INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–3–414–416
http://www.ijab.org

In Vitro Regeneration and Multiple Shoots Induction in *Citrus reticulata* (Blanco)

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ABSTRACT

Studies were initiated to explore the role of explant type, growth substances and organic additives in regeneration process of Kinnow mandarin [*Citrus reticulata* (Blanco)]. *In vitro* shoot tips and nodal segment explants were cultured on MS media supplemented with different concentrations of kinetin and benzyl aminopurine (BAP) to produce multiple shoots. The results revealed that the shoot tip explants cultured in MS media supplemented with 1 mg L^{-1} of BAP and 1.5 mg L^{-1} of kinetin showed highest shoot percentage. Average number of shoot per explant was the highest at 1.5 and 0.5 mg L^{-1} kinetin and BAP respectively. However, nodal segments cultured on MS media containing 2 mg L^{-1} NAA produce more roots.

Key Words: *In vitro*; Kinnow; Regeneration; Shoot induction

INTRODUCTION

Citrus, member of family Rutaceae refers to all edible and rootstock species and a few closely related genera. Citrus fruits are grown throughout the world and are known for their fine flavor and quality. Pakistan is one of the major citrus producing countries of the world, having an area of 0.2 million hectares with yearly production of 1995 thousands tones (Anonymous, 2004).

Among all citrus species, Kinnow mandarin [*Citrus reticulata* (Blanco)] is highly popular and commercially cultivated for its processing quality, fresh consumption and aromatic flavor. Despite its excessive cultivation, kinnow plantation still has some problems such as slow growth and long juvenility, insects, pests, diseases, alternate bearing, pre and post harvest losses, large number of seeds per fruit, short season of supply and short storage life etc.

A plant reproduces naturally through the development of zygotic embryos. Formation of the embryo begins with the division of the fertilized egg or zygote within the embryo sac of the ovule. Through an orderly progression of cell division, the embryo eventually differentiates, matures, and develops into a new plantlet. Alternatively, the plant can be derived from a single somatic cell or a group of somatic cells. This regeneration process which differs from the natural pathway is called somatic embryogenesis. Somatic embryogenesis can efficiently be used as a tool in citrus improvement programs.

In vitro propagation has therefore been a useful tool to overcome problems related with the field culture of such species (Hidaka & Omara, 1989). Multiple shoot induction and regeneration is potentially useful for the genetic improvement of fruit crops (Murashige *et al*., 1972). It is known that the tissues obtained from young plant parts have relatively more regenerative capacity than old tissues. The root and bud formation in tissue culture is dependent on a specific equilibrium between the auxins and the cytokinins, gibberellins and cytokinins ratio, which control the shoot and leaf development. (Starrantino & Caponnetto, 1990).

The research was carried out to explore the response of explants and medium composition on frequency of plant regeneration and multiple shoot induction in Kinnow mandarin.

MATERIALS AND METHODS

The mature and young shoots of Kinnow mandarin [(*C. reticulata* (Blanco)] were collected early in the morning from Experimental Fruit Garden Sq. No. 9, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The collected shoots were washed thoroughly under running tap water. Nodal segments and shoot tips were cut (1-1.5 cm in length) as described by Spripaoraya *et al*. (2003). The explants of Kinnow mandarin were surface sterilized with 70% ethanol for 1 to 2 minutes, then with 20% sodium hypochlorite (NaOCl) solution containing one drop of Tween-20 for 2 minutes and then rinsed 4 times with sterilized distilled water.

Sterilized explants of Kinnow mandarin were cultured on MS media supplemented with different (0.25, 0.5, 1.0 and 0.1 mg L^{-1}) concentrations of benzyl aminopurine (BAP). In second treatment the culturing media was supplemented with the concentrations of kinetin (0.5, 1.0, 1.5, 2.0 mg L^{-1}). The experiment was laid out in completely randomized design (CRD) and number of replications used per treatment was ten. The cultured test tubes were kept under controlled environment with 2500 lux light intensity at temperature of 25±2°C for 16 hours photoperiod (Murashige & Skoog, 1962). After the emergence of micro shoots, the shoots were separated individually and were

cultured on MS media containing different concentrations (1.0, 1.5, and 2.0 mg L^{-1}) of Naphthalene acetic acid (NAA) for root initiation. Data was collected for the multiple shoot formation, number of shoots per explant and root formation percentage.

