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In-vitro propagation of virus tolerant rootstock Carrizo citrange

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ABSTRACT

Studies on *in-vitro* propagation of virus tolerant rootstock Carrizo citrange were carried out using nodal segments of nucellar seedlings. Murashige and Skoog (1962) medium supplemented with BAP, kinetin for shoot proliferation and NAA and IBA for rooting were used in different concentration in alone or in combinations. Amongst for rooting BAP and kinetin, 1.0 mgl^{-1} BAP was found better. BAP was found better cytokinin in respect to reducing time needed to bud break and longer shoot length as compared to kinetin. Kinetin was superior cytokinin than BAP in respect to per cent survival and shoot length. In case of BAP with kinetin combinations maximum number of shoot (9.30) was ontained on BAP 2.0 mgl^{-1} + kinetin 0.5 mgl^{-1} and shoot length (2.50 cm) on BAP 0.5 mgl^{-1} + kinetin 1.0 mgl^{-1}. NAA was found as better auxin in comparison to IBA for rooting of microshoots. Interaction of NAA with IBA, the minimum time taken to root induction (17.40 days) was recorded on NAA 0.1 mgl^{-1} + IBA 0.5 mgl^{-1}, maximum number of roots (9.60) on NAA + IBA (0.5 mgl^{-1} each) and root length (7.10 cm) on NAA + IBA (1.0 mgl^{-1} each) were recorded. The rooted plantlets were successfully acclimatized in greenhouse, in pot containing soil, perlite and vermiculite in equal proportions. About 83.00 per cent success was recorded after 60 days of transfer from culture room.

Key words: Carrizo citrange, *in-vitro* propagation, nucellar seedlings.

INTRODUCTION

The importance of rootstock in fruit cultivation is well known. It influence the performance of a commercial cultivar in many ways such as increasing the adaptability of a commercial cultivar to a wide range of soil, climatic conditions and resistant to pests and diseases, *i.e.* using virus tolerant rootstock (sour orange, Cleopatra mandarin etc.), nematode tolerant (trifoliate orange) and salt tolerant (Rangpur lime, rough lemon etc.) (Agarwal, 1). There is no single rootstock which fulfill all the criteria but Carrizo citrange (*Citrus sinensis* Osbeck × *Poncirus trifoliata* (L.) is virus tolerant, dwarf, suitable for sandy soil and nematode resistant (O'Bannon and Hutchism, 9). However in India, most of the commercial citrus cultivars are grown on rough lemon which is susceptible to phytopthora disease, gummosis and nematode. Thus, the prime need is to use of tolerant rootstock to save citrus industry. The seed of Carrizo citrange is polyembryonic which produces nucellar seedlings. Nucellar seedlings are genetically uniform to mother plant and free from most of the viruses (Rangan, 12). Thus, nuceller embryos offer excellent means for production of desirable true-to-the-type planting material for commercial purpose. As a step forward towards this direction, the present study was undertaken to develop a protocol for *in-vitro* propagation of Carrizo citrange rootstock.

MATERIALS AND METHODS

In present study, nodal segment of nucellar seedlings were used as explant because they are true-to-type and uniform in growth. For this, healthy fruits of Carrizo citrange were collected from Citrus Repository of the Agricultural Research Station, Sriganganagar. They were washed in running tap water for 2-3 h. The seeds were extracted, washed thoroughly and treated with 0.2 per cent (w/v) carbendazim. The seed testa was removed under aseptic conditions. The recoated seeds were first quick rinsed with 70 per cent ethanol (10 sec) followed by 0.1 per cent (w/v) mercuric chloride ($HgCl_2$) for five minutes and three rinsings with sterile distilled water and inoculated in culture tubes (25 mm x 150 mm) containing 15-20 ml MS basal medium supplemented with 3 per cent agar and 0.8 per cent sucrose.

