OnLine Journal of Biological Science 3 (8): 718-725, 2003
ISSN 1608-4217

In vitro Rapid Regeneration of Plantlets from Leaf Explant of Native-olive (*Elaeocarpus robustus* Roxb.)

M.M. Rahman, M.N. Amin, M.A.K. Azad, F. Begum and M.Z. Karim
Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: Leaf segments from field grown mature plants of *Elaeocarpus robustus* Roxb. were used to initiate cultures for inducing callus and subsequent differentiation of shoot buds. Callus production was obtained within seven weeks and adventitious shoot formation was observed after five weeks of incubation on ½ MS medium supplemented with 0.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA + 15% CW. Complete plantlets were obtained upon transfer of shoot cuttings on ½ MS with 0.1-2.0 mg l^{-1} IBA, NAA, IAA or without any auxin. The best and healthy rooting was observed on ½ MS medium supplemented 0.2 mg l^{-1} IBA. *In vitro* regenerated plantlets were transferred to potting soil and successfully established under natural condition with about 60% survival.

Key words: Regeneration, *in vitro*, leaf explant, *Elaeocarpus robustus*

Introduction

Regeneration of plants from adventitious shoots is necessary for application of gene transfer technology and for screening plants for somaclonal variation. The latter purpose would be of particular interest in situations in which conventional breeding programmes have had relatively little impact upon commercial production. Among less important woody fruit plants of Bangladesh *Elaeocarpus robustus* is one of the most familiar ones. Although there is no well known variety of this fruit in Bangladesh, but it is cultivated in limited scale in our country. The native-olive tree is also said to be indigenous to the Chittagong and Sylhet forest of Bangladesh (Khan and Alam, 1996). The native-olive is believed to have originated in Australia. A medium sized to big tree planted for its edible fruits and timber in many areas of the country and it is specially valuable for reforestation (Ghani, 1998).

Ethanolic extract of leaves are diuretic and cardiovascular stimulant and are also used in rheumatism. Fruits are prescribed in diarrhoea and dysentery. Soup of the fruit are given for stimulating secretion from the test buds. Its is rich in vitamin C. The green fruits are eaten fresh and also used in making soup, chutney, gelly and gam. The native-olive tree is generally grown from seeds. It may also be propagated by vegetative means through stem cutting and layering. Clonal propagation of selected trees by air layering, approach grafting or budding onto seedlings rootstalks is possible, but the number of plants produced by this conventional method is relatively low (Row-dutton, 1976; Samadder, 1990). The application of tissue culture methods for improvement and large-scale propagation of fruit trees have been well demonstrated (Litz ***et al.***, 1995 and James, 1988). This plants have received very little attention for its multiplication

through ***in vitro*** culture. Regeneration of plantlets from shoot tip and nodal explants of mature native-olive trees has also been reported (Roy ***et al.***, 1993 and Rahman ***et al.***, 2001). The earlier report described ***in vitro*** plantlets regeneration through axillary shoot proliferation. In an alternative attempt to induce callus and shoot proliferation, we have achieved repetitive callus formation, adventitious shoot formation and plantlet regeneration in the cultures of leaf explants of native-olive.

Materials and Methods

Leaves were collected from the field grown mature plants of ***Elaeocarpus robustus*** Roxb. They were washed in running tap water for 7 min. and than surface sterilized with 0.1% (w/v) $HgCl_2$ solution for 5 min. followed by through washing with sterile distilled water. After the removal of petiole, three transverse cuts were made through the midrib at 1 mm interval in the middle part without serving the leaf completely. Each leaf was then placed with the adaxial side touching the medium. The leaf explants were culture on $_{½}$ MS medium supplemented with different concentrations and combinations of cytokinin and auxin such as BA, Kn, NAA, IBA were used for induction of callus.

Eight-week-old calluses were subcultured on $_{½}$ MS medium containing 0.1-1.0 mg l^{-1} BA or Kn with 0.1-0.5 mg l^{-1} of either NAA, IBA or IAA for organogenesis and plantlet formation. Effect of coconut water (CW) on shoot formation and multiplication was also determined. The calluses were subcultured at monthly interval on fresh medium of the same composition. Microcuttings prepared from ***in vitro*** grown shoots were rooted in $_{½}$ MS medium fortified with 0.1-1.0 mg l^{-1} of either NAA, IBA, IAA. The pH of the medium was adjust to 5.8 before autoclaving at 1.1 kg cm^{-2} and 121°C for 20 min. The medium was gelled with 0.7% agar. The cultures were maintained 26±1°C under 16 h photoperiod. Four-weeks-old plantlets were taken out from the culture tubes and washed to free them from agar. Then they were transplanted to plastic pots containing coco-peat and covered with transparent polyethylene lid to maintain high humidity. After 7 days polyethylene lid was removed and after two months the plants were planted in the open field.

