*Indian Journal of Agricultural Sciences* **86** (3): 297–302, March 2016/Article
https://doi.org/10.56093/ijas.v86i3.56841

# Micro propagation technique in Kinnow mandarin (*Citrus reticulata*)

RAJ KUMAR[1], M K KAUL[2], S N SAXENA[3], P KUMAR[4] and A K SINGH[5]

*Agricultural Research Station, S K Rajasthan Agricultural University, Sriganganagar, Rajasthan*

Received: 26 February 2015 ; Accepted: 28 October 2015

ABSTRACT

Study on standardization of micro propagation technique in Kinnow mandarin was carried out using nodal segments of nucelles seedling as explants. The MS medium supplemented with BAP, NAA and IAA for shoot regeneration and IBA and NAA for rooting were used in different concentration either alone or in combinations. The minimum time required to bud break (15.80 days) required was noticed on MS medium modified supplemented with BAP 1.0 mg/l + IAA 0.5 mg/l. The maximum number of shoot (4.90) on 1.0 mg/l BAP + 0.5 mg/l NAA and length of shoot (2.62 cm) on BAP 1.0 mg/l + IAA 0.5 mg/l. The maximum per cent (90 %) of micro shoots responded to rooting was observed in medium containing 1.0 mg/l either IBA or NAA or in combination of NAA (0.5 mg/l) + IBA (0.1 mg/l). The minimum time required to root induction (18.40 days) was recorded with NAA (0.5 mg/l) + IBA (0.5 mg/l). The maximum number of roots (6.70) and length (4.40 cm) were observed on MS medium supplemented with NAA (0.5 mg/l) + IBA (0.5 mg/l). The rooted plantlets were successfully acclimatized in a green house, in pots containing sterile soil, perlite and vermiculite in equal proportion. After 60 days of acclimatization under greenhouse conditions, 85.0 per cent success was noted.

**Key words**: Acclimatization, *In vitro,* Kinnow, Micro propagation, Nucellar

Kinnow, a hybrid mandarin (*Citrus deliciosa*) is a cross between the King Sweet Orange (*C. nobilis*) and Willow Leaf Mandarin (*C. deliciosa*) developed in 1915 by H B Frost, at Citrus Research Centre, Riverside, California of the University of California (Frost and Krug 1942). Kinnow fruits have a great nutraceutical value due to balance ratio between K and Na, which makes good ionic balance in the body. In citrus, Kinnow mandarin bears highest place in production, productivity, juice content and fruit quality. In India, it is being commercially grown in Punjab, Rajasthan, Haryana, Himachal Pradesh, Jammu and Kashmir and Uttar Pradesh. In Rajasthan, Sriganganagar district has a distinct position with 8650 ha area and 25 000 MT production (Anonymous 2009). The interest of farmers of North western region of the country is increasing day by day in adoption of Kinnow cultivation due to suitable agro-climatic conditions, higher crop yield and demand in international market. In kinnow, the infection by viruses and related pathogens have received attention due to decline of citrus trees, which is mainly attributed to viruses (tristeza, psorosis, greening, ring spot virus, exocortis, gummosis, xyloporosis, bacteria etc.), fungi (*Phytopthora* sp.) and bacterial canker. Besides these, other factors like rootstock incompatibility, poor management, malnutrition, marginal soils and poor quality of irrigation water also affect citrus production. Tristeza virus has wiped out the citrus industry and destroyed about 30 million trees in many countries, i.e in Argentina and Brazil, Spain, Japan and United States. The estimates indicate that Tristeza destroyed about a million trees in India (Ahlawat 1997). In North India, especially in Punjab, Byadgi and Ahlawat (1995) reported up to hundred per cent incidence of ring spot disease which cause 20.54 to 98.38 per cent yield loss in seven years old Kinnow trees. According to survey on infection of three viruses in Citrus, conducted by Kapur *et al.* (1992), revealed that highest infection was with greening followed by Exocortis and Tristeza (64.7, 2.9 and 1.5 per cent, respectively). Tissue culture technology offers an advantage over conventional methods of propagation ('T'-budding) in producing large number of true to type plants from healthy plant within a short period. The tissue cultured plants are genetically uniform in field conditions and the differences within plants either due to somaclonal variation or explants taken from more than one plant, Jayanthi *et al.* (2013). Nucellar seedlings are genetically uniform material to mother plant and free from most of the viruses. Thus nucellar embryos offer excellent means for production of desirable true to type planting material for commercial purpose. In this perspective studies were taken out to

[1]SMS (Horticulture Science) (e mail: rajhortches@gmail.com), KVK,CHES-CIAH, Godhra-Baroda Highway, Vejalpur–Panchmahals (Godhra) Gujarat 389 340; [2]Former Director of Research (e mail: drlaukmk@gmail.com), SKRAU, Bikaner, Rajasthan; [3]Principal Scientist (Plant Physiology) (e mail: shail.nrcss@gmail.com), National Research Centre on Seed Spices, Ajmer, Rajasthan; [4]Senior Scientist (Horticulture) (e mail: aksbicar@gmail.com), CHES (CIAH), Panchmahals (Godhra), Gujarat; [5]Assistant Professor (Plant Pathology) (e mail: pradeep.patho@gmail.com), ARS, Sriganganager, Rajasthan

standardize the protocol for micro propagation of Kinnow mandarin from nodal segments of nucellar seedlings which is true to type.

