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In vitro Rapid Clonal Propagation of an Ornamental Plant–*Ixora fulgens* Roxb.

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Abstract: The cultures of proliferating axillary shoots were established on MS medium with different concentrations of either BA alone or in combinations of BA with NAA or IBA and with shoot explants from field grown mature plant of *Ixora fulgens*. Nodal segments were found to be the best explants for axillary shoot formation on agar gelled MS medium with 0.5 mg l^{-1} BA+0.1 mg l^{-1} NAA. On this medium axillary buds showed sprouting within two weeks of incubation. By sequential reculturing and subculturing 15-20 usable shoots (>1 cm length) could be produced from the 2-node shoot segments after 12-15 weeks of culture. Microcuttings taken from *In vitro* proliferated shoots were rooted on ½ strength MS medium having 0.1-0.5 mg l^{-1} of either NAA, IBA and/or IAA. It was found that 90% of the plantlets could be established under *Ex vitro* conditions when they were transferred on specially made plastic tray containing coco-peet as potting mix.

Key words: *Ixora fulgens* Roxb., *In vitro* propagation, ornamental plant

Introduction

Ixora fulgens Roxb., commonly known as "Rangan or Ixora", belongs to the family Rubiaceae and is an evergreen ornamental shrub. A native of Malay peninsula and adjoining area, it is now being cultivated throughout the tropics (Bor *et al.*, 1954; Hooker, 1961). Rangan is very common and popular ornamental plants in Bangladesh as it produces bright-red, small flowers in compound cymes against the background of lush green leaves. Generally it blooms in the rainy season but it is seen in some varieties that flowering continues up to February.

In recent years Rangan is becoming popular flowers in Bangladesh. Having good potentials for marketing as cut flower and potted plant it has became one of the important floricultural crops. The Rangans are also important for different purposes because of their various colours, shapes and sizes that have made themselves demandable ornamental flowers. They are mainly used for decoration of houses, gardens, parks and office premises. The plant has both horticultural and medicinal values.

Rangan is normally propagated by cutting and layering during the rainy season (Bor *et al.*, 1954). But these processes are very slow and number of plants produced by them is very few. Moreover, the investigated species (*Ixora fulgens*) has not been seen to set fruit in Bangladesh. Under this circumstance the micropropagation method can play an important role for rapid multiplication and wide distribution of Rangan, as it produces huge number of plantlets under *In vitro* condition. There have been many reports on tissue culture propagation of ornamental plants but so far we known, there is no report on *In vitro* propagation of the *Ixora* species in Bangladesh. Considering these, the present investigation was under taken to develop the tissue culture technique for mass propagation of *Ixora fulgens* Roxb. from young shoot tip and axillary buds of mature plants.

Materials and Methods

Healthy, disease-free and actively growing shoot having nodes and tips with axillary buds (4-5 cm) were collected from the field grown mature plants. The plant material was washed thoroughly under running tap-water in the laboratory to reduce the dust and surface contaminations. Surface sterilization of the shoot segments was done by treating them with 1% Savlon for 10 minutes with constant shaking, washing with distilled water for 3-4 times, rinsing in 95% alcohol for 30 sec and finally immersing in 0.1% $HgCl_2$ for different duration of time. To remove every trace of the sterilant the material was then washed with sterile distilled water at least 4-5 changes of water. The 1.0-1.5 cm shoot segments containing nodes or shoot tips were prepared form the surface sterilized material and were used as explants. The prepared explants were cultured singly in 25x150 mm^2 culture tube containing 15-20 ml of nutrient media.

The explants were allowed to grow on agar-gelled MS medium (Murashige and Skoog, 1962) with different concentrations of cytokinins (0.2-1.0 mg l^{-1}) and auxins (0.1-1.0 mg l^{-1}). The elongated axillary shoots that proliferated on the primary explants were excised, made into nodal segments and transferred individually to the proliferating medium for further production of axillary shoots. The microcuttings were prepared from the proliferated cultures and transferred individually for rooting on ½ MS medium with NAA, IBA or IAA at a concentration of 0.1-2.0 mg l^{-1}. The pH of the medium was adjusted to 5.7 ± 0.1 before addition of agar (BDH) at 0.7% concentration. The media were dispensed into culture tubes and autoclaved at 121 °C under 1.1 kg cm^{-2} pressure for 15 minutes. Cultures were maintained at 26±1 °C with 16-h photoperiod providing light intensity of 2000-3000 lux.