Results and Discussion

Leaf explants were cultured on $_{½}$ MS medium supplemented with different concentrations and combinations of BA, Kn, NAA and IBA for inducing callus and regenerating adventitious shoots. The data on percentage of shoots formation, intensity of callus growth, number of total shoots per culture and average length of shoots per culture from different treatments were recorded after 9 weeks of culture initiation. Results obtained on morphogenic response of the cultured explants were shown in (Table 1 and 2). Among various combinations of BA and NAA, the leaf explants (Fig. A) showed good results on the medium with 1.0 mg l^{-1} BA+0.1 mg l^{-1} NAA and 1.0 mg l^{-1} BA+0.2 mg l^{-1} NAA that produce calli with shoots from 90% and 85% explants. The frequency of shoot proliferation was maximum at 1.0 mg l^{-1} BA+ 0.1 mg l^{-1} NAA and the number was 8.97±0.68 shoots per culture (Fig. C). Among the BA-IBA combinations, maximum frequency of shoot bud differentiation was observed in 75% culture at 1.0 mg l^{-1} BA with 0.2 mg l^{-1} IBA. Maximum of 5.24±0.42 shoot per culture was recorded at the same combinations. On the other

Table 1: Effect of the cytokinin (BA, Kn) and the auxin (NAA, IBA) at different concentrations and combinations in ½ MS medium on callus formation and shoot regeneration from the callus tissue of *Elaeocarpus robustus*. Data were taken after 9 weeks of culture

Growth regulators (mg l^{-1})	% of shoot formation	Intensity of callus growth	No. of total shoots per culture	Average length of shoots per culture
BA+NAA				
0.5+0.1	50	++	4.00±0.15	2.65±0.12
0.5+0.2	35	++	2.16±0.42	2.35±0.42
0.5+0.5	30	+++	1.86±0.21	2.30±0.18
1.0+0.1	90	+	8.97±0.68	3.27±0.38
1.0+0.2	85	++	6.64±0.56	3.12±0.28
1.0+0.5	45	++	3.35±0.25	2.50±0.25
2.0+0.1	60	+	4.68±0.42	2.85±0.26
2.0+0.2	45	++	2.83±0.17	2.45±0.12
2.0+0.5	30	+++	1.55±0.16	2.25±0.16
BA+IBA				
0.5+0.1	30	+	3.35±0.18	2.15±0.14
0.5+0.2	45	++	3.80±0.24	2.14±0.15
0.5+0.5	25	+++	1.66±0.21	2.10±0.18
1.0+0.1	60	+	4.52±0.34	2.85±0.14
1.0+0.2	75	++	5.25±0.42	3.15±0.18
1.0+0.5	35	++	2.85±0.16	2.10±0.15
2.0+0.1	40	++	3.55±0.23	2.15±0.25
2.0+0.2	50	++	4.05±0.15	2.23±0.13
2.0+0.5	30	+++	2.52±0.17	2.00±0.21
Kn+NAA				
0.5+0.1	40	+	3.67±0.21	2.58±0.18
0.5+0.2	25	++	3.26±0.26	2.48±0.26
0.5+0.5	20	+++	2.15±0.29	2.30±0.27
1.0+0.1	65	+	6.52±0.63	3.10±0.31
1.0+0.2	55	++	4.66±0.23	2.90±0.25
1.0+0.5	25	++	2.87±0.16	2.40±0.23
2.0+0.1	50	++	4.25±0.22	2.85±0.28
2.0+0.2	35	++	3.55±0.27	2.50±0.24
2.0+0.5	20	+++	2.50±0.25	2.35±0.22
Kn+IBA				
0.5+0.1	25	++	3.35±0.26	2.48±0.16
0.5+0.2	40	++	3.56±0.29	2.55±0.25
0.5+0.5	20	+++	2.65±0.63	2.00±0.16
1.0+0.1	50	++	4.16±0.25	2.60±0.22
1.0+0.2	60	+	4.62±0.23	2.80±0.31
1.0+0.5	30	++	3.45±0.14	2.50±0.12
2.0+0.1	30	++	3.50±0.26	2.50±0.21
2.0+0.2	45	++	3.84±0.16	2.58±0.26
2.0+0.5	25	+++	3.24±0.15	2.45±0.16

