Citrus* species studied (Baruah *et al.,* 1996). Growth medium is crucial for *In vitro* bud culture of *Citrus* (Sas-Sertkaya & Cinar, 1999). The micropropagated plantlets of *Citrus* cultivars manifest consistently superior performance when growth of *In vitro* grown micro shoots derived plantlets were compared with seedlings of *Citrus* cultivars (Singh *et al*., 2003).

Clonal plants can be obtained by stem nodal explants and subculturing of their shoot propagules. The regeneration of shoots appear to be normal and leaves were identical to parent tree. No reversion to juvenile phase was observed. The benefit of successful transfer of plantlets from tissue culture vessels to the ambient conditions by grafting *In vitro* shoots on root stock seedlings is important as disease free material. The multiplication rate that we achieved was not high to be commercially significant, but our results provide a basis for further research in micropropagation of selected Kinnow clones.

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