Results and Discussion

Proliferation of the axillary shoots was induced in two different explants, i.e. node and shoot tip segments. These explants were collected from field grown mature plant and were cultured on MS medium supplemented with different concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg l^{-1}) of BA alone and in all possible combinations with 4 different concentrations (0.1, 0.2, 0.5 and 1.0 mg l^{-1}) of NAA and IBA for selecting the best BA, BA-NAA and BA-IBA combinations. The axillary shoot proliferation from the cultured explants was remarkably influenced by type and concentration of the growth regulator. The nodal segments of *Ixora fulgens* cultured on MS medium supplemented with different concentrations of BA alone showed the best results for shoot proliferation after 7 weeks of culture. Among the cultured explants 80% nodal segments and 73% shoot tip segments responded to axillary shoot proliferation. The nodal explants produced the highest number of 6.5 ± 0.12 shoots per culture on the medium with 0.5 mg l^{-1} BA while shoot tip explants produced 4.0±0.54 shoots per culture on the same medium. The media containing 0.1, 0.2, 0.5, 1.0 and 2.0 mg l^{-1} of BA treatments induced proliferation of the axillary shoots in 40, 60, 80, 40 and 13% of the nodal explants and 33, 53, 73, 33 and 7% of the shoot tip explants, respectively. But no proliferation of shoot was observed from the nodal and shoot tip explants that were cultured on medium supplemented with 3.0 mg l^{-1} BA (Table 1).

The nodal and shoot tip explants from mature plants were also cultured on MS medium supplemented with different concentrations (0.2, 0.5 and 1.0 mg l^{-1}) of BA and in all possible combination with 4 different concentrations (0.1, 0.2, 0.5 and 1.0 mg l^{-1}) of NAA and IBA. Among these different growth regulator combinations, BA+NAA combinations showed the best proliferation results than other combinations viz. BA + IBA. The relative amounts and ratios of BA and NAA present in the culture medium influenced proliferation of axillary shoots from the nodal segments (Table 1). Normal axillary shoot formation occurred at

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Table 1: Effects of different concentrations and combinations of BA alone and with NAA and IBA on axillary shoot proliferation from *In vitro* grown nodal and shoot tip explants. There were 15 explants in each treatment and data (± SE) were collected after 8 weeks of culture.

