Journal of Biological Sciences 3 (12): 1107-1113, 2003
ISSN 1727-3048

Rapid Clonal Propagation of "Native-olive" (*Elaeocarpus robustus* Roxb.) Using Tissue Culture Technique

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Abstract: Shoot tip and nodal segments from the field grown mature plants of native-olive (*Elaeocarpus robustus* Roxb.) were used as explants and were cultured on half strength of MS medium supplemented with different types of growth regulators either alone or in combinations. Among the growth regulators and supplements (BA, Kn, NAA and coconut water) that were used in the proliferation medium the best result was observed on ½ MS medium supplemented with 0.5 mgl^{-1} BA + 0.5 mgl^{-1} Kn + 0.1 mgl^{-1} NAA + 15% CW, which promoted multiple shoot bud formation and sufficient shoot elongation. The well-developed shoots were excised to 3-4 cm cuttings and implanted individually on root induction medium. Highest percentage of micro cuttings showed root formation when they were cultured on ½ MS medium containing 0.2 mgl^{-1} IBA and incubated under 30°C in dark for initial one week. The ***in vitro*** regenerated plants were successfully established in pot holes containing coco-peat on plastic tray and maintained under polythene tents. Survival of the plantlets under ***ex vitro*** condition was 60%.

Key words: Clonal propagation, *Elaeocarpus robustus*, tissue culture technique

Introduction

The native-olive (***Eleaocarpus robustus*** Roxb.) popularly known as "Jalpai" (family-Elaeocarpaceae) is one of the fruit trees of Bangladesh. Among less important woody fruit plants of Bangladesh ***Elaeocarpus robustus*** is one of the most familiar ones. The multipurpose fruit is the most important product of the plant despite its timber value and it is specially valuable for reforestation. It is believed to have originated in Australia, however, the native-olive is well grown in Bangladesh, India, Sri-lanka, Burma and Islands of Philippine. The tree is also said to be indigenous to the Chittagong and Sylhet forests of Bangladesh (Khan and Alam, 1996). The native-olive generally grown and cultivated in limited scale in Bangladesh.

The fruits and leaves of the native-olive have considerable medicinal and food values. The fruits of olive are hard, sour and acrid. It is rich in vitamin-C and is very popular among the children. The green fruits are eaten fresh and also used in making prickle preserve, chutney, soup, jelly, squash and mixed jam. Ethanolic extract of leaves is diuretic and cardiovascular stimulant and is also used in rheumatism. Fruits are also prescribed in diarrhoea and dysentery. Soup of the fruits is given for stimulating secretions from the test buds (Ghani, 1998).

The native-olive tree is generally grown from seeds. Propagation through seeds does not

conserve true-to-type fruit in this plant due to cross pollination. Clonal propagation through conventional methods like cutting or grafting have not been successful in this plant. Clonal multiplication of superior genotypes and valuable breeding stocks can be used for establishing tree improvement and seed production orchards (Dunstan ***et al.***, 1992). Micro-propagation method is specifically applicable to species in which clonal propagation is needed (Gamborg and Phillips, 1995). The application of tissue culture methods for improvement and large-scale propagation of fruit trees have been well demonstrated (Litz ***et al.***, 1985; James, 1988). Earlier reports on regeneration of *E. **robustus*** through tissue culture (Roy ***et al.***, 1993) resulted in the production of few plantlets. So far there is no other reports on plant regeneration of native-olive. Therefore, the present investigation was under taken to establish protocols for regenerating a large number of plantlets from the shoot tip and nodal explant cultures.