RESULTS AND DISCUSSION

Effect of growth regulators on shoot formation percentage. The shoot formation percentage was found higher in shoot tip explants compared to nodal segment explants. Shoot tips cultured in MS media containing 1.0 mg L^{-1} BAP, produced more shoots (84%) compared to any other concentration of BAP. The results revealed that the shoot formation percentage increases with the increase in BAP concentration regardless of the type of explants (Fig. 1). However, shoot formation percentage was higher in both types of explants cultured in 1 mg L^{-1} BAP supplemented

Fig. 1. Effect of Benzyl aminopurine (BAP) on shoot formation %age in *C. reticulata* (Blanco)

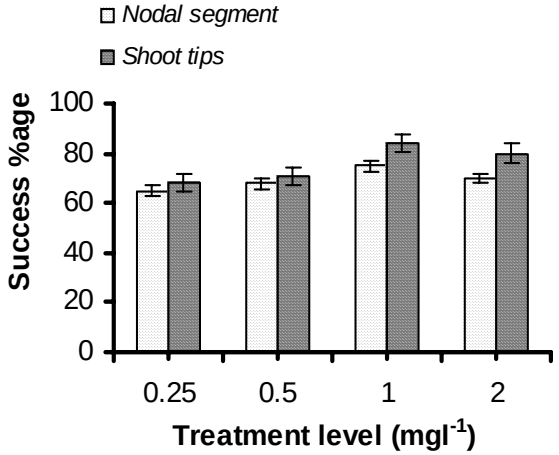


Fig. 2. Effect of Kinetin on shoot formation %age in *C. reticulata* (Blanco)

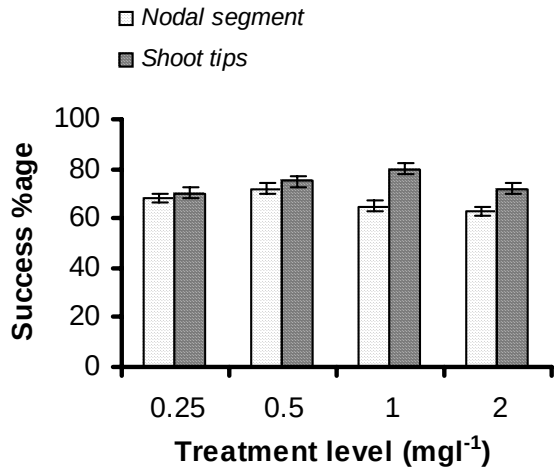


Fig. 3. Effect of Naphthalene acetic acid (NAA)on formation %age in *C. reticulata* (Blanco)

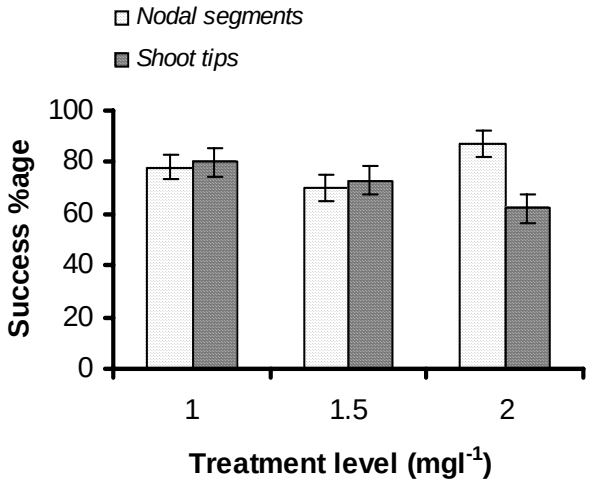
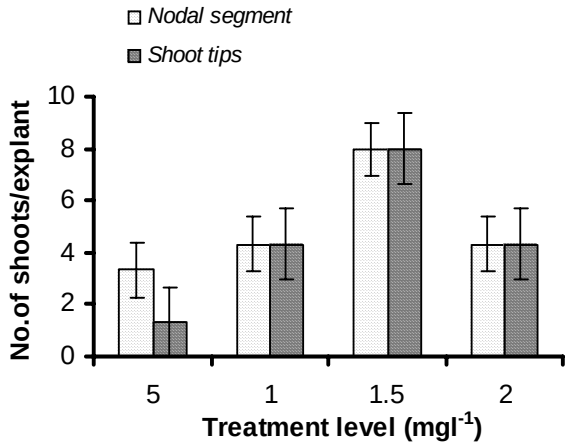