	Nodal explant			Shoot tip explant			
Growth regulators (mg l^{-1})	% of explant showing proliferation	No. of total shoots per culture	No. of usable shoots per culture	% of explant showing proliferation	No. of total shoots per culture	No. of usable shoots per culture	*Callus formation
BA							
0.1	40.0	3.0 ± 0.21	2.4 ± 0.11	33.0	2.8 ± 0.31	2.1 ± 0.21	-
0.2	60.0	5.1 ± 0.15	4.8 ± 0.37	53.0	3.9 ± 0.18	2.5 ± 0.32	-
0.5	80.0	6.5 ± 0.12	5.0 ± 0.15	73.0	4.0 ± 0.54	3.2 ± 0.41	-
1.0	40.0	3.3 ± 0.06	2.9 ± 0.38	33.0	2.5 ± 0.32	2.0 ± 0.11	-
2.0	13.0	1.8 ± 0.22	1.3 ± 0.52	7.0	1.5 ± 0.21	1.0 ± 0.10	+
3.0	-	-	-	-	-	-	+ +
BA + NAA							
0.2+0.1	47.0	4.2 ± 0.28	3.9 ± 0.51	46.0	3.8 ± 0.42	3.0 ± 0.26	-
+0.2	13.0	2.0 ± 0.32	1.5 ± 0.28	7.0	1.5 ± 0.10	1.1 ±0.13	+
+0.5	-	-	-	-	-	-	+ +
+1.0	-	-	-	-	-	-	+ +
0.5+0.1	100.0	15.7 ± 1.18	12.1 ± 1.35	93.0	12.0 ± 1.32	10.0 ± 0.92	-
+0.2	86.0	10.9 ± 0.95	8.2 ± 0.67	73.0	7.0 ± 0.41	5.9 ± 0.23	+
+0.5	27.0	3.3 ± 0.63	2.1 ± 0.29	33.0	2.5 ± 0.12	2.1 ± 0.18	+ +
+1.0	-	-	-	-	-	-	+ + +
1.0+0.1	93.0	12.6 ± 1.61	10.1 ± 1.17	86.0	9.0 ± 0.20	8.0 ± 0.56	-
+0.2	80.0	9.5 ± 0.91	7.8 ± 0.56	73.0	6.2 ± 0.13	4.1 ± 0.35	+
+0.5	60.0	6.9 ± 0.11	4.3 ± 0.55	53.0	4.0 ± 0.27	2.9 ± 0.21	+ +
+1.0	-	-	-	-	-	-	+ + +
BA + IBA							
0.2+0.1	33.0	3.1 ± 0.25	2.9 ± 0.20	27.0	2.7 ± 0.31	2.0 ± 0.14	-
+0.2	7.0	2.0 ± 0.17	1.5 ± 0.23	7.0	1.3 ± 0.10	1.0 ± 0.10	+
+0.5	-	-	-	-	-	-	+ + +
+1.0	-	-	-	-	-	-	+ + +
0.5+0.1	73.0	7.3 ± 0.38	3.8 ± 0.15	60.0	3.9 ± 0.40	3.0 ± 0.12	-
+0.2	53.0	4.0 ± 0.48	3.5 ± 0.73	46.0	3.6 ± 0.51	3.1 ± 0.41	+
+0.5	20.0	1.8 ± 0.41	1.2 ± 0.11	13.0	1.4 ± 0.30	1.1 ± 0.32	+ +
+1.0	-	-	-	-	-	-	+ + +
1.0+0.1	60.0	5.8 ± 0.55	3.1 ± 0.19	53.0	3.5 ± 0.16	3.1 ± 0.13	+
+0.2	40.0	3.5 ± 0.38	2.1 ± 0.22	33.0	2.0 ± 0.27	1.6 ± 0.19	+ +
+0.5	33.0	2.5 ± 0.13	1.0 ± 0.27	20.0	1.2 ± 0.14	1.0 ± 0.10	+ + +
+1.0	-	-	-	-	-	-	+ + +

-= no response; Callus growth rating value= (+) poor; (+ +) moderate; (+ + +) massive callus formation

Table 2: Effects of auxin in ½MS medium on adventitious root formation *In vitro* from *Ixora fulgens*. Microcuttings cultured for 4 weeks at 25 °C under 16h photoperiod. There were 25 cuttings in each treatment and the experiment was repeated once.

Hormonal supplement	% of root formation	No of roots per rooted cutting	Average length of the roots (cm)	Days to root formation	Callus formation at the cutting base
IBA					
0.1	100.0	4.7 ± 0.35	2.8 ± 0.27	8-10	-
0.2	100.0	5.6 ± 0.42	3.2 ± 0.52	8-10	-
0.5	100.0	5.1 ± 0.18	2.9 ± 0.19	8-10	+
1.0	80.0	2.5 ± 0.21	2.0 ± 0.51	12-15	+ +
2.0	-	-	-	-	+ + +
NAA					
0.1	90.0	4.5 ± 0.55	2.7 ± 0.52	10-12	-
0.2	100.0	5.0 ± 0.48	3.0 ± 0.33	10-12	+
0.5	80.0	4.2 ± 0.27	2.4 ± 0.22	10-12	+ +
1.0	70.0	3.2 ± 0.31	1.5 ± 0.33	12-15	+ +
2.0	-	-	-	-	+ + +
IAA					
0.1	40.0	1.2 ± 0.38	1.0 ± 0.33	19-24	-
0.2	50.0	2.0 ± 0.87	1.9 ± 0.53	19-24	-
0.5	70.0	2.5 ± 0.53	2.1 ± 0.55	20-25	-
1.0	40.0	1.0 ± 0.17	1.0 ± 0.53	25-30	+
2.0	-	-	-	-	+ +