All the cultures were incubated in BOD at 25 ± 2°C in dark. After 15-20 days of incubation, the nucellar seedlings were isolated and sub-cultured on fresh medium. Then 20-30 days of sub-culture, the seedlings were cut into segments, having at least two buds and then inoculated on MS medium supplemented with 0.0, 0.5, 1.0 and 2.0 mg l^{-1} BAP alone or in combinations with kinetin for axillary shoot proliferation. All the cultures were sub-cultured

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onto fresh medium after 25-30 days. The *in-vitro* generated shoots were used as micro-cuttings. The micro-shoots about 2-3 cm were incubated in culture tubes containing MS medium supplemented with 0.0, 0.1, 0.5,1.0 and 2.0 mg l^{-1} IBA alone or in combination with NAA. The rooted plantlets were removed carefully from the culture tubes and their roots were thoroughly washed under running tap water and cleaned with fine brush to remove adhering agar. The plantlets were covered with sterilized cotton wetted with half-strength MS medium for 24 h in culture room. The plantlets were treated with 0.1 per cent carbendazim for 10 min. to prevent fungal contamination. The plantlets were then transferred to pots containing sterilized soil, vermiculite, and perlite in equal proportion. The pots were kept in greenhouse at 90 per cent humidity with temperature 26 ± 2°C. The humidity was gradually lowered within 8-10 weeks upto 60 per cent. During this period, the plantlets were irrigated with Hoagland's solution at three day interval for one month. Thereafter, these were irrigated with Hoagland's solution and simple water at an interval of 2-3 days alternately.

The observations were recorded for per cent survival of explant, time required to bud break, number of shoots per culture, length of shoot, per cent micro shoot responding to root, time required for root induction, number of roots per plantlet, root length and number of plantlets survival after 60 days of planting in pots and incubated in greenhouse. The data was analysed following randomized block design.

RESULTS AND DISCUSSION

Addition of kinetin alone in MS basal medium gave maximum survival of explant (80%) at 1.0 or 2.0 mgl^{-1}. Among BAP levels, the maximum (80%) survival of explant was recorded at 1.0 mgl^{-1}. Kour *et al.* (7) has reported 100 per cent survival of explant in rough lemon on BAP 1.0 mgl^{-1} but with addition of malt extract (500 mg l^{-1}). The maximum explant survival (90%) was recorded on BAP 0.5 mgl^{-1} + kinetin 0.5 mgl^{-1} and BAP 0.5 mgl^{-1} + kinetin 1.0 mg l^{-1}. Similar findings were reported earlier by Parthasarathy and Nagaraju (10) for some citrus genotypes.

When individual levels of BAP (0.5 mgl^{-1}) and kinetin (1.0 mgl^{-1}) were added in MS basal medium the minimum time required to bud break were recorded (19.00 and 19.70 days, respectively). The higher dose of both the cytokinins had negative effect on regeneration. The BAP induced early bud break in comparison to kinetin. The combination of minimum (18.80 days) time for bud break was recorded on BAP 0.5 mgl^{-1} + kinetin 0.5 mgl^{-1}. More or less similar result has been reported by Singh *et al.* (14). They reported that the minimum number of days required to bud beak was directly dependent on *Citrus* species and combination of medium. It was also reported that the minimum days to bud beak in *Citrus reticulata* and *C. limon* was 17 and 18 days respectively, when explants of both the species were cultured on MS medium supplemented with BAP 1.0 mgl^{-1} + kinetin 0.5 mgl^{-1} + NAA 0.5 mgl^{-1}.

Among individual levels of BAP and kinetin, maximum number of shoots, *i.e.* 6.30 and 5.0 per explant were recorded with 1.0 mgl^{-1} BAP and 1.0 mg l^{-1} kinetin, respectively, while higher concentration has reverse effect on it (Table 1). In present study, BAP was found to be superior for shoot multiplication than kinetin. Baruah *et al.* (4) also observed that the BAP increased the number of shoots and also accelerate the shoot multiplication. They also reported that the lack of BAP in medium mostly produced single shoot but addition of 0.25 mg l^{-1} BAP increased shoot multiplication significantly. Similar findings have been reported by Al-Khayri and Al-Bahrany (3) and they recorded 7.0 shoots per explant, when MS medium was added with BAP 1.0 mg l^{-1}. The rate of shoot multiplication on MS medium supplemented with kinetin, the maximum number of shoots (4.60) were recorded with 2.0 mg l^{-1} BAP. Parthasarathy and Nagaraju (11) reported that when MS medium was modified with different levels of kinetin, the maximum shoots (4.60) were observed at 2.0 mg l^{-1}. In case of combination of BAP and kinetin, maximum number of shoots (9.30) was recorded at BAP 2.0 mgl^{-1} + kinetin 0.5 mgl^{-1} (Table 1; Fig. 1). This may be due to better synergistic effect of these cytokinins. The results of present studies are in line of the findings as reported by Karwa (5) who reported that the maximum number of shoots (9.11 ± 0.26) were observed when explants were inoculated on MS medium supplemented with BAP (8.88 µM) + kinetin (2.32 µM) in Nagpur mandarin. Similar findings have also been reported by Al-Bahrany (2), and Al-Khayri and Al-Bahrany (3) in lime.

