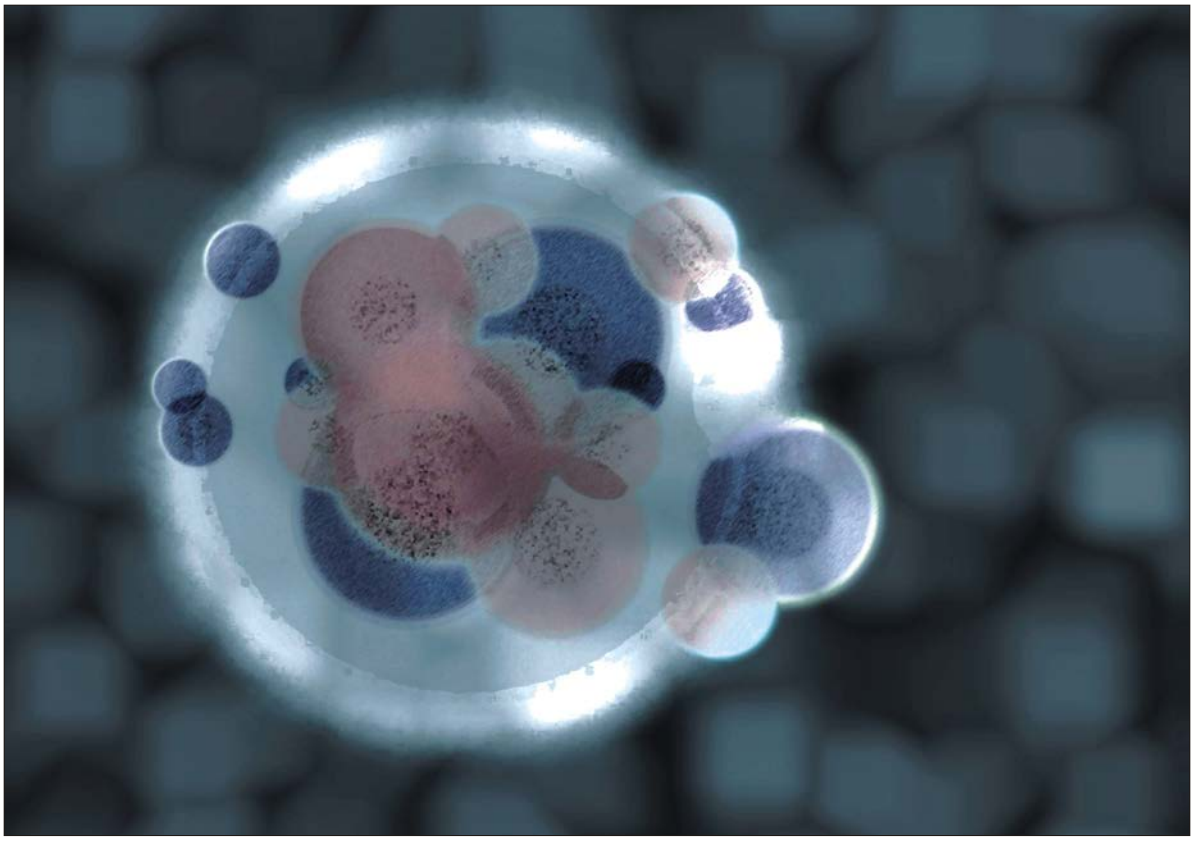

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In vitro Rooting Performance of Native-olive (*Elaeocarpus robustus* Roxb.) under Different Auxins and High Temperature Treatments

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Abstract: Micropropagation of native-olive (*Elaeocarpus robustus* Roxb.) has been proved difficult due very slow rooting of the shoot cuttings. The objective of the present investigation was to develop a technique for *in vitro* fast rooting of the aseptically grown shoots of native-olive. Rooting experiment was conducted on $^1/_2$MS medium with various concentrations and combinations of NAA, IBA and IAA along with or without incubation at higher temperature (30°C) in dark condition for initial one week. Root forming performance of IBA was proved to be the best among the three auxins tested. Highest frequency of rooting with maximum number of effective roots was observed on the hormone-free medium in normal growth room condition after treating the microcutting with 0.2 mg L^{-1} IBA and incubated under 30°C in dark for initial one week. Early emergence and fast growth of roots without any malformation were also observed at the same treatment. At this culture condition 85% of the microcuttings produced 5.80±0.15 roots per cutting where average length of roots per cutting was 4.65±0.18 cm. The plantlets originated through the above treatment established themselves under *ex vitro* condition much quicker than those originated through other treatments.