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

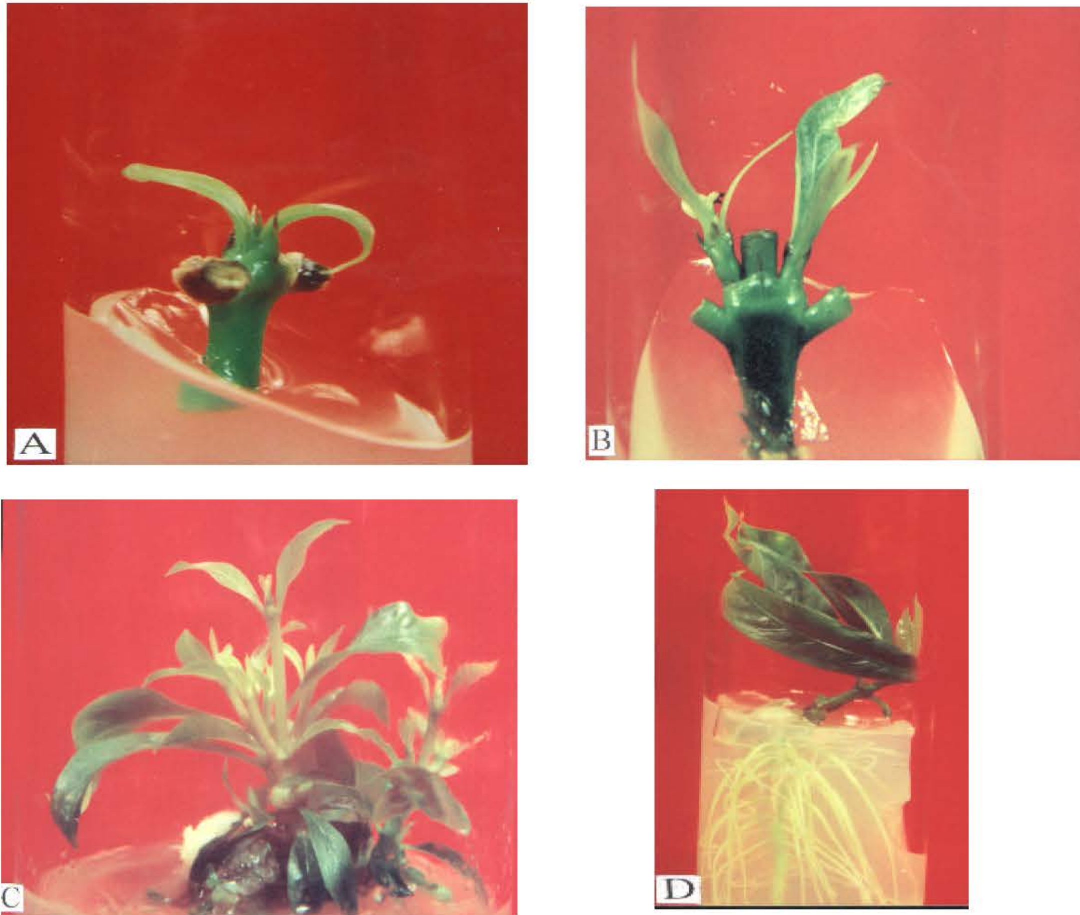

Fig. 1: Regeneration of plantlets *In vitro* from the explants of mature plants of *Ixora fulgens* Roxb., A & B) Initial development of axillary shoots from the shoot tip (A) and nodal (B) explants respectively after 2-week and 4-week of culture, C) Proliferation and elongation of the axillary shoots on a nodal explant after 10- week of culture, D) Profuse development of the adventitious roots on a regenerated shoot.

