IN VITRO PLANT REGENERATION IN KINNOW

BY

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ABSTRACT
Most of the plant regeneration processes in Citrus, through tissue culture, involve indirect somatic embryogenesis. The optimization of these processes is important for the development of in vitro plant improvement and micro-propagation studies. Thus studies were conducted with Kinnow mandarin (Citrus reticulata Blanco) using epicotyl segments from in vitro grown nucellar seedlings. Results revealed that BAP (upto 1 mg/litre was found optimum. Maximum shoot proliferation (71.66%) was observed with BAP (1.0 mg/litre) and minimum (66%) in basal MS medium. Root development was maximum (53.33%) at the concentration of NAA (0.5 mg/litre) + GA3 (0.5 mg/litre). However, root development was also better at NAA (1.0 mg/litre) alone. The complete plantlets were obtained with a survival rate of 65-70%.

Keywords: Somatic embryogenesis, Plant regeneration, Epicotyl segments, Citrus reticulata Blanco.

INTRODUCTION
The Citrus species are important fruit crops in the world. In Pakistan the Citrus fruits rank at the top in acreage as well as in production among all other fruits (Anonymous, 2005). Kinnow mandarin is the most popular Citrus cultivar because of its good flavour, delicious taste, cooling effect and high production. Inspite of several good attributes, some inherent problems in Kinnow are late maturity, high seed contents, high acidity, poor keeping quality, unsuitability for processing, alternate bearing and short season of supply (Ishfaq et al., 2004). Vegetatively reproduced fruit trees (clones) deteriorate during continued propagation because of systemic virus and mycoplasma – like organisms infection or by genetic changes (Hampton, 1972). Citrus cultivar improvement by conventional hybridization methods is hampered by the long juvenility period, high heterozygosity and a very complex reproductive biology including sexual incompatibility and sterility (Louzada and Del-Rio, 2002). This has led to more emphasis on the use of in vitro techniques for genetic improvement of particular traits in Kinnow. In vitro screening can isolate new and improved cell lines from which plants with improved traits can be regenerated (Drew, 1995).

In vitro plant regeneration technique with its multipurpose benefits could help to solve many of the problems encountered in Kinnow fruit crops improvement and cultivation. The development of efficient plant tissue culture procedures for embryogenic culture induction, maintenance and plant regeneration in Citrus is important for the application of different technologies for crop improvement. Somatic embryos have been produced from epicotyl segments (De-Almeida et al., 2006), nucellar tissue (Khan and Altaf, 2005), stigma/style explants (D-Onghia, 2001), Beloualy, 1991 regenerated complete plants from callus derived embryo cultures of C. aurantium, P. trifoliata and Carizo Citrange (C. sinensis x P. trifoliata) via somatic embryogenesis as well as organogenesis. Mishra et al., 2003 reported that among other growth regulators tested, GA3 at 1mg/l promoted formation of plantlets along with long stem. Ling and Iwamasa, 1997 induced somatic embryos from seeds of eight Citrus relatives on MS medium containing 2,4-D, BA and malt extract and they regenerated plantlets on MS medium supplemented by NAA. Gill et al. (1994) obtained 6-8 roots from somatic embryo induced shoots of Kinnow mandarin on half strength MS medium fortified with NAA (0.5 mg/litre).
The objective of this study was to regenerate plantlets via direct organogenesis from epicotyl segments obtained from *in vitro* germinated nucellar seedlings of Kinnow mandarin.

**MATERIALS AND METHODS**

The experiment was conducted at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The seeds were extracted from mature fruits of Kinnow mandarin (*Citrus reticulata* Banco). The testa of freshly extracted seeds was removed. The seeds were surface sterilized with 0.1% Mercuric chloride for 3-6 minutes in laminar air flow cabinet. They were then rinsed 3 times with sterile distilled water and inoculated on MS (Murashige and Skoog, 1960) basal medium. Epicotyl segments, 1.0-1.5 cm in length were excised from two weeks old seedlings and cultured on MS medium supplemented with varying levels of BAP (0.00, 0.25, 0.5, 1.0, 2.0 and 3mg/litre). Five culture flasks (4 seeds/flask) in each level of BAP formed one replication. The experiment was replicated thrice. The data on shoot proliferation, shoot number, shoot length (cm) and leaf number were recorded 4 weeks after seed culture.

The regenerated shoots were rooted on MS medium containing different concentrations of GA₃ (0.25, 0.5 and 1.0 mg/litre) in combination with and without NAA (0.5, 1.0 and 2.0 mg/litre). Five culture flasks in each concentration formed one replication. The experiment was replicated thrice. Observations on number of regenerated shoots, developed roots, number of roots and root length (cm) were recorded 3 weeks after root initiation.

The basal and modified MS media for shoot regeneration/rooting contained 2% sucrose and 10 g/litre agar at pH 5.8 before autoclaving at 1.05 Kg/cm² pressure for 5 minutes. The cultures were incubated at 25 ± 2 °C with photoperiodic regimes of 16 hr light and 8 hr dark. The data were tested by factorial experiment in completely randomized block design.