Callus growth rating value = (+) poor, (++) moderate and (+++) profuse callus formation

Table 2: Effect of different concentrations and combinations of the cytokinin (BA, Kn) and the auxin (NAA, IBA) with 15% coconut water (CW) in ½ MS medium on callus formation and shoot regeneration from the callus tissue of *Elaeocarpus robustus*. Data were taken after 6 weeks of culture

Growth regulators (mg l^{-1})	% of shoot formation	Intensity of callus growth	No. of total shoots per culture	Average length of shoots per culture
BA+NAA+CW				
0.5+0.1+15%	60	+	6.85±0.56	3.46±0.27
0.5+0.2+15%	50	++	5.65±0.35	3.15±0.29
1.0+0.1+15%	100	+	10.60±1.76	3.71±0.32
1.0+0.2+15%	80	++	8.60±0.42	3.56±0.45
2.0+0.1+15%	75	+	7.55±0.42	3.50±0.25
2.0+0.2+15%	55	++	6.55±0.28	3.25±0.28
BA+IBA+CW				
0.5+0.1+15%	70	+	6.55±0.12	2.96±0.13
0.5+0.2+15%	55	++	5.44±0.42	2.52±0.25
1.0+0.1+15%	85	+	8.76±0.86	3.12±0.15
1.0+0.2+15%	65	++	6.52±0.23	2.70±0.31
2.0+0.1+15%	50	++	4.36±0.28	2.41±0.24
2.0+0.2+15%	45	++	3.64±0.19	2.12±0.17

Callus growth rating value = (+) poor, (++) moderate and (+++) profuse callus formation

Table 3: Effect of different concentration and combination of auxins on adventitious root formation from the *in vitro* grown micro-cutting cultured on ½ MS medium. There were 15-20 micro-cuttings in each treatment. Data ($\bar{x}$± S.E) were recorded after 6-8 weeks of culture

Type of auxin	Different concentration of auxin (mg/l)	% of micro-cutting rooted	Number of root per micro-cutting	Average length of the root (cm)
IBA				
	0.1	45	1.40±0.12	3.50±0.21
	0.2	80	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	70	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	-	-	-

hand, among different combinations of Kn with NAA, maximum frequency of shoot bud formation was observed in 65% and maximum number of shoot per culture was 6.52±0.63 recorded at the 1.0 mg l^{-1} Kn+0.1 mg l^{-1} NAA.

Among the Kn-IBA combinations, maximum frequency 60% of shoot bud differentiation and maximum number of shoots (4.62±0.23) per culture was observed at 1.0 mg l^{-1} Kn + 0.2 mg l^{-1} IBA.

