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Priyanka Singh
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

BK Singh
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

SP Singh
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Minakshi Padhi
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Priyanka Singh
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Micropropagation of Kinnow mandarin using nodal segments as explants

Priyanka Singh, BK Singh, SP Singh and Minakshi Padhi

Abstract
In the present investigation nodal segments were used as explant for *in vitro* shoot regeneration in Kinnow. The Murashige and Skoog (1962) basal medium was fortified with BAP and NAA to examine the different shoot regeneration parameters. BAP (2.9 mg/L) and NAA (0.06 mg/L) when added together to the medium produced the maximum sprouting percent, number of shoots, shoot length, and number of leaves. The *in vitro* regenerated shoots were transferred in to half strength WPM medium enriched with IBA and NAA for initiation of root.

Keywords: kinnow, nodal segments, *in vitro*, cytokinin

Introduction
Kinnow is one of the most important citrus cultivars belonging to the family Rutaceae. It is a hybrid from the cross of *Citrus nobilis* and *Citrus deliciosa* and is widely grown in North India. It has proved to be quite promising in place of mandarins because of its wide adaptability to variable agro-climatic conditions.
It is commonly propagated by seeds and budding, which restrict the production of true to type plants all the year the round. The infection by viruses and related pathogens in association with rootstock –scion incompatibility have further posed impedimentation in production of quality planting materials and successful cultivation of Kinnow. These problems can effectively be addressed by adoption of plant tissue culture. A number of works have been published on the micropropagation and tissue culture of citrus cultivars and rootstocks (Al Bahrany 2002; Carimi and De Pasquale 2003; Da Silva *et al*. 2008; Pérez-Tornero *et al*. 2010) [13, 10, 14, 15].
Considering the above facts the present study was conducted to develop micro propagation protocol of Kinnow mandarin using nodal segments.

Materials and Methods
The work entitled Micropropagation of Kinnow mandarin using nodal segments as explants was carried out in the Plant Tissue Culture Laboratory, Department of Horticulture, Institute of Agricultural Sciences, BHU, Varanasi. Nodal segments of 1-2cm in length containing at least one axillary bud were taken from Kinnow tree in the experimental orchard.
Nodal segments was cleaned in running tap water for 15 to 20 minutes. Then a few drops of liquid detergent (Tween 20) were added in beaker containing water. It was then kept on rotator shaker for 7 minutes. Thereafter it was again rinsed under running tap water for 20 minutes. Explants were immersed in fungicide solution- SAAF® (carbendazim) (100 mg/100 ml water) +bactericide solution- Streptocycline (20-40 mg/100 ml water) for 20 minutes in rotator shaker and then washed with double distilled water 4-5 times.
The explants were then transferred into Laminar Air Flow Chamber and were then surface sterilized with 0.1 % mercuric chloride for different durations. Basal MS medium was fortified with different plant growth regulators (BAP and NAA) for culture initiation. The pH of the medium was adjusted to 5.8 with 0.1 NaOH or HCl before autoclaving. The culture medium was autoclaved at 121ºC at 15 psi for 20 min. After autoclaving growth medium was allowed to cool at room temperature before use.
Upon inoculation of explants culture tubes were maintained under the photoperiod of 16/8 hours light and dark cycle at 25±2°C and 2-3 weeks interval subculturing was performed. *In vitro* regenerated shoots were transferred to half strength WPM medium supplemented with IBA and NAA in combination for root initiation. The experimental data was analysed following completely randomized design (CRD) with four replications

Results and Discussion
Cytokinins are very important during tissue culture of plants as they induce division and organogenesis (Howell *et al*., 2003) [1].