## MATERIALS AND METHODS

Present study, was carried out during 2007-09 to standardize the protocol for micro-propagation of Kinnow mandarin. The healthy fruit of Kinnow mandarin were collected from genuine healthy Kinnow plants from citrus repository of Agricultural Research Station, Sriganganager. They were washed under running tap water. The seeds were extracted and washed thoroughly treated with 0.2% (w/v) Bavistin (a systemic fungicide of BASF India Ltd, Mumbai). The seed testa was removed under aseptic condition. These decoated seeds were first quick rinsed with 70% ethanol followed by mercuric chloride ($HgCl_2$ @ 0.1% w/v) for five minutes and inoculated in MS basal medium. After 15-20 days of incubation, the nucellar seedlings were isolated and sub cultured on fresh medium. After 20-30 days of sub culture, these seedlings were cut into segments so as to have two buds at least and then inoculated on MS medium supplemented with BAP @ 0.0, 0.5, 1.0, 3.0, 5.0 mg/ l and auxin (NAA, IAA) @ 0.0, 0.1, 0.5 and 1.0 mg/l alone or in combination on axillary shoot proliferation was studied. The *in vitro* generated shoots were used as micro cuttings. The dried, under sized leaves and multiple shoots were removed. The micro shoot about 2-3 cm were incubated in culture tube (25 × 150 cm) containing 15-20 ml MS medium supplemented with various concentrations of auxin (IBA and NAA) @ 0.0,0.1,0.5,1.0 and 2.0 mg/l alone or in combination. The rooted plantlets were carefully removed from the culture tubes and their roots were thoroughly washed under running tap water and cleaned with fine brush to remove adhered agar. The plantlets were covered with sterilized cotton wetted with half strength MS medium for 24 hr in culture room. The plantlets were treated with Bavastin @ 0.2 per cent for 10 minutes to prevent fungal contamination. These plantlets were transferred to pots containing sterilized soil, vermiculite and perlite in equal proportion. These pots were kept in green house at 90 per cent humidity with temperature $26 \pm 2^0$C. The humidity was continuously lowered within 8-10 weeks up to 60%. During this period the plantlets were irrigated with Hoglands solution at three days interval for one month. After that, these were irrigated with Hoglands solution and simple water at an interval of 2-3 days alternately. The observations were recorded for number of plantlets survival after 60 days of planting in pots and incubated in green house. The test of significance of variation in experimental results obtained from various treatments, the data were statistically analyzed by Factorial Randomized Block Design.

## RESULTS AND DISCUSSION

*Influence of BAP and in combination with NAA and IAA on regeneration parameters of Kinnow mandarin*

*Per cent survival of explants:* The maximum (80.00 per cent) survival of explants was observed when the MS medium was supplemented with BAP 1.0 mg/l (Table 1). Further increase in BAP concentration decreased the survival. Kour *et al.* (2007) has reported 100 per cent survival of explants in rough lemon on BAP 1.0 mg/l but with addition of malt extract (500 mg/l). The similar results are also reported by Kumar *et. al.* (2012) in Rough lemon and Parthasarathy and Nagaraju (1996c) in *Citrus* species. Different concentrations of NAA were at par in their effect on explant survival, except 0.5 mg/l, 80 per cent survival was observed at IAA at 0.5 mg/l (Table 1). Among NAA levels along with BAP, under different concentrations had synergistic effect on survival percentage. The maximum (90 per cent) survival was observed on MS medium containing BAP 1.0 mg/l + NAA 0.1 mg/l (Table 1). Kanjilal *et al.* (2006) who observed 100 per cent survival of explants in Rangpur lime on 0.5 mg/l, 1.0 mg/l, 2.0 mg/l, and 3.0 mg/l BAP with 0.5 mg/l NAA. A maximum 90 per cent explants survival was obtained when MS medium was supplemented with BAP 1.0 mg/l + IAA 0.5 mg/l (Table 1). Edriss and Burger (1984a) observed low frequency of shoot formation (30 per cent) on MS media containing BAP (0.5 mg/l) with NAA (0.1 mg/l).