The roots of *in vitro* propagated plantlets were washed under running tap water to remove adherent agar. Initially plugs of flasks were partially loosened and later completely removed. This helped in partial hardening of the plantlets. The plantlets were then transferred to pots containing soil mixture. The pots were kept in partial light and covered with polythene bags until they acclimatized to lower humidity. The plants were watered with tap water at 2-3 days interval. The data regarding soil survival of plants was recorded after three months of pots transfer.

**RESULTS AND DISCUSSION**

Significant interaction was observed between BAP levels and Kinnow mandarin for all the characters studied (Table 1). Maximum shoot proliferation (71.66%) was noted when the explants (epicotyl segments) were cultured on BAP (1.0 mg/litre) and minimum shoot proliferation (1.66%) in basal medium. The number of shoots and leaves was also maximum (2.06, 0.91 respectively) on BAP (1.0 mg/litre). However, maximum shoot length was observed at low concentration of BAP (0.25 mg/litre). Almost similar findings were reported in Khasi mandarin (*C. reticulata* Blanco), which indicated that BAP (0.75 mg/litre) was optimum for shoot proliferation (Parthasarathy and Nagaraju, 1996). Duran-Vila et al. (1989) reported that BAP (3 mg/litre) was optimum for shoot and bud proliferation. Maggon and Singh (1995) reported that BAP (up to 2 mg/litre) had a promotive effect while higher concentration of BAP had a depressive effect. Rahman et al. (1996) obtained 4.72 leaves/shoot on MS medium supplemented with BAP (1 mg/litre). The variation might be due to endogenic levels of growth regulators.

Also significant interactions between auxins (GA₃ and NAA) and explants of Kinnow mandarin were observed (Table 2). It was observed that all the regenerated shoots developed the roots. Maximum number of regenerated shoots (53.33%) developed roots and number of roots was noted maximum (4.20) when explants were cultured on NAA (0.5 mg/litre) + GA₃ (0.5 mg/litre) and both these were noted minimum (1.66% and 0.16 respectively) when cultured on basal MS medium. NAA (1 mg/litre) alone was observed optimum for these characters. However, maximum root length (2.45 cm) was observed on NAA (0.5 mg/litre) + GA₃ (1.0 mg/litre). The current results are parallel with the findings of Chen et al., 1990 and Mishra et al., 2003, that GA₃ at 1mg/l was effective for embryo to plantlet regeneration. Among various regeneration media tested, MS medium containing GA₃ (2mg/l) and AdSO₄ (25mg/l) responded excellently in regeneration of somatic embryos into plantlets (Khan and Altaf, 2005). The growth performance of the regenerated cum grafted Kinnow mandarin plants is being monitored.
Table-1: Effect of different BAP concentration on morphogenetic response in Kinnow mandarin

<table>
<thead>
<tr>
<th>BAP (mg/litre)</th>
<th>No. of explants showing shoot proliferation</th>
<th>Shoot number</th>
<th>Shoot length</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.33 (1.66)</td>
<td>0.33</td>
<td>0.31</td>
<td>1.50</td>
</tr>
<tr>
<td>0.25</td>
<td>6.83 (34.16)</td>
<td>1.09</td>
<td>1.30</td>
<td>4.31</td>
</tr>
<tr>
<td>0.50</td>
<td>11.50 (57.50)</td>
<td>1.43</td>
<td>1.18</td>
<td>4.28</td>
</tr>
<tr>
<td>1.00</td>
<td>14.33 (71.66)</td>
<td>2.06</td>
<td>0.91</td>
<td>4.56</td>
</tr>
<tr>
<td>2.00</td>
<td>5.50 (27.50)</td>
<td>1.31</td>
<td>0.83</td>
<td>14.11</td>
</tr>
<tr>
<td>3.00</td>
<td>4.16 (20.83)</td>
<td>1.13</td>
<td>0.73</td>
<td>3.89</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentage of shoot proliferation

Table-2: Effect of different concentration of GA3 and NAA on development of roots in Kinnow mandarin

<table>
<thead>
<tr>
<th>Concentration (mg/litre)</th>
<th>No. of regenerated shoots developed roots</th>
<th>Number of roots</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA3 (0.25)</td>
<td>0.16 (1.66)</td>
<td>0.16</td>
<td>0.11</td>
</tr>
<tr>
<td>GA3 (0.50)</td>
<td>1.16 (11.66)</td>
<td>2.41</td>
<td>0.99</td>
</tr>
<tr>
<td>GA3 (1.0)</td>
<td>1.50 (15.00)</td>
<td>2.91</td>
<td>1.20</td>
</tr>
<tr>
<td>NAA (0.5) + GA3 (0.25)</td>
<td>1.66 (16.66)</td>
<td>3.41</td>
<td>1.93</td>
</tr>
<tr>
<td>NAA (0.5) + GA3 (0.5)</td>
<td>5.33 (53.33)</td>
<td>4.20</td>
<td>2.45</td>
</tr>
<tr>
<td>NAA (0.5) + GA3 (1.0)</td>
<td>2.50 (25.00)</td>
<td>3.84</td>
<td>3.33</td>
</tr>
<tr>
<td>NAA (2.0)</td>
<td>2.00 (20.00)</td>
<td>3.55</td>
<td>1.74</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentage of shoots produced roots
REFERENCES