0.1-0.2 mg l^{-1} of NAA with a higher proportion (0.5-1.0 mg l^{-1}) of BA. Among different treatments, 0.5 mg l^{-1} BA with a low concentration of NAA (0.1 mg l^{-1}) showed the highest frequency of proliferation. In this combination 93% of the shoot tip explants (Fig. 1A) and 100% of the nodal explants (Fig. 1B) responded to shoot proliferation. On this medium highest number of total shoot per culture was 15.7±1.18 and 12.0±1.32, and number of usable shoots per culture was 12.1±1.35 and 10.0±0.92 respectively for nodal and shoot tip explants. The lowest results were observed on the 0.2 mg l^{-1} BA + 0.2 mg l^{-1} NAA supplemented medium where only 13 and 7% explants showed proliferation with 2.0±0.32 and 1.5±0.10 usable shoot per culture respectively for nodal and shoot tip explant. At the combination of 0.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA the proliferated axillary shoots were green and vigours in growth that ultimately produced healthier plantlets (Fig. 1C). Among the different BA-NAA combinations 0.5 mg l^{-1} BA+ 0.1 mg l^{-1} NAA was found to be the best formulation for proliferating the axillary shoots of *Ixora fulgens*.

In case of BA and IBA combinations maximum of 73% nodal explants and 60% shoot tip explants produced normal shoot proliferation when the explants were cultured with 0.5 mg l^{-1} BA and 0.1 mg l^{-1} IBA. Other combinations of BA and IBA in the initial media greatly altered the growth behaviour of the cultured explants that proliferated axillary shoots. Among the different formulations of BA-IBA used in this experiments, 0.5 mg l^{-1} BA and 0.1 mg l^{-1} IBA combination produced maximum number of 7.3±0.38 shoots per culture in nodal explants, where the shoot tip explants produced 3.9±0.40 shoots per culture. At rest of the combinations of BA+NAA and BA+IBA callus began to form at cut ends of explants within 15-20 days of culture. Small amount (+) of callus had formed on 0.2 mg l^{-1} NAA or IBA with all levels of BA, whereas large amount of (+ + +) callus produced when 0.5-1.0 mg l^{-1} NAA or IBA added with all levels of BA. Addition of 1.0 mg l^{-1} NAA or IBA to the medium containing all levels of BA failed to induce proliferation of any axillary shoot but they produced only callus.

Microcuttings (2.0-4.0 cm long) were prepared from the *In vitro* proliferated usable shoots and cultured on ½ MS medium supplemented with 0.1-2.0 mg l^{-1} of either NAA, IBA or IAA for adventitious root induction (Table 2). Percentage of root induction and number of roots per shoot were remarkably activated by the concentration and type of auxins. Among three types of auxin IBA was found to be comparatively more effective than other two auxins, NAA and IAA at different concentrations tested for producing roots. On the medium with 0.2 mg l^{-1} of auxins the cultured shoot cuttings produced the highest number of healthy roots per microcuttings (Fig. 1D). The rooting frequencies were 100% for IBA and NAA and 50% for IAA (Table 2). The maximum length of the longest root was 3.2 ± 0.52 cm on 0.2 mg l^{-1} IBA supplemented medium, 3.0 ± 0.33 cm on 0.2 mg l^{-1} NAA supplemented medium and 1.9 ± 0.33 cm on 0.2 mg l^{-1} IAA supplemented medium (Table 2). When the shoots cuttings were cultured on ½ MS medium supplemented with the highest

concentration (2.0 mg l^{-1}) of either IBA, NAA or IAA, they could not form any root but produced callus at their cut bases (Table 2). Besides, malformation and slow growth of roots were also observed at higher concentrations of NAA and IAA supplemented media. On the other hand IBA in the medium promoted root length and number by inhibiting basal callusing and growth deformities.

After shoot and root development, attempts were made to establish regenerated plantlets into soil. Plants propagated *In vitro* are not transferred to an open soil environment directly and a hardening stage is usually used in which the plantlets are transferred from *In vitro* condition into humid *Ex vitro* condition in the growth room. The regenerated plants of Rangan were taken out from *In vitro* condition and planted into plastic pots containing either garden soil and compost in a ratio of 1:1, garden soil, compost and sand in a ratio of 2:2:1 or 100% coco-peet. The *In vitro* derived plants acclimated better under *Ex vitro* conditions when they were transferred on specially made plastic tray containing coco-peet as potting mix and 95% of the plantlets could be established on the soil.