Key words: *In vitro, Elaeocarpus robustus*, rooting

INTRODUCTION

The native-olive (*Elaeocarpus robustus* Roxb.) is one of the most familiar fruits among the potentially important woody fruit plants of Bangladesh. It belongs to the family Elaeocarpaceae and is believed to have originated in Australia. A medium to big-sized tree planted for its edible fruits and timber in many areas of Bangladesh[1]. The improvement of fruit tree like native-olive through the application of conventional breeding methods has been extremely slow. The difficulty in rooting the cuttings from elite tree species has further complicated the process of their clonal propagation. Accumulation of inhibiting substances (phenolic compounds) in the growth medium is a major problem more frequently associated with micropropagation and root formation of woody perennials. Another problem of *in vitro* rooting is the callus formation at the microcutting base in auxin enrich medium that subsequently results in malformation and failure of vascular connection between the root and shoot. So, many commercial laboratories do not root microcuttings *in vitro* because it is labour-intensive and expensive. The process of rooting *in vitro* has been estimated to account for approximately 35 to 75% of the total cost of micropropagation[2].

To overcome of these problems of fruit plants many researchers have been successful in root formation using different techniques. Addition of activated charcoal (AC) to the auxin-containing medium markedly improved rooting behaviour of the microcutting and checked callus formation at the microcutting base[3]. Dark treatment during initial stage is an important factor for rooting where normal condition was not effective. Apple microcutting also showed increased rooting in response to dark treatment[4]. Ambient temperature during induction period plays an important role in rooting of microcuttings. It has been observed that rooting frequency and root number per cutting increased significantly 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[5] and in apple[6,7].

The *in vitro* propagation technique of native olive has been reported earlier but the results are not reproducible[8-10]. The *in vitro* shoot multiplication of this plant can be achieved easily but root formation is found to be very difficult. Keeping this view in mind, the present research work was under taken as a new approach in the advancement of native-olive micropropagation. In this study, an effort was made to establish a reproducible protocol for *in vitro* rooting of the native-olive microcuttings through manipulation of rooting environment.

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MATERIALS AND METHODS

The *in vitro* grown shoots of native-olive were used as source of starting material to conduct different experiments in the present investigation. The study was carried out in the Plant Tissue Culture Laboratory, Department of Botany, University of Rajshahi. The experiments for adventitious root formation on the shoots proliferated *in vitro* were conducted only after having sufficient amount of shoot cultures. Different rooting experiments were carried out to determine the suitable media composition, auxin requirement, dark and temperature treatment and other culture conditions. After 10-12 weeks of proliferation, the usable shoots (with length more than 3 cm) from the multiple shoot masses were separated and individual shoots were placed on ½ MS medium supplemented with different concentrations and combinations of auxins and without any auxin. The adventitious roots were produced from the cut bases of microcuttings within 2-3 weeks of culture on suitable medium when the microcuttings were treated under appropriate temperature and complete dark condition. The well prepared media were adjusted to pH 5.7±0.1 and sterilized by autoclaving at 121°C for 20 min at 1.1 kg cm^{-2} pressure. The cultures were maintained in an air-conditioned culture room maintained at 16 h photoperiod with a light intensity of 2000-3000 lux (50-70 $\mu Em^{-2} s^{-1}$) provided by 40 W cool-white florescent tubes where temperature was maintained at 26±1°C but humidity was not controlled for the experiments. For dark and temperature treatments rooting cultures were also grown in a incubator maintained at different temperature regimes.

RESULTS AND DISCUSSION

Successful rooting of the *in vitro* grown shoots is an important factor for establishing tissue culture derived platelets on the soil. Without establishment of the regenerated plantlets *in vitro* propagation is virtually meaningless and it depends upon efficient rooting of the shoots. For this purpose, the present experiments were carried out on adventitious root formation in shoot cuttings taken from the *in vitro* proliferated shoots.

Effects of single auxin treatment: Microcuttings (3-4 cm) were isolated from the *in vitro* proliferated usable shoots and cultured on ½ MS medium supplemented with 0.05-2.0 mg L^{-1} either of IBA, NAA or IAA for adventitious rooting (Fig. 1A and B). Among the three types of auxin, IBA at a concentration of 0.2 mg L^{-1} was found to be the best for root formation. The percentage rooted shoots, number of roots and length of roots were not markedly affected by the auxin type in the normal growth room condition (Table 1). Superiority of IBA to other auxins for inducing adventitious roots has been proved in many fruit trees like apple, guava and jackfruit[3,5,11,12].