Materials and Methods

Eight to ten cm shoot pieces collected from the mature plants of *E. **robustus*** were first defoliated and treated with 1% Savlon (an antiseptic plus surfactant) for about 10 min, then washed thoroughly under tap water and finally surface sterilized with 0.1% $HgCl_2$ for 7 min followed by washing thrice in autoclaved distilled water. The explants consisting of (1-1.5 cm) shoot tip and nodal segments were prepared from the surface sterilized material. They were then implanted on agar-gelled half strength of MS medium (Murashige and Skoog, 1962) containing a series of concentrations of either BA alone or in different combinations of BA, Kn, NAA and 15% coconut water (CW) for shoot bud initiation and multiplication. Micro cuttings prepared from ***in vitro*** proliferated shoots were cultured in half MS medium fortified with 0.1-2.0 mgl^{-1} of either IBA, NAA or IAA and incubated under 30°C in darkness for initial one week. All media were supplemented with 3% sucrose, adjusted to pH 5.7±0.1, gelled with 0.6% agar and steam sterilized for 20 min at 121°C under 1.1 kg cm^{-2} pressure. The cultures were grown at 26±1°C under 16 h photoperiod with a photon flux density of about 60 μ $mol.m^{-2}s^{-1}$ (2500 lux).

Results and Discussion

Two types of explant showed different responses when they were cultured on ½ MS medium with various concentrations and combinations of cytokinins and auxins (Table 1). Of the two cytokinins (BA and Kn) used, BA was found to be comparatively batter for shoot proliferation. Similarly, the nodal segments responded better than the shoot tip explants and 85% culture of which produced shoots on ½ MS medium with 0.5 mgl^{-1} BA + 0.5 mgl^{-1} Kn + 0.1 mgl^{-1} NAA. The explants produced two to three shoots in 3-4 weeks after inoculation (Fig. A). On the other hand, about 60% cultures of shoot tip explants produced shoots on the medium with same combinations of hormones (data not shown in the table).

For subculturing, individual shoots grown on ½ MS containing 0.5 mgl^{-1} BA + 0.5 mgl^{-1} Kn + 0.1 mgl^{-1} NAA were excised, made into nodal segments and reinoculated onto fresh medium with same combination of growth regulators (Fig. B). In each subculture axillary and adventitious shoots grew up from each shoot segment and lateral branches developed from basal nodes of a

Table 1: Effect of growth regulators in ½ MS medium on shoot proliferation from the native-olive explants. Data were collected from 15-20 nodal explants after 8 weeks of their culture

Growth regulators (mgl^{-1})	% of shoot proliferation	No. of total shoots per culture	No. of usable shoots per culture	Average length (cm) of of shoots per culture
BA				
0.5	30	1.65±0.16	1.08±0.11	1.55±0.14
1.0	55	3.76±0.18	1.83±0.17	2.24±0.12
1.5	40	2.35±0.25	1.86±0.21	1.95±0.18
Kn				
0.5	20	1.35±0.12	1.00±0.16	1.25±0.13
1.0	45	3.10±0.18	1.45±0.12	2.00±0.10
1.5	35	2.07±0.25	1.20±0.26	1.55±0.17
BA+Kn				
0.5+0.2	55	2.45±0.12	1.96±0.15	2.23±0.13
0.5+0.5	75	5.56±0.42	3.25±0.21	2.85±0.18
1.0+0.2	60	3.25±0.16	2.38±0.13	2.34±0.14
1.0+0.5	50	2.15±0.18	1.87±0.24	2.10±0.23
BA+Kn+NAA				
0.5+0.2+0.1	60	4.92±0.29	3.64±0.19	3.12±0.17
0.5+0.5+0.1	85	6.78±0.27	4.36±0.28	3.26±0.25
1.0+0.2+0.1	40	2.88±0.23	2.36±0.26	2.21±0.14
1.0+0.5+0.1	50	3.25±0.15	2.45±0.12	2.36±0.13
BA+Kn+NAA+CW				
0.5+0.2+0.1+15%	80	5.56±0.42	4.27±0.26	3.12±0.15
0.5+0.5+0.1+15%	90	11.33±1.30	8.76±0.86	4.27±0.31
1.0+0.2+0.1+15%	60	4.52±0.24	4.10±0.19	2.86±0.28
1.0+0.5+0.1+15%	85	8.64±0.56	5.44±0.42	3.61±0.24

Table 2: Effect of different concentration and combination of auxins on adventitious root formation from the *in vitro* grown micro cuttings cultured on ½ MS medium. There were 15-20 micro cuttings in each treatment. Data (x̄±SE) were recorded after 6-8 weeks of culture