Micropropagation of *Ixora fulgens* through tissue culture technique was undertaken considering its beautiful flowers and medicinal values. *In vitro* plant regeneration depends on a number of factors including the composition of culture medium, proper concentration of growth regulators and the response of the explants as well as the genotype of the plant material. Commonly it has been shown that the basic regulatory mechanism underlying plant organ initiation involves a balance between auxin and cytokinin. Most plants exhibit varied degrees of responses to full strength MS medium, the herbaceous and semi-woody species like Rangans respond better than the woody ones (Bhojwani and Razdan, 1983). Many authors reported that other ornamental plant species could be micropropagated on MS medium; e.g., *Bougainvillea* sp. (Sharma and Dhir, 1985), *Ficus auricaulata* (Amatya and Rajbhandary, 1989), *Dianthus caryophyllus* (Dohare, 1992), *Pancratium biflorum* (Begum and Hadiuzzaman, 1993), etc. These findings also support that herbaceous and semi woody species respond better to full MS medium than to ½ MS medium.

To obtain plantlets with uniform characteristics that is with clonal fidelity in terms of growth characteristics and habits, the direct regeneration is essential. It is a useful means of producing plantlets from young and mature trees with a lower risk of genetic instabilities than the other routes of plantlet regeneration (Rao and Lee, 1986). In the present investigation plantlet regeneration was achieved through axillary shoot proliferation system. Between the two explants nodal explant showed comparatively better response for axillary shoot proliferation. The technology of plant multiplication by *In vitro* process from nodal explant is simple, efficient and also economical (Choudhary and Prakash, 1992). Lim-Hu and Kong (1985) and Zobayed (1991) also succeeded in the mass production of *Lagerstromia* sp. using nodal segments of mature trees.

Different concentrations and combinations of BA with different auxins viz. NAA, IBA IAA were used in shoot proliferation media. Among these different media combinations, BA-NAA combinations produced only axillary shoots but no roots from nodal and shoot tip explants. On the shoot proliferation medium, a comparatively higher concentration of BA (0.5-1.0 mg l^{-1}) along with a lower concentration of NAA (0.1-0.2 mg l^{-1}) showed the best result. This is in agreement with the results of *Ficus auriculata* (Amatya and Rajbhandary, 1989), *Pancratium biflorum* (Begum and Hadiuzzaman, 1993), *Eucharis grandiflora* (Mujib *et al.*, 1993) and other plant species. It was also reported that many plant species like *Rosa hybrida* cv. Queen Elizabet (Rout *et al.*, 1989) and *Vanilla walkeriae* (Agrawal *et al.*, 1992) produced multiple shoots from nodal explant on MS medium supplemented with BA, GA_3 and casein hydrolysate.

Induction and development of roots at the bases of the *In vitro* grown shoots is an indispensable step to establish tissue culture derived plantlets on the soil. The most effective auxins are definitely IBA and NAA for rooting (Pierik, 1987). Roy *et al.* (1987), Jaiswal and Amin (1987), Niraula and Rajbandary (1988) reported that roots obtained in ½ MS with IBA, NAA, IAA either individually of in combinations from the proliferated shoots of *Mitrayana parvifolia, Psidium guajava* and *Poncirus trifoliata,* respectively. Regarding the optimum concentrations, auxin requirement was more for root initiation than for shoot development (Audus, 1959). The highest percentage (100%) of root regeneration was obtained in ½ MS with 0.2 mg l^{-1} IBA within 8-10 days and the lowest rooting (40%) was obtained with 0.1 and 1.0 mg l^{-1} IAA. The findings are in agreement with those observed in *Adhatoda vasica* (Azad *et al.*, 1999), African marigold (Kothari and Chandra, 1984) and *Chrysanthemum morifolium* (Hoque *et al.*, 1995), nonetheless in many other plant species NAA was also found to be efficient.

References

Agrawal, D.C., G.C. Morwal and A.F. Mascarenhas, 1992. *In vitro* propagation and slow growth storage of shoot cultures of *Vanilla walkeriae* Wight- an endangered orchid. Lindleyan, 7: 95-99.

Amatya, N. and S.B. Rajbhandary, 1989. Micropropagation of *Ficus auriculata* Lour. In: Application of biotechnology in forestry and horticulture. V. Dhawan (Ed.) Plenum Press, New York, pp: 157-163.

Audus, L.J., 1959. Plant Growth Substance. Interscience Publ. New York.