Effects of double auxin treatments: In this experiment, excised microcuttings were also cultured on ½ MS medium supplemented with different combinations (two auxins at a time) of three auxins (IBA, NAA and IAA) at different concentrations. The percentage of cutting rooted, number of roots per rooted cutting and average length of roots reveal that only some specific combinations of IBA and NAA produced increased number of roots than rest of the treatments (Table 2). Among the various combinations of IBA and NAA, best rooting result was observed on the medium supplemented with 0.1 mg L^{-1} IBA+0.1 mg L^{-1} NAA after 6-8 weeks of culture. On this growth regulator combination 70% of the microcuttings produced 3.55±0.12 roots per culture, where average length of the roots was 4.50±0.32 cm (Fig. 1C). When the medium contained higher concentrations of IBA and NAA, then the percentage of root formation, number of roots per microcutting and average length of roots decreased gradually with the increase of the growth regulator concentration and produced mass of callus at the end of cutting base. Synergistic effect of IBA+NAA was also observed during root induction for guava[13], *Averrhoa carambola*[14] and jackfruit[12].

Effects of auxin, dark and higher temperature treatments: Other phase of the experiment was to determine the effect of different concentrations of the better auxin (IBA) on induction and growth of adventitious roots if incubated under 30°C in dark for initial one week (Table 3). The highest percentage of root formation was 70%, highest number of roots per microcuttings was 4.00±0.30 and average length of roots per microcuttings was 4.25±0.15 cm, which were observed in ½ MS with 0.2 mg L^{-1} IBA and incubated under 30°C in dark for initial one week (Fig. 1E). In this experiments all growth parameters of root formation were improved but excessive callus formation at the cut base of microcuttings and malformation of roots were observed. In the other experiment, microcuttings were cultured on ½ MS medium supplemented with different concentrations of IBA with incubated under normal growth chamber temperature (26±1°C) in dark for initial one week, where maximum root formation was 50%, number of roots per microcutting was 3.20±0.16 and average length of roots per culture was 4.00±0.26 cm when medium having 0.2 mg L^{-1} IBA (Fig. 1D). In the another experiment, microcuttings were cultured on ½ MS medium supplemented with different concentrations of IBA, incubated under 30°C in dark for initial one week and then transfer to the hormone-free

Table 1: Effects of different concentrations of single auxins on adventitious root formation from the *in vitro* grown microcuttings cultured on ½ MS medium. There were 20 microcuttings in each treatment and data (x̄±SE) were recorded after 6-8 weeks of culture

Conc. of auxins (mg L^{-1})	% of cutting rooted	No of roots per rooted cutting	Average length of the roots (cm)	Callus formation at the cutting base
IAA				
0.05	-	-	-	-
0.1	30	1.52±0.27	2.52±0.32	-
0.2	35	1.71±0.16	2.84±0.30	-
0.5	40	2.05±0.20	3.45±0.20	-
1.0	20	1.22±0.25	2.25±0.24	-
1.5	15	1.00±0.21	2.00±0.20	+
2.0	-	-	-	++
IBA				
0.05	-	-	-	-
0.1	40	2.00±0.55	3.60±0.32	-
0.2	50	2.40±0.23	4.00±0.26	-
0.5	35	1.80±0.22	3.20±0.30	
1.0	30	1.55±0.20	3.00±0.22	+
1.5	-	-	-	++
2.0	-	-	-	++
NAA				
0.05	-	-	-	-
0.1	35	1.80±0.20	3.52±0.35	-
0.2	50	2.22±0.23	3.84±0.21	-
0.5	30	1.51±0.13	3.00±0.32	-
1.0	30	1.20±0.24	2.80±0.28	++
1.5	-	-	-	++
2.0	-	-	-	+++

(-) indicate no response; (+) slight callusing; (++) considerable callusing and (+++) profuse callusing

Table 2: Effects of three auxins in combinations on adventitious root formation from the *in vitro* grown microcuttings cultured on ½ MS medium. There were 20 microcuttings in each treatment and data (x̄±SE) were recorded after 6-8 weeks of culture

