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MICROPROPAGATION OF KINNOW THROUGH NODAL EXPLANTS

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ABSTRACT

This study was conducted to develop protocol for micropropagation of kinnow from nodal explants. These nodal explants were cultured on MS medium fortified with different concentrations and combinations of BAP and NAA. Best shoot multiplication was obtained on MS medium supplemented with BAP (3 mg/L) and NAA (0.5 mg/L). Transfer of shoots to a rooting medium induced highest percentage of rooting on MS supplemented with NAA (2.0 mg/L).

INTRODUCTION

Kinnow is one of the most important citrus cultivar belonging to family Rutaceae. The first generation hybrid between King Mandarin (*C. nobilis* Lour) × Willow leaf mandarin (*C. deliciosa* Tenora) (Joshi *et al.*, 1997; Sharma *et al.*, 2007) developed by H.B. Frost at California, Regional fruit station USA (Rashid *et al.*, 2005). It was first introduced in India during early 1940's at the fruit experiment station of Punjab Agriculture College and Research Institute Lyallpur by S.Bhadur Lal Singh (Singh *et al.*, 1978). Since then it has assumed great importance among north Indian growers and a large acre is being brought under its cultivation particularly in Punjab, Haryana, Rajasthan and Himachal Pradesh (Khurdiya and Lotha, 1994).

Kinnow is very useful citrus fruit and because of being superior to orange in yield and quality, it has great demand. The rind of kinnow containing numerous oil glands comes off easily with bare hands since it is loosely bound compared to other types of citrus fruit (Mukhtar *et al.,* 2005; Singh *et al.*, 2008). Kinnow provides sufficient vitamin C to fulfill daily need. Its pulp is used to make delicious desserts, jams and sauces and skin can be used to make cosmetics and essence. It have vitamin c, lycopene and flavonoides, which are known to reduces prostate and breast cancer, viral affects and cholesterol level and improve capillary activity. It is rich in fiber, which is imported for production and maintenance of collagen (Sharma *et al.*, 2007 and Altaf, 2006).

The present investigation was undertaken on an important hybrid fruit crop, kinnow mandarin with a view to develop a reliable protocol for its clonal propagation under *in vito* conditions. Conventional method of propagation of kinnow has several problems like time consuming, polyembryony (Cameron and Frost, 1968) and cross pollination etc. The citrus seedlings are perennial and take 5-6 years to grow. It has inherited polyembryony from cultivar King Mandarin. Polyembryony accompanied by high ovule fertility becomes responsible for unwanted apomictic seedy trait (Altaf and Rehman, 2008). Citrus also stands among difficult to root crops and micropropagation offers rapid propagation of such crops in limited space and time under controlled conditions throughout the year (Mohammad *et al.*, 2005). Citrus propagation by conventional means is restricted to particular season and availability of plant material. It doesn't guarantee trueness of cultivars and mass production of certified citrus plants throughout the year.

Therefore, *in vitro* micro propagation constitutes a feasible alternative to overcome these problems. Such process involves bud multiplication from shoot tips, nodes or axillary buds, possesses less probability of somaclonal variation among regenerates in comparison with callus mediated regeneration pathway. Several research work on plant regeneration, callusing, embryogenesis have been achieved in number of citrus species like *C. reticulata* Blanco (Gill *et al.,* 1995), Kinnow (Praveen *et al.*, 2003), (Altaf, 2006), *Citrus jambhiri* (Ali and Mirza, 2006), etc. The aim of this work was to set a protocol for establishment, regeneration, rooting and hardening of kinnow from nodal explants.

MATERIALS AND METHODS

Nodal explants (1-1.5 cm in diameter and 3-4 cm in length) were collected from healthy lateral branches of adult plants

growing at field (Forestry Department of Sam Higginbottom Institute of Agriculture Technical and Sciences). These explants were washed with running tap water for 1h followed by washing with detergent for five minutes. Further washed with tap water and rinsed with distilled water 2-3 times.

Disinfection was conducted in laminar airflow with 20% Sodium hypochlorite (NaOCl) for 5 min., supplemented with two drop of Tween 20 (Sigma) per 100mL followed by 0.1% $HgCl_2$. Finally washed with autoclaved distilled water 3-5 times.

After treatment explants were cut from both ends leaving 1 cm (in length) stem with bud in centre and cultured vertically on Murashige and Skoog media supplemented with 3% w/v Sucrose (Himedia), different concentration of plant growth regulators including BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5mg/L), NAA (0.5mg/L) and solidified with 8% agar (Himedia). The pH of media adjusted to 5.6- 5.8 with 1N NaOH, 1N HCl and autoclaved at 121°C temperature and 15 lbs pressure for 20 minutes.

Inoculated explants were incubated at 24±2°C under white fluorescent light (10.9 μmol m^{-2} s^{-1}) with 18h photo period. Ten explants were used for each treatment. To maintain healthy cultures, these cultures were subculture to fresh medium of same composition every one week. Visual observations were taken every 3 days and effect of different treatments was quantified on the basis of percentage of explants showing responses for shoot regeneration. Data were collected after 3 weeks including shoot were separated from shoot cluster and incubated in test tube containing 20mL. of medium 3% sucrose and solidified with 8% agar, having different concentration of NAA.

These cultures were maintained under identical condition. Observations were noted after 3 weeks including number of roots and root length. After 4 weeks, regenerated plantlets were carefully removed from test tube, residual agar at root region was washed with Bavestein and transferred to culture bottles having mixture of perlites, solarite and vermiculite (3:1:1) and again cultures maintained under identical conditions for 2-3 weeks. After this, these complete plantlets were planted in pots with potting mixture (1soil: 1peat: 1moss). Then data were collected after 5 week.