Azad, M.A.K., M.N. Amin and F. Begum, 1999. *In vitro* rapid regeneration of plantlets from cotyledon explant of *Adhatoda vasica* Nees. Plant Tissue Cult., 9: 121-126.

Begum, S. and S. Hadiuzzaman, 1993. *In vitro* multiple shoot regeneration from bulb scale and leaf segment of *Pancratium biflorum* Roxb. Plant Tissue Cult., 3: 41-45.

Bhojwani, S.S. and M.K. Razdan, 1983. Plant Tissue Culture: Theory and Practice. Elsevier Sci. Publ. Amsterdam, pp: 520.

Bor, N.L., M.B. Raizada and A. Raizada, 1954. Some Beautiful Indian Climbers and Shrubs. The Bombay Natural History Society, Bombay, India, pp: 93-101.

Choudhary, M.L. and P. Prakash, 1992. Influence of kinetin and NAA on shoot root growth of carnation (*Dianthus caryoehyllus* L.) *In vitro.* Agric. Sci. Digest., 12: 105-108.

Dave, Y. and V. Thomas, 1991. Anatomy and histochemistry of stipular collectors in *Ixora coccinea* L. J. Indian Bot. Soc., 70: 51-54.

Dohare, S.R., 1992. Commercial exploitation of micropropagation of carnation (*Dianthus caryophyllus*). Nat. Sem. on Commercial Floric. in India: Present and potential, IAHS, Bangalore: July 1992, pp: 12-13.

Hooker, J.D., 1961. Ixoreae. In: Flora of British India. CB and KCSI (Eds.) 3: 137-149.

Hoque, M.I., M. Fatema, R. Hashem and R.H. Sarker, 1995. *In vitro* plant regeneration in *Chrysanthemum morifolium* Ramat. Annual Tissue Cult. Conf. (Dhaka, Mach 19). Plant Tissue Cult., pp: 92.

Jaiswal, V.S. and M.N. Amin, 1987. *In vitro* propagation of guava from shoot cultures of mature trees. J. Plant Physiol.,130: 7-12.

Kothari, S.L. and N. Chandra, 1984. *In vitro* propagation of African marigold. Hort. Sci., 19: 703-705.

Lim-Hu, C.L. and L.S. Kong, 1985. Micropropagation of *Lagerstroemia speciosa* (Lythraceae). Botanic gardens (Singapore). Gardens Bulletin, 38: 175-189.

Mujib, A., S. Bandyopadhyay, B.K. Jan and P.D. Ghosh, 1993. Direct embryogenesis from bulb-scale explant of *Eucharis grandiflora*. Pl. Tissue Cult., 3: 5-9.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Pl., 15: 473–497.

Niraula, R. and S.B. Rajbhandry, 1988. *In vitro* propagation of trifoliate organ (*Porcirus trifoliat*). National Conf. Sci. Technol., pp: 141-144.

Pierik, R.L.M., 1987. *In vitro* culture of higher plants. In: Tropical Crops Dicotyledon. J.W. Purseglone (Ed.). Martinns Nijhoff Publ. Derdrecht, Boston, Lancaster., pp: 364-370.

Rao, A.N. and S.K. Lee, 1986. An overview of the *In vitro* propagation of woody plants and plantation crops. In: L.A. Withers and P.G. Alderson (Eds). Plant Tissue Culture and its Agricultural Application, pp: 123-138.

Rout, G.R., B.K. Debata and P. Das, 1989. Induction of somatic embryogenesis in *Rosa hybrida* cv. Landora. Orissa J. Hort., 17: 46-49.

Roy, S.K., S.K.L. Rahman and P.C. Datta, 1987. *In vitro* propagation of *Mitragyna parviflolia* Korth. Pl. Cell Tissue and Organ Cult., 12: 75-80.

Sharma, R. and K.K. Dhir, 1985. *In vitro* culture of axillary buds of *Bougainvillea* cultivar 'Marypalmer'. Indian J. Plant Physiol., 28: 169-176.

Zobayed, S.M.A., 1991. Direct and indirect *In vitro* regeneration in *Lagerstroemia* spp. M.Sc. Thesis, Dept. Bot. Uni. Dhaka, Bangladesh.