Maximum shoot length (1.60 cm) was recorded on 1.0 mgl^{-1}. The higher dose of BAP had adverse effect (Table 1). This may be due to the inhibitory effect. The findings of present study are similar to the results reported by Kour *et al.* (7) in rough lemon. In case of kinetin levels, the maximum shoot length (2.20 cm) was observed at 1.0 mgl^{-1}. Kinetin was found to be better cytokinin for enhancing the shoot length as compared to BAP (Table 1). The maximum shoot length (2.50 cm) was observed in MS medium supplemented with BAP 0.5 mgl^{-1} + kinetin 1.0 mgl^{-1} (Table 1; Fig. 1). This may be due to better synergistic effect of the two cytokinins. The results of present study are similar to the findings of Al-Khayri and Al-Bahrany (3). The results of present study are in line with those of Karwa (5) in Nagpur mandarin.

Table 1. Effect of BAP & kinetin, added singly and in combination in basal medium, on different regeneration parameters of Carrizo citrange.

Treatment (mg l^{-1})		Explant survival (%)	No. of days taken to bud break	No. of shoots/ explant	Shoot length (cm)
BAP	Kinetin				
0.0	0.0	50 (45.00)	21.20	1.20	1.50
0.0	0.5	70 (56.79)	21.00	4.20	1.90
0.0	1.0	80 (63.43)	19.70	5.00	2.20
0.0	2.0	80 (63.43)	20.40	4.30	1.60
0.5	0.0	70 (56.79)	19.00	4.20	1.40
1.0	0.0	80 (63.43)	19.10	6.30	1.60
2.0	0.0	60 (50.77)	20.70	3.40	0.90
0.5	0.5	90 (71.57)	18.80	7.00	1.90
0.5	1.0	90 (71.57)	19.80	8.00	2.50
0.5	2.0	80 (63.43)	19.80	5.50	2.30
1.0	0.5	70 (56.79)	20.50	8.10	1.65
1.0	1.0	80 (63.43)	20.40	7.20	1.80
1.0	2.0	80 (63.43)	21.70	3.50	1.40
2.0	0.5	60 (50.77)	20.80	6.30	1.30
2.0	1.0	60 (50.77)	21.20	6.40	1.50
2.0	2.0	50 (45.00)	22.80	3.10	1.20
CD at 5%		0.75	0.86	0.49	0.27

*Figures given in parentheses are angular transformed values

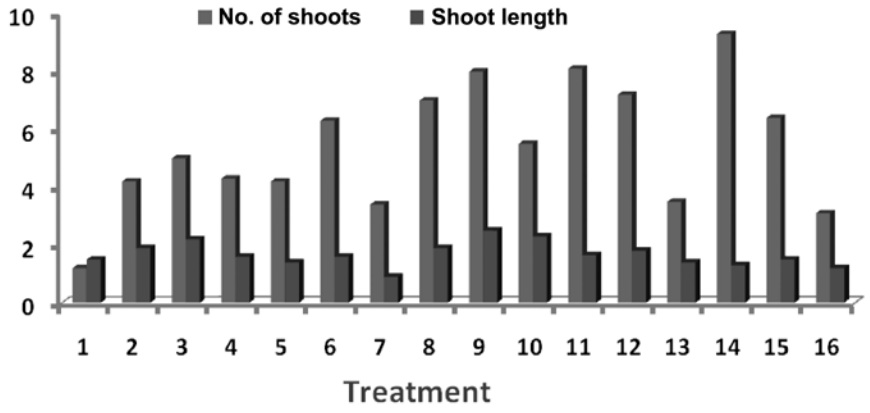

Fig. 1. Effect of cytokinin (BAP and kinetin) levels added singly and in combination in MS basal medium on No. of shoots and shoot length (cm) in Carrizo citrange.

