In vitro Shoot Development from Juvenile Cuttings of Field-Grown Olive (Olea europaea L.) cv. Leccino

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Abstract: Effects of different growth regulators on uniconal explants of field-grown olive (Olea europaea L.) cv. Leccino were investigated. Single-node cuttings were obtained from 8th bud pair. Disinfection protocols were standardized by using 0.1% mercuric chloride for five min followed by 50% Clorox for 15 min. sterilized cuttings were tested on MS medium supplemented with various plant growth regulators viz. gibberelic acid kinetin benzylaminopurine, and isopentenyladenine. These growth regulators were used singly in different combinations ranging from 1.0 to 8.0 µM and in vitro. GA3 @ 4 µM was found to be more effective in enhancing shoot elongation. A higher concentration of kinetin, BAP and 2IP induced more callusing and hyoperhydrated microshoots. Inclusion of polyvinylpyrrolidone @ 0.2 g/l in media was found to be successful to eliminate phenolic exudates.

Key words: In vitro. Olea europaea, explant, disinfection, growth regulators

Introduction
Olive is one of the oldest and most important crops found in the Mediterranean area and its cultivation is presently expanding into areas of South America, Australia and South Africa (Rugini and Fedeli, 1990; Rugini and Lavee, 1992). Thousands of different olive genotypes are currently under cultivation and a high level of morphological variability is present. Nevertheless, to optimize the future sustainability of this crop certain problems, which severely limit the broad application of this crop such as low efficiency production and high cultivation cost must be solved.

Olive breeding through conventional and modern genetic methods has not yet resulted in significant improvement of this crop (Menecuccini and Rugini, 1993; Lavee, 1990; Rugini and Caricato, 1996). Failure to achieve this objective is due to its long juvenility phase (10-15 years), slow developmental stages and self-incompatibility in many olive cultivars (Canas et al., 1987a,b; Canas and Benbadis, 1988; Cozza et al., 1997; Lavee, 1990; Menecuccini et al., 1991; Rugini, 1998). Thus breeding of this species is difficult and time consuming, severely restricting breeders from quickly developing and propagating varieties.

In vitro propagation presents a very important alternative, also because it has higher rates of multiplying clean (pest and disease-free) planting material and the small amount of space required to multiply large number of plants. Micropropagation techniques were developed during the past two decades and are now well established (Batrollini et al., 1990; Cozza et al., 1997; Dimassi-Theriou, 1994; Rama and Pontikis, 1990; Rugini, 1984, 1988; Rugini and Fontanazza, 1981; Troncoso et al., 1999). Micropropagation from axillary buds is not a common practice in many olive cultivars (Cozza et al., 1997; Rugini and Caricato, 1996, Rugini and Lavee, 1992). This is because growth is slow, characterized by poor proliferation and delayed and insufficient in vitro rooting. Most recently in vitro propagation from axillary buds was reported in olive (Otero and Docambo, 1998). Growth regulators and media manipulations are the key factors for in vitro proliferation and regeneration in olive (Cozza et al., 1997; Dimassi-Theriou, 1994). The aim of this study was to study the effects of different growth regulators on shoot development from juvenile cuttings of field-grown olive (Olea europaea L.) cv. Leccino.

Materials and Methods
Research work was conducted at National Agricultural Research Centre, from Sep. 2001 to Jan. 2002, Agricultural Biotechnology Institute, NARC provided the experimental facilities. The explants of olive (Olea europa L.) cv. Leccino were kindly provided by Horticultural Research Institute, NARC, Islamabad.