*Time required to bud break:* The increasing concentration of BAP significantly decreased the number of days taken to bud break. A minimum of 17.20 days were required to bud break when MS medium was fortified by BAP 1.0 mg/l. (Table 1) Further increase in concentration of BAP delayed the bud break. The similar results are also reported by Kumar *et. al.* (2011) in Acid lime. In case of NAA, minimum 18 days was needed to bud break at 0.1 mg/ l (Table 1). The probable reason for this may be that BAP suppressed apical dominance and stimulate the lateral buds. Early bud bursting within 15.70 days was observed when MS medium supplemented with BAP 1.0 mg/l + NAA 0.5 mg/l (Table 1). It may be due to optimum ratio of cytokinin and auxin required to complete any physiological process. In case of IAA, minimum 17.80 days were required to bud break at 0.5 mg/l and further increase in concentration delayed it. In case of interaction between BAP and IAA, bud break took place in 15.80 days when MS medium was modified with BAP 1.0 mg/l + IAA 0.5 mg/l (Table 1). These findings are in line with those reported by Singh *et al.* (2007) in ker (*Capparis decidua*) who reported that increase in concentration of BAP, IAA in MS medium significantly delayed time required to bud break.

*Number of shoot:* BAP 1.0 mg/l produced maximum 4.0 shoots and further increases in concentration reduced number of shoot, while in control only single shoot (Fig 1). Similar observations were made by Parthasarathy and Nagaraju (1993a) in Khasi mandarin where proliferation of shoots proportionally increased with increasing BAP levels up to 0.75 mg/l and above this, BAP reduced number of shoot and increased callusing, while NAA alone did not have significant effect on number of shoot (Fig 1). Interaction between BAP + NAA induced maximum number of shoots (4.90/explants) in MS medium containing 1.0 mg/l BAP +

Table 1 Effect of cytokinin (BAP and Kinetin) and Auxin (NAA and IAA) added alone and in combination in basal medium, explant survival and time required to bud break of Kinnow mandarin

| Treatments (mg/l) | | Per cent ex-plants survival | | | | | No. of days to bud break | | |
|---|---|---|---|---|---|---|---|---|---|
| Cytokinin | Auxin | BAP:NAA | BAP: IAA | Kinetin:NAA | Kinetin: IAA | BAP:NAA | BAP: IAA | Kinetin:NAA | Kinetin: IAA |
| 0.0 | 0.0 | 60(50.77) | 60(50.77) | 60(50.77) | 60(50.77) | 19.00 | 90.80 | 19.00 | 19.00 |
| 0.0 | 0.1 | 70(56.79) | 70(56.79) | 60(50.77) | 80(63.43) | 18.00 | 18.80 | 18.40 | 18.50 |
| 0.0 | 0.5 | 80(63.43) | 80(63.43) | 80(63.43) | 80(63.43) | 18.80 | 17.80 | 18.60 | 18.00 |
| 0.0 | 1.0 | 70(56.79) | 60(50.77) | 70(56.79) | 60(50.77) | 20.20 | 21.30 | 20.90 | 21.20 |
| 0.5 | 0.0 | 70(56.79) | 70(56.79) | 60(50.77) | 70(56.79) | 17.70 | 17.70 | 18.00 | 17.90 |
| 1.0 | 0.0 | 80(63.43) | 80(63.43) | 80(63.43) | 80(63.43) | 17.20 | 17.20 | 17.80 | 18.70 |
| 3.0 | 0.0 | 60(50.77) | 60(50.77) | 70(56.79) | 70(56.79) | 18.80 | 18.30 | 19.20 | 19.00 |
| 5.0 | 0.0 | 50(43.00) | 60(50.77) | 60(50.77) | 60(50.77) | 20.10 | 20.20 | 20.30 | 20.00 |
| 0.5 | 0.1 | 80(63.43) | 70(56.79) | 70(56.79) | 70(56.79) | 17.30 | 17.80 | 18.00 | 18.30 |
| 0.5 | 0.5 | 90(71.57) | 80(63.43) | 80(63.43) | 80(63.43) | 17.00 | 16.80 | 17.85 | 18.20 |
| 0.5 | 1.0 | 70(56.79) | 70(56.79) | 80(63.43) | 70(56.79) | 18.70 | 19.00 | 20.00 | 18.40 |
| 1.0 | 0.1 | 90(71.57) | 80(63.43) | 80(63.43) | 70(56.79) | 17.10 | 17.20 | 17.30 | 18.60 |
| 1.0 | 0.5 | 90(71.57) | 90(71.57) | 90(71.57) | 90(71.57) | 15.70 | 15.80 | 17.10 | 17.60 |
| 1.0 | 1.0 | 70(56.79) | 70(56.79) | 80(63.43) | 80(63.43) | 17.00 | 18.70 | 20.00 | 18.80 |
| 3.0 | 0.1 | 60(50.77) | 70(56.79) | 70(56.79) | 80(63.43) | 16.80 | 19.20 | 18.40 | 19.20 |
| 3.0 | 0.5 | 70(56.79) | 80(63.43) | 80(63.43) | 80(63.43) | 18.30 | 17.80 | 17.90 | 19.80 |
| 3.0 | 1.0 | 70(56.79) | 60(50.77) | 70(56.79) | 70(56.79) | 15.80 | 20.20 | 19.20 | 19.40 |
| 5.0 | 0.1 | 60(50.77) | 60(50.77) | 60(50.77) | 70(56.79) | 18.30 | 20.80 | 19.30 | 19.20 |
| 5.0 | 0.5 | 70(56.79) | 70(56.79) | 70(56.79) | 70(56.79) | 18.00 | 18.00 | 18.20 | 20.00 |
| 5.0 | 1.0 | 60(50.77) | 60(50.77) | 70(56.79) | 60(50.77) | 20.40 | 22.20 | 20.80 | 20.20 |
| SEm ± | | 0.43 | 0.41 | 0.38 | 0.21 | 0.15 | 0.22 | 0.24 | |
| CD (P=0.05) | | | 1.20 | 1.14 | 1.06 | 0.60 | 0.43 | 0.61 | 0.66 |