RESULTS

Shoot multiplication

Nodal explants were inoculated on MS media supplemented with different concentrations of BAP and NAA for optimization of the best phytohormonal combination and concentrations for shoot multiplication (Table 1). The results revealed that the MS media containing BAP (3mg/L) + NAA (0.5mg/L) was found to have the maximum (Fig. a and b) and MS media containing BAP (4.5mg/L) + NAA (0.5mg/L) the minimum shoot multiplication based on the percent response. Shoot multiplication increases when the concentration of BAP increased from 1.5- 3.0mg/L with 0.2mg/L NAA. A further increase in BAP concentration reduces the shoot multiplication.

Rooting

The proliferated shoots were individually separated and transferred to rooting media consisting of MS basal medium supplemented with different concentrations of NAA (Table 1.2). Among the various concentration tested, NAA (2.0 mg/L) showed the maximum percentage of root formation (Fig. C).

These regenerate plantlets (Fig. d) were transferred to culture bottles having mixture of perlites, solarite and vermiculite (3:1:1).

DISCUSSION

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. Tissue culture and micropropagation protocols have been described for a number of citrus species and explants sources (Grinblat, 1972, Chaturvedi and Mitra, 1974).

The MS medium supplemented with 3 mg/L BAP and 0.5 mg/L NAA proved to be best medium for shoot multiplication. Table 1 showed that multiplication increases with increase in BAP concentration up to 3mg/l but further increase in BAP concentration reduces the shoot multiplication. The data revealed that high concentration of cytokinin and low concentration of auxin is required for shoot multiplication. Similarly, shoot regeneration was also observed using internodal explants cultured on MS medium fortified with 2.5 mg/L BAP and 0.2 mg/L NAA (Praveen *et al.,* 2003) and the formation of shoots and shoot buds in sweet orange took place on MS medium containing NAA at 0.1mg/L and BAP at 0.25 mg/L using internodal as explants (Thirumalai and Thamburaj, 1996). On the contrary, Shoot regeneration was the highest in stem segment explants on MS medium supplemented with 3 mg/L BAP (Ali and Mirza, 2006).

For rooting in regenerated shoots different concentrations of NAA were tested. Best rooting were observed on MS medium supplemented with 2 mg/L NAA Table 2. Similarly, the regenerated shoots (2-3 cm) were rooted in MS medium supplemented with either only NAA (0.75 mg/L) or NAA (0.50

Table 1: Effect of different growth regulators supplemented with MS medium on shoot multiplication. (data represent average of three experiments with three replicates in each)

S. No.	BAP (mg/L)	NAA (mg/L)	Percent response
1	0.5	0.5	10
2	1.0	0.5	16
3	1.5	0.5	25
4	2.0	0.5	39
5	2.5	0.5	56
6	3.0	0.5	60
7	3.5	0.5	49
8	4.0	0.5	22
9	4.5	0.5	12

Table 2: Effect of Different concentration of NAA in MS medium on rooting (data represent average of three experiments with three replicates in each)

S. No.	NAA	Rooting (%)
1	1.0	25
2	1.5	40
3	2.0	55
4	2.5	30
5	3.0	23

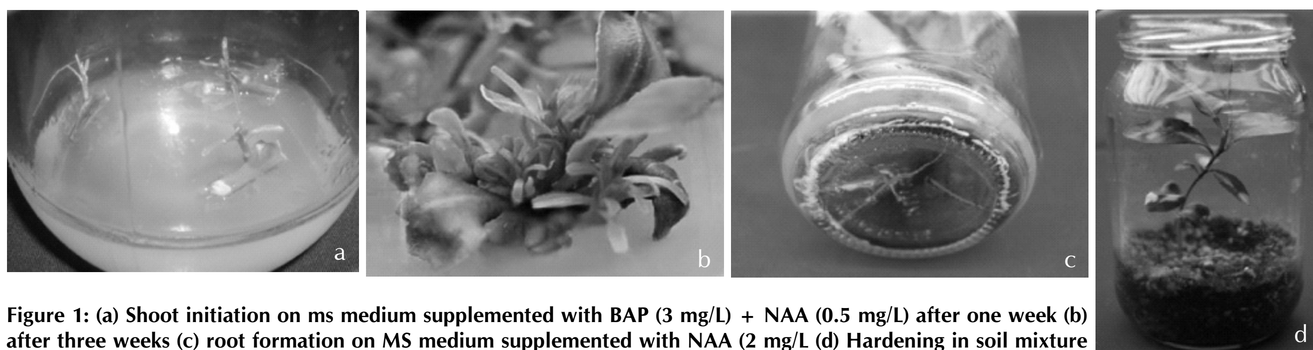

Figure 1: (a) Shoot initiation on ms medium supplemented with BAP (3 mg/L) + NAA (0.5 mg/L) after one week (b) after three weeks (c) root formation on MS medium supplemented with NAA (2 mg/L (d) Hardening in soil mixture

mg/L) and IBA (2.0 mg/L) in sweet orange (Das *et al.,* 2000) and roots were initiated in *in vitro* derived shoots on 3/4 strength MS medium supplemented with NAA at 3 mg/L in sweet orange (Thirumalai *et al.,* 1996). On the contrary, rooting was promoted by a supplement of 1 mg GA_3 (Beloualy *et al.,* 1991).

This report provides a simple protocol for *in vitro* micro propagation of kinnow by using nodal explants of field grown plant. These plantlets were transferred to soil mixture containing perlites, solarite and vermiculite (3:1:1). The rooted plantlets of *Stevia rebaudiana* were hardened in 1:1:1 ratio of sand: soil: vermicompost and were successfully established in soil (Sairkar *et al.*, 2009).

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