Explants were obtained from the growing shoot apices of 10-15 years old field-grown Leccino plants. Single-node cuttings were obtained from 8th bud pair. Expanded leaves were removed and 4-5 cm long pieces were washed thoroughly in running tap water for 2-3 h and immersed for 30 min in a solution of 10% (v/v) of H2O2, 100 mg l-1 plus citric acid (150 mg l-1). Explants were disinfected by immersion in 0.1% mercuric chloride (HgCl2) for 5 min and rinsed three times with sterile distilled water. For complete sterilization explants were dipped in 60% Clorox (NaOCl) for 15 min and again washed with sterile distilled water. 5 min for each wash. The explants were then placed on MS (Murashige and Skoog, 1962) media supplemented with µM (0, 1, 2, 4, and 8) of GA3, Kn, BAP and 2IP. Medium in all the treatments contained 0.2 g l-1 polyvinylpyrrolidone (PVP) to prevent browning by phenolic exudates. The pH of the medium was adjusted to 6.8 prior to autoclaving at 121° C for 20 min. For solidification gelrite of Sigma Chemical Co @ 2 g l-1 was used. Culture conditions were 23-26°C, 16 hr photoperiod at 48 µmol s-1 m-2. Visual observations were taken after every week and subculture period was maintained after every 20-25 days. After 3 subcultures in the same media the data were collected for shoot height, extent of callus formation and incidence of hyperhydration.

Results and Discussion
Among different growth regulators tested for shoot growth, GA3 affected the shoot height greatly (Fig. 1a, b). GA3 @ 4 µM gave the maximum shoot height (32mm) (Fig. 1c). On the other hand BAP was least effective in enhancing the shoot height. Shoot height was reduced with an increase in the concentration levels of different cytokinins (Kn, BAP and 2IP). Hyperhydration incidence was increased with BAP, with a maximum of 40% at 8 µM GA3 and 2IP treated cultures as well as the control did not experience any hyperhydricity symptoms. Increased concentration of BAP also enhanced callusing at the base portion of shoots. Kinetin showed low callusing at higher concentration. No callusing was recorded in the control, 2IP and GA3 treated cultures throughout the period of study (Table 1). Dismassi-Theriou (1994) reported a shoot height of 10.4 mm in 'Kalamos' olive on woody plant medium (VPM) containing 4.4 µM BA, 4.9 µM IBA (indolebutyric acid) and 0.3 µM GA3. Zeatin riboside was reported effective in the micropropagation of 'Dolce Agroia' olive and was used @ of 45.6 µM in combination with 0.5 µM IBA and 0.3 µM GA3 (Rugini and Fontanazza, 1981). In contrast to these results Rugini (1984) reported that zeatin was the most effective cytokinin for shoot proliferation. These findings are in exact corroborations with Shibli et al. (2000) who reported a height of 48 mm in 'Nabali' olive on MS medium containing 6 µM GA3. Shoot proliferation was also reported to be genotype (cultivar) dependent (Rama and Pontikis).
Table 1: Influence of different growth regulators on shoot height, incidence of hyperhydration and callusing in olive (Olea europaea L.) cv. Leccino.

<table>
<thead>
<tr>
<th>Cytokinin(s)</th>
<th>Shoot height (mm)</th>
<th>Hyperhydration (%)</th>
<th>Callusing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>GA₃</td>
<td>1</td>
<td>30</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>20</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>28</td>
<td>0.0</td>
</tr>
<tr>
<td>Kinetin</td>
<td>1</td>
<td>24</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>16</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>13</td>
<td>14.0</td>
</tr>
<tr>
<td>BAP</td>
<td>1</td>
<td>28</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>14</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>14</td>
<td>20.0</td>
</tr>
<tr>
<td>2IP</td>
<td>1</td>
<td>27</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25</td>
<td>0.0</td>
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<tr>
<td></td>
<td>8</td>
<td>28</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Data represent mean of 10 replicates/treatment in two repeated experiments.

Diasambo (1981), Rugini (1980) and Canas and Benbadis (1990), where explants from adult cuttings suffered from rapid oxidation, despite preventive treatments. Tannin exudation (which causes blackening, explant necrosis and low incidence of sprouting) was stopped at all stages of micropropagation (Rugini, 1984). The use of ascorbic acid and citric acid at the end of sterilization does not improve the explant survival (Martino et al., 1989, Khan et al., 2002).

Based on these results, it is concluded that the single-node cuttings from the 6th bud pair of shoots were suitable for the in vitro culture of olive cv. Leccino. GA₃ affected the shoot height greatly. This cultivar is also suitable for cryopreservation, which makes it possible to conserve this valuable olive genetic resource for future.

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References


Khan et al.: In vitro shoot development of olive