*Figures given in parentheses are angular transformed values

0.5 mg/l NAA (Fig 1). Karwa (2003) reported 6.53 ± 0.95 shoots/explant at BAP 4.44 µM + NAA 2.69 µM in Nagpur mandarin. The similar findings are also reported by Syamal *et al.* (2007) in Khasi mandarin. Kanjilal *et al.* (2006) reported induction of 5.6 ± 0.69 shoots by BAP 1.0 mg/l + NAA 0.5 mg/l in Rangpur lime. In case of different levels of

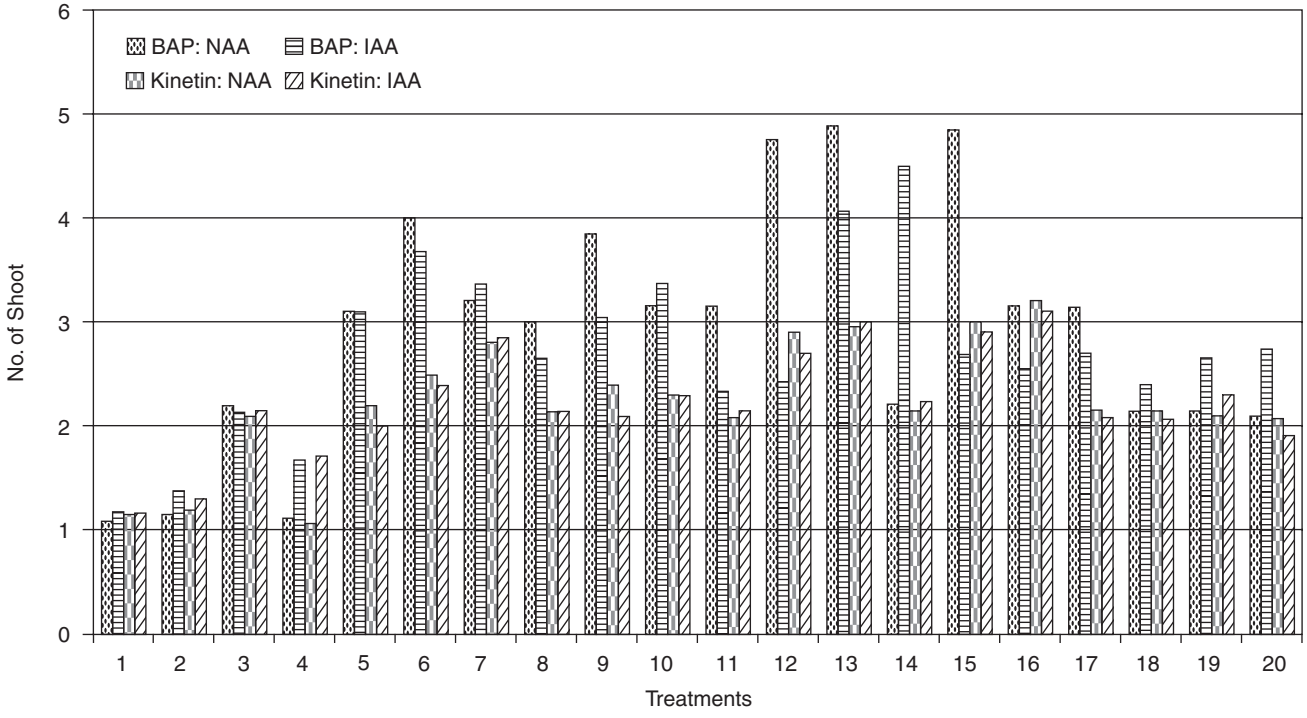


Fig 1 Effect of cytokinin (BAP, kinetin) and auxin (NAA, IAA) alone and in combination in MS basal medium on number of shoots in Kinnow mandarin

IAA, the maximum number of shoots (2.13/explant) were observed at 0.5 mg/l IAA (Fig 1). There was significant interaction between BAP and IAA for number of shoots. The maximum (4.50 shoots/explant) was observed on BAP 1.0 mg/l + IAA 1.0 mg/l (Fig 1). The results of the study are in accordance with the findings as reported by Singh *et al.* (2007) on mulberry.