The success of a culture is influenced by the type and concentration of applied cytokinins, as their uptake, transport, and metabolism differ between varieties and they can interact with endogenous cytokinins of an explant (Werbrouck *et al*., 1996; Strnad *et al.* 1997; Van Staden *et al*., 2008) [2, 3, 4].
Similarly, the superiority of BA over other cytokinins on multiple shoot bud differentiation has also been established in a number of cases (Jeong *et al*., 2001; Loc *et al.*, 2005, Phulwaria *et al*., 2012) [5, 6, 7]. The addition of auxin to the medium has been found to be useful for shoot production in some cases (Zhang *et al.*, 2000) [9].
Nodal segments of Kinnow showed maximum sprouted shoots (76.23%), shoot length (2.65 cm) and number of leaves (5.00) when cultured in medium containing BAP (2.9 mg/L) and NAA (0.06 mg/L) in combination. Increase in concentration of BAP further had inhibitory effect on sprouting of nodal segments and the induction of NAA in the medium assisted in nullifying the effect of higher cytokinin concentration. BA, at various concentrations, has been the most commonly used plant growth regulator for the citrus shoot proliferation (Carimi and De Pasquale 2003) [10].
The MS medium enriched with 2.9 mg/L BAP and 0.06 mg/L NAA were judged to be best medium for shoot multiplication and recorded maximum (3.25) number of shoots. The cytokinin concentration (BAP) augmented the number of shoots up to a certain level and then decreased with further increase in cytokinin concentration (Table 1). These results are in agreement with Murkute *et al*. (2008) [9] and Kaur *et al*. (2015) [12]. Likewise a promotive effect of BAP on shoot regeneration at low concentration and toxic effect at higher concentration have also been reported for different citrus genotypes (Costa *et al*., 2004) [11].

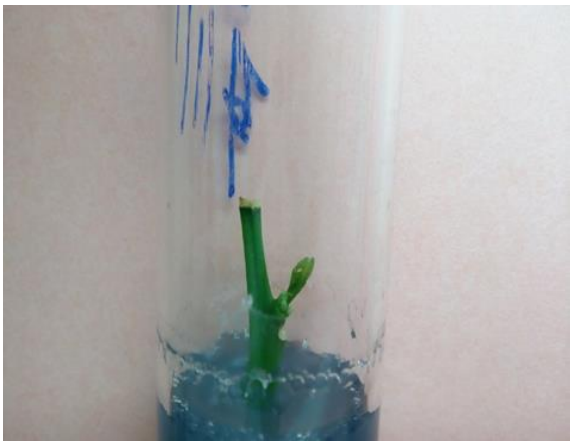
Table 1: Effect of cytokinin and auxin on different shoot regeneration parameters of Kinnow

Concentration of growth regulators mg/L (BAP +NAA)	Sprouted shoot %	Number of shoots	Shoot length (cm)	Number of leaves
T1(0.0+0.0)	0.0	0.0	0.0	0.0
T2(0.4+.001)	44.70	1.00	1.00	2.50
T3(0.9+0.02)	53.36	1.50	1.18	2.75
T4(1.4+0.03)	58.39	1.75	1.85	3.00
T5 (1.9+0.04)	65.75	2.00	2.28	3.50
T6 (2.4+0.05)	70.63	2.75	2.43	4.25
T7 (2.9+0.06)	76.23	3.25	2.65	5.00
T8(3.4+0.07)	67.62	2.50	2.30	3.75
T9(3.9+0.08)	49.37	1.00	1.73	2.25
T10(4.4+0.09)	37.28	0.75	1.20	1.75
S.Em (±)	0.556	0.204	0.142	0.285
C.D.	1.615	0.592	0.413	0.827

After 4 to 5 weeks, the *in vitro* regenerated shoots were transferred into root induction medium comprising half strength of WPM basal medium with IBA and NAA. Similar findings have also been reported by Kanwar *et al.* (2016) [16] and Kaur *et al*. (2015) [12].

a. Bud break in nodal segment at 8 days after inoculation in MS media fortified with BAP and NAA

b. Shoot initiation



c. Shoot elongation

d. Leaves proliferation

Fig 1

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