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Short Communication

In vitro propagation of *Citrus reticulata* Blanco (Nagpur mandarin)

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In Central India Nagpur mandarin is the most important commercially grown *Citrus* cultivar, which accounts for 41% of total citrus production in the country [1]. Nagpur mandarin occupies a position of dominance due to its suitability for eating as fruits or for serving as dessert fruits, excellent fresh juice with pleasant flavor and preparation of various processed food products, confectioneries and cosmetics. However trees were noticed to be declining on a large scale due to diseases like Citrus Triesteza Virus, Greening disease and other unknown factors, from 1957 [2]. Later on, it was identified to be citrus die back [3]. For a viable and productive citrus industry, disease free foundation stock is necessary and there is an urgent need to develop disease free planting material of Nagpur mandarin for future multiplication to prevent losses incurred in absence of disease free planting stock.

Healthy and disease free Nagpur mandarin orchards were selected from Amravati region as the source of fruit in the year 2000-2001. Mature and bold seeds were collected from ripened fruits and washed thoroughly under tap water. Seeds were immersed in 70% ethanol for 5 min. and washed with distilled water. Both the seed coats were removed aseptically in the laminar flow hood with minimum damage to the cotyledons and immersed in 1 % freshly prepared NaOCl solution for 5 min. followed, by 4 washes with sterile double distilled water. One seed per tube was cultured aseptically on Murashige and Skoog's MS basal medium [4] enriched with different combinations of 6-Benzyl Amino Purine (2.22μM - 8.88μM), Kinetin (2.32μM - 4.65μM) and a-Naphthol acetic acid (1.34μM - 5.37μM), Sucrose 3 % and solidified using 0.25% Phytagel (Sigma).

There were three replications for each treatment with 10 tubes in each replicate. Experiments were repeated three times and data obtained was analyzed statistically [5]. Approximately 30 days after initiation of multiple shoot formation, the shoots were individually subcultured in the rooting medium comprising MS medium + BAP (1.11μM - 2.22μM) + IBA (2.46μM - 9.84μM).

All the cultures were maintained at 25 ± 2°C with relative humidity of 60% and illumination from cool white fluorescent tubes (45μ mol m^{-2} S^{-1} 16/8 hr). Observations pertaining to average number of shoots per explant and number of roots obtained per shoot were recorded at regular intervals and stored in terms of photographs and observation tables.

The study demonstrates use of different hormones alone or in combination for multiple shoot formation from nucellar embryos. MS medium fortified with BAP (8.88μM), NAA (2.69μM) and Kin(2.32μM) induced maximum shoot induction (80%) when compared to other hormonal combinations and a maximum number of multiple shoots (16.8 ± 0.96) per explant were also regenerated in the same combination. When the cytokinin / auxin ratio was varied it exhibited frequent drop in induction of shoot formation. However Edriss and Burger [6] reported increase in BAP concentration increased number of shoots formed from root segment. Where as Gill [7] noted maximum shoot regeneration in MS medium supplemented with BAP (1mg/1) and GA3 (2mg/1).

Multiple shoots thus obtained were individually rooted in vitro in MS medium fortified with BAP and IBA. It was noted that MS medium without auxins showed very poor or no rooting. However IBA alone induced upto 50% rooting. But the critical combination of IBA (4.92μM) + BAP (1.11μM) induced maximum

Table 1. Effect of various hormone concentrations on shoot induction and number of shoots regenerated per explant in *Citrus reticulata*.

MS medium + (μM)	% Shoot induction	Avg. number of shoots/explant
No hormones	00	00
BAP 4.44	25.99	2.33±0 65
BAP 4.44 + NAA 2.69	32.18	6.53±0.95
BAP 8.88 + NAA 2.69	67.36	11.6±0.48
BAP 8.88 + NAA 2.69 + Ki 2.32	80.46	16.8±0.96
BAP 8.88 + Kin 2.32	53.82	9.11±0.26
BAP 8.88 + NAA 5.37 + Ki 2.32	40.33	7.35±0.33

Table 2. Effect of various media on rooting of *in vitro* regenerated *Citrus reticulata* shoots

MS + (μM)	% root formation	Avg. no. of root/shoot
No hormones	04.35	1.0±0.33
IBA 2.46	35.12	2.2±0.33
IBA 4.92	50.25	2.9±0.65
IBA 2.46 + BAP 1.11	51.08	2.9±0.95
1BA 4.92 + BAP 1.11	78.44	5.8±0.86
IBA 4.92 + BAP 2.22	61.50	4.0±0.32
IBA 9.84 + BAP 2.22	55.28	3.6±0.11

Note: In both the tables ± denotes values of standard error

78% rooting with an average of 5.8 ± 0.86 roots / shoot. Vijayakumari [8] reported plantlet formation in MS medium enriched with Kin (0.1 mg/1) and Kin + IAA (2mg/l each). The plants with well-developed roots were transferred to earthen pots containing sterilized sand: soil (2:1) mixture. Pots were kept at 25 ± 2°C and 80% humidity for 10-12 days and then transferred to green house. These plants may also be used for micrografting or *in vivo* grafting on a healthy and disease free citrus rootstock to produce true to type clones, which is an urgent need for improvement and maintenance of Nagpur mandarin.

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