Combination of auxin (mg L^{-1})	% of cutting rooted	No. of roots per rooted cutting	Average length of the roots (cm)	Callus formation at the cutting base
IBA+NAA				
0.1+0.05	45	2.85±0.25	4.00±0.12	-
0.1+0.1	70	3.55±0.12	4.50±0.32	-
0.1+0.2	60	3.25±0.21	4.20±0.25	-
0.2+0.05	55	3.00±0.23	4.00±0.26	-
0.2+0.1	40	2.66±0.20	3.68±0.21	-
0.2+0.2	30	1.82±0.18	3.00±0.15	+
IBA+IAA				
0.1+0.05	40	2.75±0.26	3.60±0.15	-
0.1+0.1	50	3.10±0.24	3.80±0.20	-
0.1+0.2	65	3.30±0.15	4.00±0.33	-
0.2+0.05	30	2.44±0.22	3.25±0.24	-
0.2+0.1	35	2.55±0.20	3.45±0.22	-
0.2+0.2	20	1.45±0.16	2.85±0.20	++
NAA+IAA				
0.1+0.05	30	2.35±0.12	3.10±0.21	-
0.1+0.1	40	2.40±0.14	3.25±0.20	-
0.1+0.2	45	2.60±0.20	3.30±0.32	-
0.2+0.05	30	2.25±0.14	3.00±0.24	-
0.2+0.1	-	-	-	+
0.2+0.2	-	-	-	+++

(-) indicate no response; (+) slight callusing; (++) considerable callusing and (+++) profuse callusing.

medium. In this case, maximum root formation was observed on the hormone-free medium in normal growth room condition after treatment of 0.2 mg L^{-1} IBA with incubation under 30°C in dark for initial one week. In this condition 85% of the cultured microcuttings produced 5.80±0.15 of roots per microcutting and average length of roots per culture was 4.65±0.18 cm (Fig. 1F).

Complete darkness and comparatively higher temperature (30°C) during root initiation phase was found to promote the rooting frequency in *Elaeocarpus robustus* microcuttings. Similar result was obtained by Rahman *et al.*[9]. However, root formation in true-olive (*Olea europea*) depended mainly on shoot quality and environment conditions[15,16]. Rugini *et al.* [15] also reported that darkness during the rooting phase caused an increase in efficiency of rooting and root growth, this increase is similar to the results caused by placing the entire culture in total darkness for one week. The findings are also in agreement with those observed in Jackfruit[17] and Carambola[18]. Hammerschlag[19] stated that a 2-week dark period was essential for maximum rooting *in vitro* of Calita plum. Apple microcuttings also showed

Table 3: Effects of dark and higher temperature treatments during initial one week of rooting of microcuttings on media containing different concentrations of IBA. Each treatment consisted of 15-20 microcuttings and data (x̄±SE) were recorded after 6 weeks of total culture

Culture condition	Conc. of auxins (mg L^{-1})	% of microcutting rooted	No. of root per microcutting	Average length of the root (cm)	Callus formation at the cutting base
26±1°C plus dark treatment for initial	IBA				
1 week and then maintained at same	0.05	-	-	-	-
temperature	0.10	45	2.74±0.24	3.75±0.35	-
	0.20	50	3.20±0.16	4.00±0.26	+
	0.50	40	2.45±0.12	3.60±0.24	++
	1.00	-	-	-	+++
30±0.1°C plus dark treatment for	IBA				
initial 1 week and then maintained in	0.05	-	-	-	-
auxinmedinm at 26±1°C	0.10	60	3.00±0.12	4.00±0.24	-
	0.20	70	4.00±0.30	4.25±0.15	++
	0.50	50	2.86±0.24	3.80±0.18	++
	1.00	-	-	-	+++
30±0.1°C plus dark treatment for	IBA				
initial 1 week and then transferred	0.05	-	-	-	-
to auxinfree medium and maintained	0.10	60	3.25±0.12	4.50±0.24	-
at 26±1°C	0.20	85	5.80±0.15	4.65±0.18	-
	0.50	50	3.00±0.20	4.20±0.25	-
	1.00	-	-	-	++

(-) indicate no response; (+) slight callusing; (++) considerable callusing and (+++) profuse callusing.

increased rooting in darkness[4]. Reasons for the enhanced rooting in darkness is not clear; however, it has been reported that levels of certain natural growth inhibitors are lower in plant tissue grown under dark than that grown under light[20,21]. Percentage of cuttings rooted and number of roots per cutting 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[5] and apple[6,7].

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