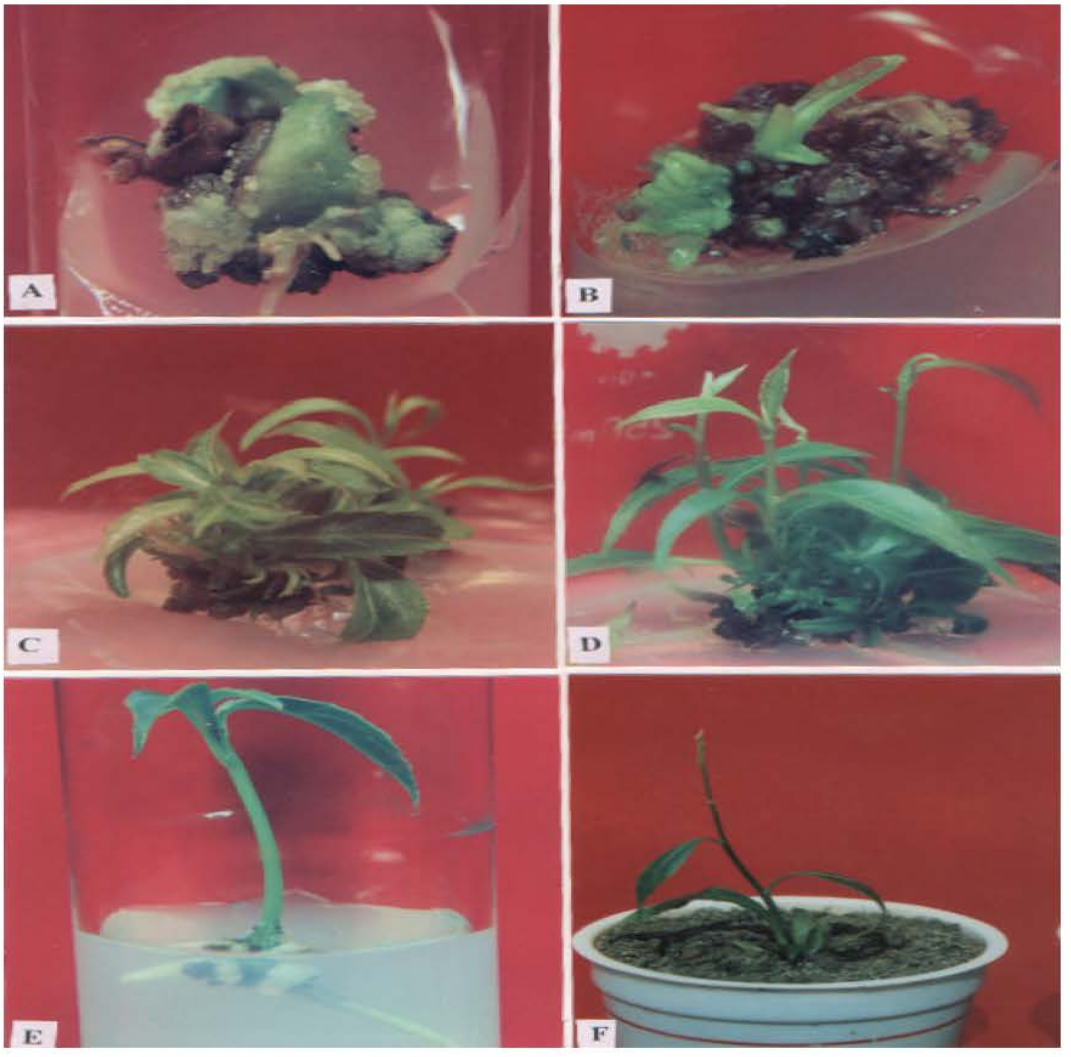

Fig.1 A-F: Callus induction and organogenesis in *Elaeocarpus robustus*.

A: Induction of callus on leaf explants on ½ MS supplemented with 1.0 mg l^{-1} BA+ 0.1 mg l^{-1} NAA after nine weeks in culture.

B: Shoot bud formation from callus on ½ MS containing 1.0 mg l^{-1} BA+ 0.1 mg l^{-1} NAA after nine weeks in culture.

C: Development of shoots from shoot bud primordia after six weeks in culture.

D: Development of shoots on ½ MS supplemented with 1.0 mg l^{-1} BA+ 0.1 mg l^{-1} NAA+ 15%CW after six weeks in culture.

E: Development of roots from the base of excised shoot on ½ MS fortified with 0.2 mg l^{-1} IBA after six weeks in culture.

F: Regenerated plantlet on the soil after eight weeks of transfer under *in vitro* condition.

Other combination with moderate response were 1.0 mg l^{-1} BA+0.2 mg l^{-1} NAA, 1.0 mg l^{-1} BA+0.1 mg l^{-1} IBA, 1.0 mg l^{-1} Kn+0.2 mg l^{-1} NAA and 1.0 mg l^{-1} Kn+0.1 mg l^{-1} IBA that produced calli with shoots from 85, 60, 55 and 50% explants. Among the treatment 2.0 mg l^{-1} BA or Kn with 0.5 mg l^{-1} NAA and IBA produce fast growing callus, but failed to induce any shoot. Out of BA with NAA or IBA and Kn with NAA or IBA combinations, 1.0 mg l^{-1} BA with 0.1 mg l^{-1} NAA showed maximum results in the frequency of shoot regeneration from leaf explant. Combination of 2.0 mg l^{-1} BA/Kn with 0.5 mg l^{-1} NAA/IBA resulted the best callus growth from the leaf explant. Comparatively higher levels of the cytokinin (1.0-2.0 mg l^{-1}) with a lower level of the auxin (0.1-0.2 mg l^{-1}) was essential for the differentiation of adventitious shoots. Similar observation were made on seedling leaf segments culture of carambola (Litz and Conover, 1980) and stem callus culture of Jackfruit (Amin, 1991). Superior effect of BA-NAA combination on adventitious bud proliferation from leaf explant has also been reported by Islam ***et al.*** (1992) for ***Aegle marmelos***. Organogenesis and plants formation in presence of BA with NAA was reported from cotyledon explants (Bornman, 1983; Niedz ***et al.***, 1989; Dong and Jia, 1991; Jahan and Hadiuzzaman, 1996 and Kouider ***et al.***, 1985).