The maximum (90%) microshoot rooting was observed at 0.5 mg l^{-1} either IBA or NAA and further increasing concentration further had negative effect. These findings are in accordance with the results reported by Kitto and Young (6) in Carrizo citrange. In case of combination of 1.0 mg l^{-1} IBA and 0.1 mg l^{-1} NAA, the maximum rooting of micro-shoot (90%) was recorded. It may be due to synergistic effect between the auxins. The results of present study are in close conformity with the findings of Singh *et al.* (14) in lemon.

The minimum time required to root induction was recorded (17.40 days) was observed with NAA 0.1 mg l^{-1}+ IBA 0.5 mg l^{-1}. The results of present study are

in agreement with the findings as reported by Singh *et al.* (14) in lemon. The maximum number of roots (6.30) was recorded with NAA (0.5 mg l^{-1}) compared IBA levels. It is also revealed from Table 2 that auxin NAA exhibited better results in rooting than IBA. The findings of the study are in concurrence with Kitto and Young (6) in Carrizo citrange with 10.2 roots / microshoot in 1.0 mgl^{-1} NAA. The maximum number of roots (9.60) was recorded with NAA + IBA (each 0.5 mg l^{-1}) (Table 2; Fig. 2). This is may be due to better synergistic effect between both the auxins. The similar finding was earlier reported by Singh *et al.* (13). Kour *et al*. (7) observed 2.47 roots per micro-shoot when cultured on MS medium modified by BAP (1.5 mg l^{-1}) + malt extract (500 mg l^{-1}) + NAA (0.25 mg l^{-1})

With IBA, the maximum root length (4.50 cm) was recorded with 0.5 mgl^{-1}. Combination of both auxins gave maximum root length (7.10 cm) with 1.0 mg l^{-1} NAA + 1.0 mg l^{-1} IBA (Table 2; Fig. 2). This may be due to better synergetic effect of both the auxins. The results of present study are in close conformity with Syamal *et al.* (15) in Kagzi lime, and Singh *et al.* (14) in lemon.

In-vitro propagated plantlets were successfully acclimatized by transferring them in small plastic pots containing a mixture of soil: perlite: vermiculite in equal proportion. About 90 per cent survival of plantlets was observed. It may be due to higher number of roots, high porosity, cation exchange capacity (CEC) and water holding capacity of potting medium. According to Baruah *et al.* (3) the survival per cent of *in-vitro*

Table 2. Effect of IBA and NAA added singly and in combination on rooting of Carrizo citrange microshoots.

Treatments (mg l^{-1})		Rooting (%)	No. of roots/ shoot	Root length (cm)
NAA	IBA			
0.0	0.0	00 (0.00)	0.00	0.00
0.1	0.0	70 (56.79)	1.70	2.70
0.5	0.0	90 (71.57)	6.30	3.20
1.0	0.0	80 (63.43)	3.40	3.80
2.0	0.0	80 (63.43)	2.30	1.80
0.0	0.1	70 (56.79)	1.50	1.00
0.0	0.5	90 (71.57)	2.20	4.50
0.0	1.0	90 (71.57)	2.60	3.60
0.0	2.0	70 (56.79)	2.00	3.20
0.1	0.1	80 (63.43)	2.30	3.00
0.5	0.1	80 (63.43)	4.10	4.10
1.0	0.1	90 (71.57)	2.20	5.20
2.0	0.1	80 (63.43)	2.60	4.60
0.1	0.5	70 (56.79)	8.20	4.40
0.5	0.5	80 (63.43)	9.60	4.80
1.0	0.5	80 (63.43)	4.60	6.20
2.0	0.5	70 (56.79)	2.30	6.30
0.1	1.0	80 (63.43)	2.10	4.90
0.5	1.0	80 (63.43)	5.80	6.70
1.0	1.0	60 (50.77)	6.00	7.10
2.0	1.0	60 (50.77)	2.20	4.00
0.1	2.0	60 (50.77)	2.00	2.40
0.5	2.0	60 (50.77)	3.70	2.10
1.0	2.0	60 (50.77)	6.80	1.80
2.0	2.0	50 (45.00)	2.40	1.60