Types of auxin	Different conc. of auxin (mgl^{-1})	% of micro-cutting rooted	No. of root per micro-cutting	Average length of the root (cm)	Callus formation at the cutting base
IBA					
	0.1	45	1.40±0.12	3.50±0.21	-
	0.2	90	2.50±0.21	4.00±0.46	-
	0.5	60	2.35±0.15	3.25±0.18	++
	1.0	-	-	-	+++
NAA					
	0.1	40	1.25±0.21	3.00±0.24	-
	0.2	80	2.00±0.23	3.45±0.14	-
	0.5	55	1.90±0.18	2.90±0.23	++
	1.0	-	-	-	+++
IAA					
	0.1	30	1.40±0.23	2.35±0.18	-
	0.2	50	1.65±0.26	3.30±0.16	-
	0.5	45	1.50±0.21	1.85±0.13	++
	1.0	-	-	-	+++

Table 3: Effects of dark treatment and temperature during initial one week of rooting of micro-cuttings on media contains 0.2 mgl^{-1} of IBA. Each treatment consisted of 15-20 micro cuttings and data (=±SE) were recorded after 6 weeks of total culture

Culture condition	% of micro-cutting rooted	No. of root per micro-cutting	Average length of the root (cm)	Callus formation at the cutting base
Temperature 30°C plus dark treatment 1 week	100	2.30±0.21	4.63±0.31	+
Temperature 25°C plus dark treatment 1 week	85	2.00±0.18	4.15±0.28	+
Temperature 25°C plus culture under 16-h photoperiod for 6 weeks	75	1.70±0.15	3.80±0.24	++

good number of these newly formed shoots. A clump of five to seven shoots was thus produced after 3-4 subcultures (Fig. C and D). But the growth and length of the regenerated shoots on above media were not sufficient enough for their transfer into the rooting medium.

In an attempt to enhance shoot proliferation and elongation, coconut water (CW) was added to the medium. Addition of 15% CW to the proliferation medium increased the number of useable shoots per culture. Thus the more effective medium supplementation determined for multiplication of large number of shoots with proper length was 0.5 mgl^{-1} BA + 0.5 mgl^{-1} Kn+0.1 mgl^{-1} NAA+15 % CW. On this media combination 90% of the nodal explants produced 11.34±1.30 shoots/ culture, where number of usable shoots/ culture was 8.76±0.68 and average length of shoots/culture was 4.27±0.33 cm (Fig. E). Roy ***et al.*** (1993) and Rahman ***et al.*** (1999) reported that addition of 10% CW increased the number of shoot in ***Eleaocarpus robustus*** and ***Emblica officinalis*** cultures, respectively. There are other reports on the effects of the addition of complex organic substances on the growth of culture of woody species (Gamborg ***et al.***, 1976; Thrope, 1982; Sen ***et al.***, 1992).

Micro cuttings (3-4 cm) prepared from the ***in vitro*** proliferated shoots were cultured on ½ MS medium with 0.1-1.0 mgl^{-1} of either IBA, NAA or IBA for adventitious rooting. The effect of these three auxins on percentage of root formation, number of roots per shoot and length of the longest roots were recorded after six weeks of culture. The rooting responses to different auxins and treatments are shown in Tables 2 and 3.

Both the concentration and nature of the auxins used markedly influenced percentage of root formation and number of roots per shoots. Among the three types of auxins used, IBA was found to be the best for root induction. Ninety percent of shoot cuttings produced roots when they were cultured on the medium with 0.2 mgl^{-1} IBA. Higher concentration (1.0 mgl^{-1}) of IBA, NAA or IAA could not form any root but produced callus at the cut bases of the shoots. The free-hand sections of the calloid cutting bases showed that there were rudimentary roots deep into the callus tissue at the surface of shoot cuttings. It was an indication that high concentration of the auxins induced root formation but they hampered root emergence and elongation by producing callus tissue at the site. Cent percent shoot produced roots when they were cultured on ½ MS medium containing 0.2 mgl^{-1} IBA and incubated under 30°C in dark for initial one week. In this treatment, the highest number of roots per micro cutting was 2.80±0.21 and their maximum length was 4.63±0.46 cm (Fig. F). Complete darkness for initial one week and comparatively higher temperature (30°C) during root initiation phase was found to promote