In an attempt to enhance shoot proliferation, coconut water (5-15% v/v) was added to the medium. Addition of 15% CW to the medium increased the number of shoots per culture (Table 2). Thus the more effective medium determinate for multiplication of large number of shoot with proper length was ½ MS + 1.0 mg l^{-1} BA+0.1 mg l^{-1} NAA+15% CW. In this combination, the highest percentage of shoot proliferation was 100%, highest number of shoot per culture was 10.60±1.76 and average length of shoot per culture 3.71±0.32 cm (Fig. D). Roy ***et al.*** (1993) and Rahman ***et al.*** (2001) reported that addition of coconut water increase the number of shoots in ***Elaeocarpus robustus*** culture. There are other reports on the effect of the addition of complex organic substance on the growth of culture of woody species (Gamborg ***et al.***, 1976; Thrope, 1982 and Sen ***et al.***, 1992). Rahman ***et al.*** (1999) reported that the addition of 15% CW to the medium for the culture of shoot tip and nodal segments of ***Emblica officinalis*** resulted in significant increase in growth of axillary shoot.

Micro-cuttings prepared from ***in vitro*** proliferated shoots with more than 1.5 cm length were rooted on agar gelled medium with 0.1-1.0 mg l^{-1} of either NAA, IBA and IAA supplemented or omitted. Among different types of auxin used, IBA was found to be the best for root induction. Among concentration of NAA, IBA and IAA the maximum percentage of culture that produce roots was 80% when the shoots were cultured in medium having 0.2 mg l^{-1} IBA (Fig. E). Media containing NAA and IAA also resulted in root formation but the rooting response was not as good as in the media containing IBA. Maximum rooting frequency was 70% on media supplemented with 0.2 mg l^{-1} NAA and 50% on medium supplemented with 0.2 mg l^{-1} IAA. Percentage of root induction and number of roots per shoot were also highly influence by concentrations and types of auxin. No rooting found on auxin omitted media and poor rooting was obtained with IAA and NAA at the concentration tested compared to IBA supplemented media (Table 3). Being stable nature IBA has been the preferred auxin for adventitious root initiation in many species (Amin ***et al.***, 2001; Rahman ***et al.***, 2001 and Khatun ***et al.***, 2001). Hustchinson (1981) found IBA as superior auxin to IAA or NAA for ***in vitro*** rooting of apple shoots, while Amin and Jaiswal (1987) observed its superiority for rooting of guava and jackfruit micro-cuttings.

References

Amin, M.N., 1991. Callus culture and plant regeneration from an economically important tropical tree-jackfruit. In : Intl. Conf. Genetic Engineering and Biotechnology, Kathmandu, Nepal., pp: 50.

Amin, M.N. and V.S. Jaiswal, 1987. Rapid clonal propagation of guava through in vitro shoot proliferation of nodal explants of mature trees. Plant Cell Tissue Organ Cult., 9: 235-245.

Amin, M.N., M.A. Habib, M.A.K. Azad and M. Akhteruzzaman, 2001. Large scale plant regeneration in ***Ficus religiosa*** L. through tissue culture. 4th Intl. Plant Tissue Cult. Conf. (1-3 Nov. Dhaka)., pp: 9.

Bornman, C.H., 1983. Possibilities and constraints in the regeneration of tree from cotyledonary muddles of ***Picea abies in vitro***. Physiol. Plant., pp: 57-116.

Dong, J.Z. and S.R. Jia, 1991. High frequency plant regression from cotyledon of watermelon (***Citrullus vulgaris*** Schrad.) Plant Cell Rep., 9: 559-562.