*Shoot length:* BAP significantly increased shoot length but only up to a certain level after that it had reverse effect. The maximum shoot length (2.35 cm) was observed on BAP 1.0 mg/l (Fig 1). This may be due to the reason that cytokinin promotes shoot proliferation by inducing cell division and enlargement. BAP has been reported to be the best cytokinin for citrus shoot proliferation (Kumar *et al.* 2012) in Rough lemon and (Kumar *et al.* 2013) in Carrizo citrange. The levels of NAA were not having significant effect on length of shoot (Fig 1). Interaction effect of BAP with NAA resulted in maximum length of shoots 2.51 cm in MS medium modified by BAP 1.0 mg/l + NAA 0.5 mg/l (Fig 1). The efficacy of BAP was further enhanced when small amount of NAA was added in the medium. These results are in agreement with the earlier findings of Kumar *et al.* (2013) in Carrizo citrange, Rana and Singh (2002) in Kagzi lime and Parthasarathy *et al.* (2001) in *Citrus* species and reported that the BAP at 2.0 mg/l or above suppressed length of shoot. The results of the present studies are strengthened by the findings of Kanjilal *et al.* (2006) in Rangpur lime, who had reported that the maximum length of shoot was observed in MS medium supplemented with BAP 1.0 mg/l + NAA 0.5 mg/l. Parthasarathy and Nagaraju (1996a) also reported that effect of NAA on length of shoot was none. Kumar *et al.* (2011) in Acid lime. The effect of

Table 2 Effect of IBA and NAA, alone and in combination in basal medium, on Per cent micro-shoot responded to rooting and No. of days taken to root induction in Kinnow mandarin

| Treatments (mg/l) NAA | IBA | Per cent micro-shoot responded to rooting | No. of days taken to root induction |
|---|---|---|---|
| 0.0 | 0.0 | 00 (0.00) | 0.00 |
| 0.1 | 0.0 | 60 (50.77) | 24.10 |
| 0.5 | 0.0 | 80 (63.43) | 23.30 |
| 1.0 | 0.0 | 90 (71.57) | 21.20 |
| 2.0 | 0.0 | 70 (56.79) | 21.40 |
| 0.0 | 0.1 | 80 (63.43) | 24.10 |
| 0.0 | 0.5 | 80 (63.43) | 21.50 |
| 0.0 | 1.0 | 90 (71.57) | 23.40 |
| 0.0 | 2.0 | 60 (50.77) | 24.40 |
| 0.1 | 0.1 | 80 (63.43) | 24.10 |
| 0.5 | 0.1 | 90 (71.57) | 22.30 |
| 1.0 | 0.1 | 70 (56.79) | 21.10 |
| 2.0 | 0.1 | 60 (50.77) | 23.80 |
| 0.1 | 0.5 | 80 (63.43) | 23.70 |
| 0.5 | 0.5 | 70 (56.79) | 18.40 |
| 1.0 | 0.5 | 70 (56.79) | 19.90 |
| 2.0 | 0.5 | 60 (50.77) | 21.40 |
| 0.1 | 1.0 | 80 (63.43) | 22.00 |
| 0.5 | 1.0 | 70 (56.79) | 21.20 |
| 1.0 | 1.0 | 70 (56.79) | 22.10 |
| 2.0 | 1.0 | 60 (50.77) | 24.10 |
| 0.1 | 2.0 | 50 (45.00) | 19.80 |
| 0.5 | 2.0 | 60 (50.77) | 23.30 |
| 1.0 | 2.0 | 60 (50.77) | 24.40 |
| 2.0 | 2.0 | 50 (45.00) | 24.90 |
| SEm ± | | 0.44 | 0.23 |
| CD (P=0.05) | | 1.22 | 0.65 |