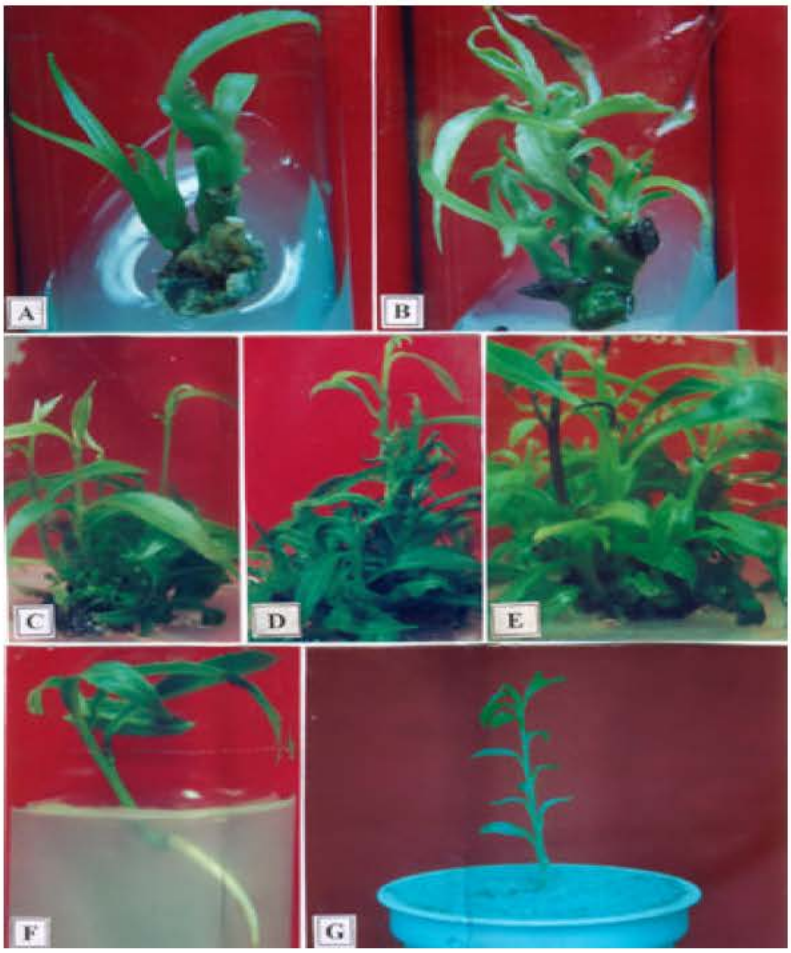

Fig. 1: A-G. *In vitro* proliferation of shoots and development of complete plantlets in *Elaeocarpus robustus* Roxb.

A: Two shoots proliferated from a nodal explant on ½ MS + 0.5 mgl^{-1} BA + 0.5 mgl^{-1} Kn + 0.1 mgl^{-1} NAA after three weeks of culture.

B: Multiple shoot proliferation from a nodal explant on same medium after five weeks of culture.

C, D: Multiplication and elongation of shoots from nodal explants on same medium after eight weeks of culture.

E: A clump of proliferated shoots from nodal explants on ½ MS + 0.5 mgl^{-1} BA + 0.5 mgl^{-1} Kn + 0.1 mgl^{-1} NAA + 15% CW after twelve weeks of culture.

F: Root formation in the regenerated shoot on ½ MS + 0.2 mgl^{-1} IBA + incubation under 30°C in dark for initial one week.

G: *In vitro* regenerated plantlets transferred in small plastic pot

rooting frequency in ***E. robustus*** micro cutting . The findings are in agreement with those observed in jack fruit (Amin, 1990) and carambola (Amin ***et al.***, 1992). Apple micro cutting also showed increased rooting in response to dark treatment (Welander, 1983). Percentage of cuttings rooted and number of roots per cuttings were improved by raising the ambient temperatures of the culture from 25 to 30°C similar increased rooting frequency at 30°C than at 25°C has also been observed in guava (Amin and Jaiswal, 1989) and apple (Zimmerman, 1984; Zimmerman and Fardhan, 1985). In the present experiment different attempts were made to establish ***in vitro*** regenerated plantlets but the survival rate was only 60% (Fig. G).

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