*Figures given in parentheses are angular transformed values

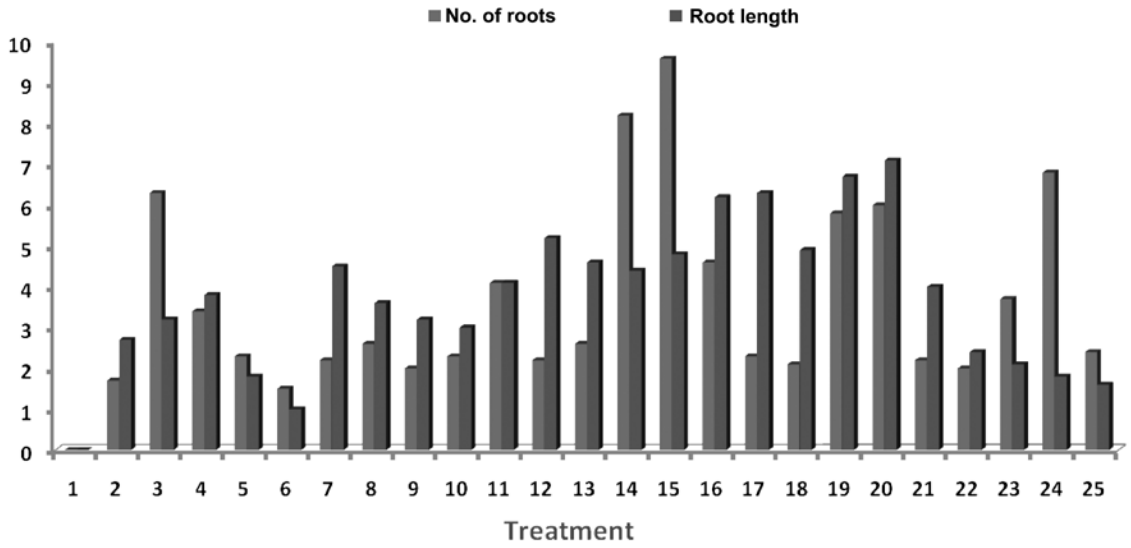

Fig. 2. Effect of auxin levels (NAA and IBA) added singly and in combination in MS basal medium on No. of roots and root length (cm) in Carrizo citrange.

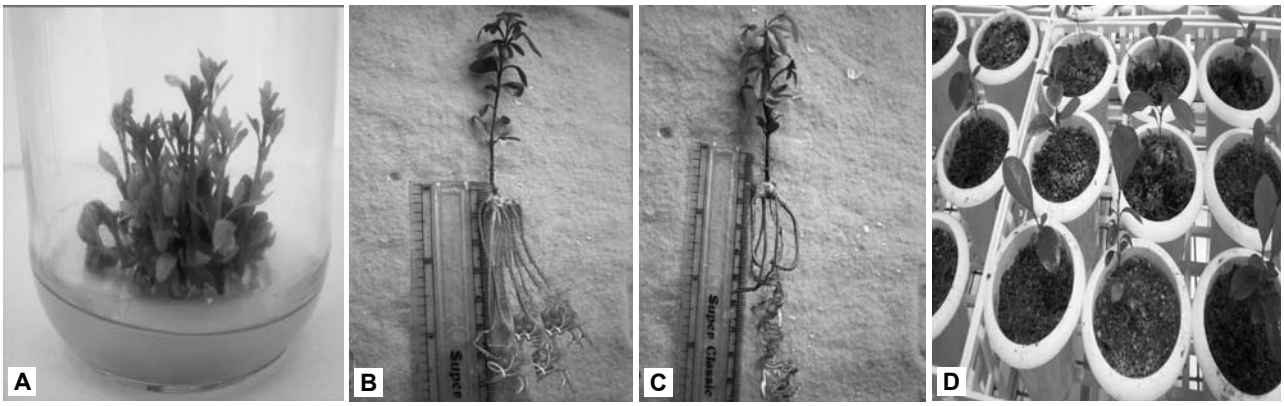

Fig. 3. Micropropagation stages in Carrizo citrange rootstock. (a) Shoot proliferation MS 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} kinetin, (b) Rooting on MS + 0.5 mgl^{-1} IBA, (c) Rooting on 1.0 mgl^{-1} NAA + 1.0 mgl^{-1} IBA, (d) Plantlets in acclimatization.

raised plantlets is directly related to number of roots. The results of the present study are in line with the findings reported by Singh *et al.* (14) in *Khasi* mandarin, Singh *et al.* (13) in rough lemon, and Kumar *et al.* (8) in citrus.

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