*Figures given in parentheses are angular transformed values

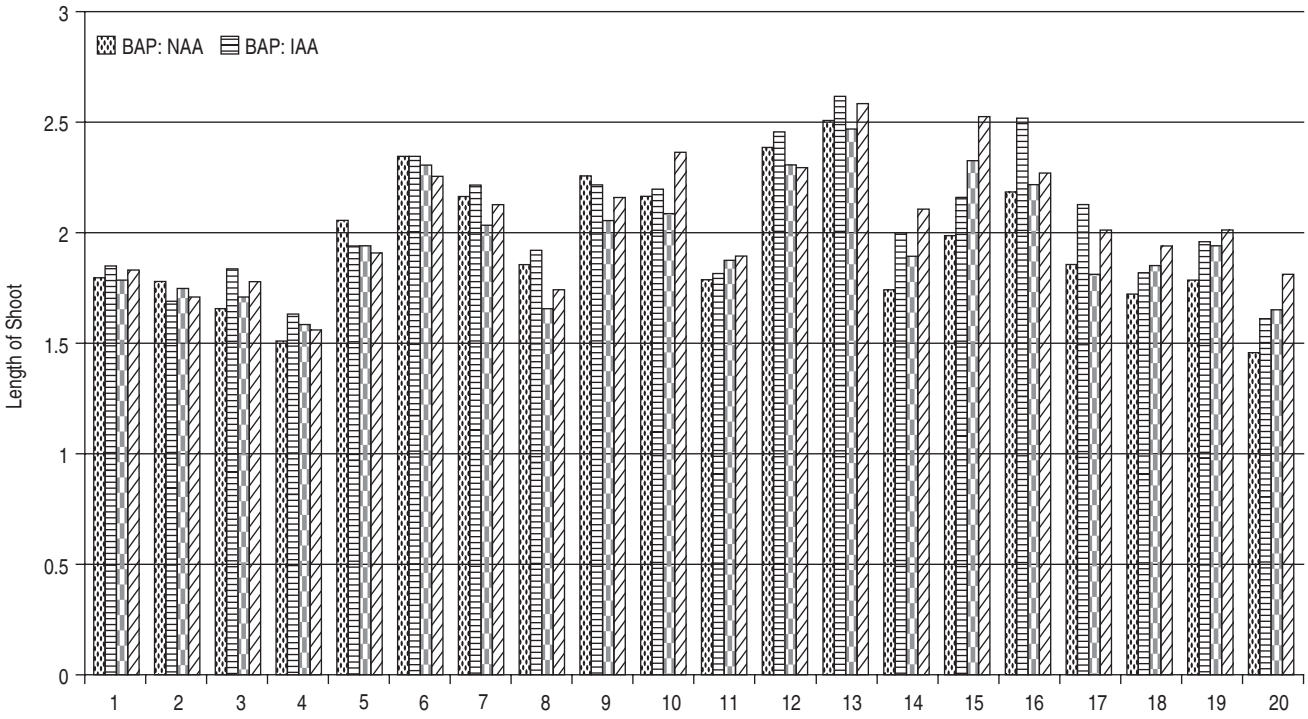


Fig 2 Effect of cytokinin (BAP, kinetin) and auxin (NAA, IAA) alone and in combination in MS basal medium on length of shoots in Kinnow mandarin

IAA on length of shoot was not significant over control (Fig 1). The interaction effect of BAP with IAA, the maximum shoot length (2.62 cm) was recorded in MS medium supplemented by BAP 1.0 mg/l + IAA 0.5 mg/l (Fig 1). These findings are supported by the similar results of Singh *et al.* (2007) in ker (*Capparis decidua*).

*Influence of IBA and NAA alone or in combination on rooting parameters of Kinnow mandarin*

*Per cent survival of micro shoots for rooting:* The survival per cent of micro shoot for rooting was significantly affected by individual and combination effect of IBA and NAA levels. The maximum (90%) survival of micro shoot responded for rooting was observed at 1.0 mg/l either IBA or NAA and in combination (0.5 mg/l) + IBA (0.1 mg/l) (Table 2). The higher concentration of alone or in combination has negative effect on it. It means the higher levels auxins are not comprehensive for increase survival per cent of shoot for rooting. The findings of present investigation are in concurrent with Kitto and Young (1981) reported in Carrizo, the maximum (80 per cent) micro shoot responded to rooting on 1.0 mg/l NAA. The results of present study are in close conformity with Singh *et al.* (1994) in lemon who reported maximum (80%) micro shoot responded to root induction and Kumar *et al.* (2013) in Carrizo citrange.

*Time required for root induction:* Among individual levels of IBA and NAA the minimum time 21.50 days and 21.20 were recorded on 1.0 mg/l respectively (Table 2). The results of present study are in agreement with Syamal *et al*. (2007) who reported minimum 15.32 days for root induction in khasi mandarin at 2.0 mg/l. The similar results have also been reported by Singh *et al.* (1994) and Kumar *et al.* (2011) in Acid lime. In case of combination of both auxin the minimum time (18.40 days) to root induction was recorded at NAA (0.5 mg/l) + IBA (0.5 mg/l). It may be due to auxin promoted adventitious root formation on intact plants as well as excised stem cutting. The results of present study are in agreement with Syamal *et al.* (2007) who reported in Kagzi lime that minimum 18.79 days were required to root induction with NAA 0.5 mg/l+ IBA 0.5 mg/l. The similar results are also reported by Singh *et al.* (1994) and Kumar *et al.* (2013) in Carrizo citrange.

*Number of roots:* The different levels of IBA significantly increase the number of roots up to 1.0 mg/1. The maximum numbers of roots (3.50 per micro shoot) were observed on 1.0 mg/l IBA (Fig 3). Kumar *et al.* (2001) reported that the maximum of 3.91 roots per micro shoots on MS medium modified with 1.0 mg/l IBA Kumar *et al.* (2012) in Rough lemon. Among various concentrations of NAA (0.0, 0.1, 0.5, 1.0, and 2.0 mg/l), the maximum number of roots (2.70 per micro shoot) were observed on 1.0 mg/l NAA and no root formation was recorded on MS medium, devoid of auxin (Fig 3). Syamal *et al*. (2007) also reported 2.98 roots per micro shoot when micro shoot were inoculated in MS culture medium fortified by NAA 1.0 mg/l. In case of interaction effect of NAA with IBA, maximum 6.70 roots