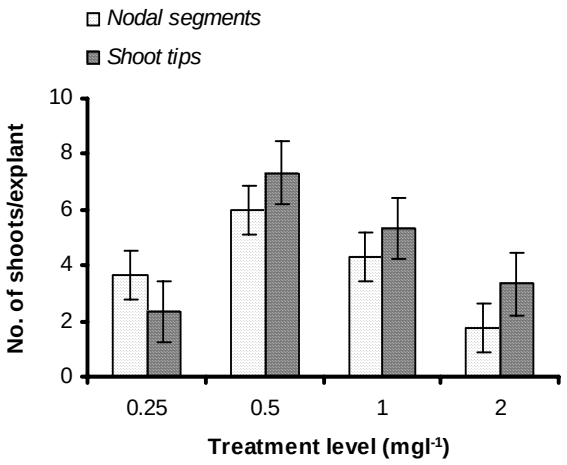
Fig. 4. Effect of Kinetin on number of shoots per explant in *C. reticulata* (Blanco)



culturing media. Similarly, percentage of shoot formation was also higher (80%) in shoot tip explants cultured in MS media supplemented with 1.5 mg L^{-1} kinetin (Fig. 2). However, the shoot formation percentage was not increased with the increase in concentration of kinetin compared to BAP. This indicated that concentration of growth regulators have significant effect on the successful shoot induction. The results are in accordance with the findings of Oh *et al.* (1991), Vestri *et al.* (2003) and Kim *et al.* (2002) that variations in concentration of different growth hormones significantly affect the shoot formation percentage in citrus cultivars.

Effect of growth regulator on number of shoots per explant. The number of shoots per explant was found higher in shoot tip explants compared to nodal segments (Fig. 4 & 5). Shoot tips cultured in MS media supplemented with 1.5 mg L^{-1} kinetin produced number of shoots per explant (7.99). More number of shoots per explant was also

Fig. 5. Effect of Benzyl aminopurine (BAP) on number of shoots per explant in *C. reticulata* (Blanco)



observed in shoot tips (7.33) cultured in MS media containing 0.5 mg L^{-1} BAP (Fig. 5). In case of nodal segment explants number of shoots was found higher (6.33) in MS media supplemented with 1 mg L^{-1} kinetin (Fig. 4) followed by 0.5 mg L^{-1} BAP. The best optimum concentration of growth hormone was 0.5 mg L^{-1} BAP, for the number of shoots per explant. Similar results were obtained by Al- Khayri and Al- Bahray (2001) that best results for multiple shoot formation (8 shoots per node) were obtained with 1 mg L^{-1} BAP and 0.5 mg L^{-1} kinetin in lemon. Chandra *et al.* (2003), also observed varying degree of success percentage at different hormonal levels of BAP and and got similar results by culturing same explant on MS media

Effect of NAA on root formation. The percentage of root formation was high in nodal segments compared to shoot tips explant (Fig. 3). The nodal segments, cultured in MS media supplemented with 2.0 mg L^{-1} of NAA, formed maximum roots (87%) compared to any other treatment. However, in shoot tip explant root formation was higher in MS media supplemented with 1 mg L^{-1} NAA. These results are in conformity with the Kim (2002), who noted that MS media supplemented with 1.5 mg L^{-1} NAA was most effective for root induction in Yooza mandarin. Similarly, Cheong *et al.* (2003) also got increased root formation percentage (70 - 80%) with increasing level of auxins NAA and indole-3-butalic acid in the media. De-Almedia *et al.* (2004) also got best result with 1 mg L^{-1} BAP for shoots induction and 1 mg L^{-1} IBA for rooting of sweet orange cultivars.

CONCLUSION

It is concluded that the *in vitro* propagation method have a great potential and promise for clonal propagation of citrus trees. The optimization of different concentrations of growth regulators could also be helpful to increase the number of shoots per explant and to induce the root formation in regenerated plantlets.

ACKNOWLEDGEMENT

We are gratefully acknowledged the Ministry of Science and Technology, Government of Pakistan for the financial assistance.

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(Received 09 January 2005; Accepted 10 February 2005)