Gamborg, O.L., T. Murashige, T.A. Thorpe and I.K. Vasil, 1976. Plant Tissue Culture media. ***In vitro***, 12: 473-478.

Ghani, A., 1998. Monographs: ***Elaeocarpus serratus*** Linn. In: Medicinal plants of Bangladesh: Chemical constituents and Uses. Asiatic. Soc. Bangladesh Publ., pp: 167-168.

Hutchinson, J.F., 1981. Tissue culture propagation of fruit trees. In: A. N. Rao (Ed.). Proc. Symp. on tissue culture of economically important plants, Singapure, pp: 113-120.

Islam, R.M., R. Karim, M. Hossain and O.I. Joarder, 1992. Plant regeneration from leaf-derived callus in ***Aegle marlelos*** Corr. Plant Tissue Cult., 2: 55-59.

Jahan, M.A.A. and S. Hadiuzzaman, 1996. Callus induction and plant regeneration from different explants of ***Solanum nigrum*** L. seedlings. Plant Tissue Cult., pp: 57-62.

James, D.J., 1988. Cell and tissue culture technology for the genetic manipulation of temperate fruit trees. In: Biotechnology of higher plants. G. E. Russell (Ed.). Intercept. Dorset. England., pp: 33-80.

Khan, M.S. and M.K. Alam, 1996. ***Elaeocarpus floribundus*** In: S.M.R. Amin and F.U. Ahmed (Eds.), Homestead flora of Bangladesh. Forestry Div. BRAC. Dhaka, Bangladesh., pp:123.

Khatun , A. and M.T. Hossain, 2001. ***In vitro*** micropropagation of pomegranate (***Punica granatum***). 4th Int. Plant Tissue Cult. Conf. (1-3 Nov. Dhaka)., pp: 27.

Kouider, M.S.S. Korban, R.M. Skirvin and H. Joung, 1985. The relationship of apple embryos and their cotyledon to maturity dormancy and the potential to form shoots ***in vitro***. J. Amer. Soc. Hort. Sci., 110: 93-96.

Litz, R.E. and R.A. Conover, 1980. Partial organogenesis in tissue cultures of ***Averrhoa carambola***. Hort. Sci., 15: 735.

Litz, R.E., G.A. Moore and C. Srinivasan, 1985. ***In vitro*** systems for propagation and improvement of tropical fruits and palms. Hort. Rev., 7: 157-198.

Niedz, R.P., S.S. Smith, K.B. Dunbar, C.T. Stephens and H.H. Murakishi, 1989. Factors influencing shoot regeneration from cotyledonary explants of ***Cucumis melo***. Plant Cell,Tissue and Organ Cult., 18: 313-319.

Rahman, M.M, M.N. Amin and M.A.K. Azad, 2001. Micropropagation of a dwarf variety of Native-Olive (***Elaeocarpus robustus*** Roxb.). 4th Intl. Plant Tissue Cult. Conf. (1-3 Nov. Dhaka)., p: 10.

Rahman, M.M., P.K. Roy, M.A. Mannan and S.K. Roy, 1999. Clonal propagation of ***Emblica officinalis*** through ***in vitro*** culture. Plant Tissue Cult., 9: 17-23.

Rowe-Dutton, P., 1976. ***Artocarpus heterophyllus*** - Jackfruit. In : The propagation of Tropical Fruit Trees. R.J. Garner and S.A. Chaudhuri (Eds.). FAO, CAB, London, pp: 269-290.

Roy, S.k., M.S. Islam and J. Sen, 1993. Clonal propagation of ***Elaeocarpus robustus*** through ***in vitro*** culture, Intl, Plant Tissue Cult. Conf. (12-19 December, Dhaka), pp: 44.

Samaddar, H.N., 1990. Jackfruit. In : Fruit: Tropical fruit and Subtropical. T.K. Bose and S.K. Mitra (Eds.), Naya Prokash, Calcutta., pp: 638-639.

Sen, J., M.S. Islam and S.K. Roy, 1992. Micropropagation of juvenile and adult ***Gmelina arborea***. Plant Tissue Cult., 2: 89-95.

Thorpe, T.A., 1982. Carbohydrate utilization and metabolism. In: Tissue Culture in Forestry, J.M Bonga and D.J. Durzan (Eds.), Martinus Nijhoff, The Hague, pp: 325-368.