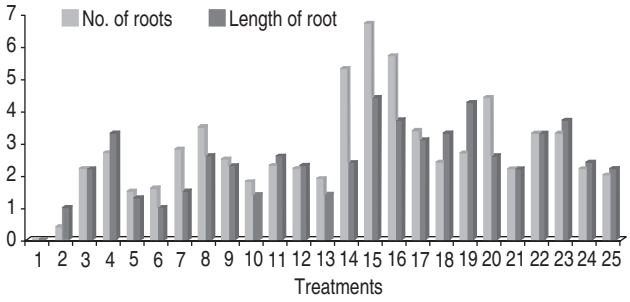


Fig 3 Effect of auxin (NAA and IBA) alone and in combination in MS basal medium on number of roots and length of roots (cm) in Kinnow mandarin

were observed on 0.5 mg/l NAA + 0.5 mg/l IBA (Fig 3). Singh *et al.* (1999a) obtained 4.30 to 7.30 roots per micro shoot (1-2 cm long) cultured on MS medium containing IBA 1.0 mg/l + NAA 0.5 mg/l + BAP and adenine sulphate each 0.1 mg/l. Kour (2007) observed 2.47 roots per micro shoot when the cultured in MS medium modified by BAP 1.5 mg/l + malt extract 500 mg/l + NAA 0.25 mg/l. The similar results are also reported by Kumar *et al.* (2013) in Carrizo citrange.

*Length of root:* The length of root was significantly influenced by different concentrations of NAA and IBA. The length of root significantly increased with increasing concentration of IBA up to 1.0 mg/l level and higher than this had reverse effect. The maximum length of roots (2.60 cm) was observed when MS medium was modified by 1.0 mg/l IBA. The similar results are also reported by Syamal *et al.* (2007). The increase in level of NAA significantly increased length of root up to 0.5 mg/l and further increase in concentration decreased it. The maximum length of roots (3.80 cm) was observed when micro shoots were cultured on MS medium modified with 1.0 mg/l NAA. Kitto and Young (1981) in Carrizo obtained 2.30 cm length of roots when micro shoot inoculated in MS medium fortified by 1.0 mg/l NAA. In interaction effect of IBA and NAA, on length of root, a maximum of length of root (4.40 cm) was observed on MS culture medium supplemented with NAA and IBA each 0.5 mg/l (fig 4). Syamal *et al*. (2007) reported maximum length of root 2.16 cm when micro shoots were cultured in MS medium supplemented with IBA 1.0 mg/l NAA 0.5 mg/l. The findings of study are in agreement with the findings as reported by Al-Khayri and Al-Bahrany (2001), Kumar *et al.* (2011) in Acid lime, Kumar *et al.* (2012) in Rough lemon and Kumar *et al.* (2013) in Carrizo citrange.

*Acclimatization of in-vitro generated plantlets*

*In-vitro* prepared plantlets were successfully acclimatized by transferring them in small pots containing a potting mixture of soil: Perlite : vermiculite in equal proportion. The 82.65 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.* (1996b) survival per cent of *in-vitro* plantlets is directly

related to number of roots. The results of the present study are in line with earlier reporters, Singh *et al.* (2007) reported 92.00 per cent success of *in-vitro* plantlets *in vivo* condition when vermiculite and coco peat (3:1) ratio used as potting medium. The similar findings are also reported by Kumar *et al.* (2010) who reported 82.50% in Kinnow, 83.00% in Carrizo citrange, 90.00 in rough lemon and 75.00% in cleopatra. Singh *et al.* (1994) who reported that 60 per cent plantlets of khasi mandarin established in soil. Perez-Molphe-Balch and Ochoa-Alejo (1997) reported 85 per cent survival rate in Musambi, Singh *et al.* (1999a) reported 80% in Rough lemon plantlets in soil. The acclimatized plantlets were planted in screen proof net house for taking bud wood.

## REFERENCES


Ahlawat Y S. 1997. Viruses, greening bacterium and viroids associated with citrus (*Citrus* species) decline in India. *Journal of Agricultural Science* **67**(2): 51–7.

Al-Khayri J M and Al-Bahrany A M. 2001. *In vitro* micro-propagation of *Citrus aurantifolia*. *Current Science* **81**( 9-10): 1 242–46.

Anonymous. 2009. Director of Horticulture, Sriganganagar.

Baruah A, Nagaraju V and Parthasarathy V A. 1996b. Micro-propagation of three endangenered citrus species-2, rooting ex vitro. *Annals of Plant Physiology* **10**: 129–32.

Byadgi A S and Ahlawat Y S. 1995. A new viral ring-spot disease of citrus (*Citrus* species) in India. *Indian Journal of Agricultural Sciences* **65**(10): 763–70.

Edriss M H and Burger D W 1984a. *In vitro* propagation of 'Troyer' citrange from epicotyl segments. *Scientia Horticulturae* **23**:159–62.

Frost H B and Krug C A. 1942. Diploid tetraploid Perielinal chimeras as bud variants in citrus. *Genetics* **27**:619.

Jayanthi M, Sarika N, Sujatha G, Mathur R. K, Rao C S and Mandal P K. 2013 Evaluation of SSRs (microsatellites) for detecting genetic variability in oil palm (*Elaeis guineensis*) clone. *Current Horticulture* **1**(1): 3–6.

Kanjilal B, Allaya S, Gurung A, Lama Y, Sharma J, Thapa K and Mukhopadhyay S. 2006. Micropropagation of Rangpur lime (*Citrus limonia* Osbeck) using modal stem segments as explants source. *Proceedings of National Symposium on Citriculture,* NRC, Nagpur, pp 18–23.

Kapur S P, Thind S K, Cheema S S and Sohi H S. 1992. Citrus greening in the Punjab: status and management. *Proceeding of Twelfth Conference of International Organization of citrus Virologists*, held during 23-27 November 1992 at New Delhi, p 52 (abstr.).

Karwa A. 2003. *In vitro* propagation of *Citrus reticulata* Blanco (Nagpur mandarin). *Indian. Journal of Genetics* **63**(2):187–8.

Kitto S L and Young M J. 1981. *In vitro* propagation of Carrizo citrange. *Hort. Science* **16**(3): 305 –6.

Kour K, Bakshi, P. and Kher, R. 2007. Studies on micro-propagation of rough lemon. *Indian Journal of Horticulture*. **64**(4):454–5.

Kumar, Krishan, Dhatt A S and Gill M T S. 2001. *In vitro* plant regeneration in sweet orange (*Citrus sinensis*, L. Osbeck cv. Musambi and Jaffa). *Indian Journal of Horticulture.* **58**:11–3.

Kumar R, Kaul M K, Saxena S N, Bhargava S and Singh S S. 2010. Acclimatization of *In-vitro* generated Citrus plantlets. *Indian Journal of Horticulture* (67) (special issue): 423–5.

Kumar R, Kaul M K, Saxena S N, Bhargava S, Singh A K and Singh J. 2011.

Standardization of micro propagation technique for Acid lime *Citrus aurantifolia* Swingle. *Progressive Horticulture*. **43**(1):25–9.

Kumar R, Kaul M K, Saxena S N, Bhargava S and Singh A K 2013. *In-vitro* propagation of virus tolerant root stock Carrizo Citrange *Indian Journal of Horticulturae*. **70**(2):179–84.

Kumar R, Kaul M K, Saxena S N, Bhargava S and Singh S. 2012. Protocol standardization for micropropagation of *Citrus jambhiri* Lush. using nodal segments of nucellar seedlings. *Progressive Horticulture* **44**(1):101–9.

Parthasarathy V A and Nagaraju V. 1993a. Effect of growth regulators in transplanted embryoids of *Citrus reticulata* Blanco. *Annals of Plant Physiology* **7**:21–4.

Parthasarathy V A and Nagaraju V. 1996c. *In vitro* response of shoots of citrus species to basal media and benzylamino purine. *International Journal of Tropical Agriculture* **14**:14–4.

Parthasarathy V A, Barus A, Nagaraju V and Parthasarathy V. 2001. Quadratic response of citrus species to cytokinins and comparative efficiency on morphogenetic characters of *in vitro* proliferated shoots. *Indian Journal of Horticulture* **58**:336–41.

Parthasarathy V A and Nagaraju V. 1996a. *In vitro* shoot proliferation in certain citrus species. *Indian Journal of Horticulture* **53**:171–4.

Perez- Molphe-Balch E and Ochoa-Alejo N. 1997. *In vitro* plant regeneration of Mexican lime and mandarin by direct organogenesis. *Hort Science* **32**:931–4.

Rana J S and Singh R. 2002. *In vitro* clonal propagation of Kagzi lime (*Citrus aurantifolia* Swingle) through shoot tips. *Progressine Horticulture.* **34**:27–34.

Singh D, More T A and Awasthi O P. 2007. Studies on micropropagation of ker (*Capparis decidua*). *National Seminar on Opportunities and Challenges of Arid Horticulture for Nutrition and Liveli hood,* 8-9 March, 2008.

Singh D, More T A, Awasthi O P, Niwas R and Srivastava S. 2007. *In vitro* morphogenesis in mulberry. *National Seminar on Opportunities and Challenges of Arid Horticulture for Nutrition and Liveli hood*, 8-9 March 2008.

Singh S, Ray B K and Deka P C. 1999a. Micropropagation of *Citrus jambhiri* cultivar Rough lemon. *J. Interacad* **3**:140–5.

Singh S, Roy B K, Bhattacharya S and Deka P C. 1994. *In vitro* propagation of *Citrus reticuleta* Blanco and *Citrus limon* Burm. *Hort Science.* **29**(3): 214–6.

Syamal M M, Upadhyay S and Biswas B 2007. *In vitro* clonal propagation of kagzi lime (*Citrus aurantifolia* Swingle). *Indian Journal of Horticulture* **64**(1): 84–6