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REGENERATION OF *FICUS GLOMERATA* ROXB., USING SHOOT TIPS AND NODAL EXPLANTS

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Abstract

Shoot tips and nodal explants from *in vitro* growing seedlings of *Ficus glomerata* Roxb. (Moraceae). showed best shoot induction (88%) on MS medium supplemented with 0.5 mg/l BAP, where maximum number of shoots were produced per culture. *In vitro* raised shoots rooted well on half strength of MS medium with 2.0 mg/l IBA + 0.1 mg/l NAA. The survival rate of regenerated plantlets was 82%.

Introduction

Ficus glomerata Roxb. (syn. *Ficus racemosa* Linn.) commonly known as 'Jogyadumur', belongs to Moraceae, grows throughout Bangladesh in moist localities, e.g. along the banks of streams and sides of ravines (Ghani 2003). It is found also in rocky slopes, sometimes growing gregariously. It is often cultivated in villages for its edible fruits. Leaves, fruits and extracts of barks are used against various types of diseases (Ghani 2003). The bark contains 14% tannin and root is reported to be useful in dysentery (Anon. 1956). The plant has the capacity to reduce the blood sugar level significantly (Rahman *et al.* 1994).

In vitro propagation of woody plant species was successful (Deshpande *et al.* 1998, Hassan *et al.* 2009, Munshi *et al.* 2004, Rahman *et al.* 2004). But there is no report on the micro-propagation of *F. glomerata* using shoot tip and nodal explants. The present study was therefore, undertaken to develop a protocol for mass clonal propagation of *F. glomerata* through *in vitro* culture.

Materials and Methods

Shoot tips and nodal explants from aceptically grown mature seeds of *Ficus glomerata* were cultured on MS medium (Murashige and Skoog 1962) following normal *in vitro* culture procedures (Hassan *et al.* 2999) for adventitious shoot regeneration.

Half strength MS medium was used for *in vitro* rooting. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 15 × 150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 1.9 kg/cm^2 pressure at 121°C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2°C and under 1200 lux/m^2 fluorescent light.

Shoot proliferation from shoot tips and nodal explants of *in vitro* growing seedlings were obtained in two separate sets of experiments. In the first experiment 0 - 2.0 mg/l BAP and 0 - 2.0 mg/l Kn were added into MS to select the best cytokinin for shoot induction. In the second set, combination of BAP (0 - 2.0 mg/l) with NAA (0.1 - 0.5 mg/l) and BAP (0 - 2.0 mg/l) with IAA (0.1 - 0.5 mg/l) were assessed for shoot multiplication. Number of new shoot proliferated in each culture was recorded every week after inoculation.

For *in vitro* rooting, individual shoots (3 - 5 cm) were excised from the proliferated shoots cultures and implanted onto half strength MS with different concentrations and combinations of NAA, IBA and IAA.

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The rooted plantlets were taken out from the culture tubes, washed to remove agar gel and transplanted to plastic pots with soil and compost (1 : 1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2° C under a 12 hr photoperiod (1500 lux/m^2 sun light) for acclimatization. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

The shoot tips and nodal explants were found to be swollen and produced three to four shoots within three - four weeks of inoculation (Fig. 1a) on MS containing BAP (0.4 mg/l) alone but the number of shoots increased up to 11 with 0.5 mg/l BAP. Both the explants responded in the same medium but highest number of microshoots were induced from nodal explants. Combinations of BAP with NAA or IAA were not found to be suitable than BAP alone for shoot induction (Table 1) and combinations of Kn with NAA or IAA were also not found to be suitable for shoot induction. Newly initiated shoots were separated and subcultured repeatedly in fresh MS with 0.5 mg/l BAP, where the number of shoots increased up to 16.2 ± 0.86 per culture (Table 1). Deshpande *et al.* (1998) reported that dormant axillary buds of nodal explants collected from a

Table 1. Effects of growth regulators on morphogenic response of shoot tips and nodal segments of *Ficus glomerata*. n = 15, ± = Standar error.

MS medium with growth regulators (mg/l)			Shoot tips		Nodal segments	
BAP	NAA	IAA	% of explants forming shoots	Mean No. of shoots/explant	% of explants forming shoots	Mean No. of shoots/explant
Control			-	-	-	-
0.1			41.2 ± 2.47	4.4 ± 0.45	48.8 ± 1.77	5.4 ± 0.45
0.2			56.8 ± 2.14	7.6 ± 0.77	68.8 ± 2.35	8.0 ± 0.39
0.5			**72.4 ± 2.89**	**12.2 ± 0.37**	**88.2 ± 2.80**	**16.2 ± 0.73**
1.0			63.4 ± 1.57	10.6 ± 0.45	71.4 ± 2.38	15.6 ± 0.72
1.5	0.1		57.6 ± 2.16	9.4 ± 0.72	67.6 ± 2.16	13.4 ± 1.18
2.0	0.2		34.8 ± 2.58	7.8 ± 0.76	33.6 ± 1.84	12.4 ±0.82
0.5	0.5		61.4 ± 2.87	9.2 ± 0.59	68.6 ± 1.70	13.6 ± 1.14
1.0	0.5		42.6 ± 0.87	7.6 ± 0.77	43.6 ± 0.51	12.6 ± 0.91
1.5			28.2 ± 1.66	6.0 ± 0.63	41.2 ± 2.47	10.4 ± 0.66
2.0			22.2 ± 1.96	4.4 ± 0.45	32.2 ± 0.66	8.8 ± 0.95
0.5		0.1	48.8 ± 1.77	8.0 ± 0.39	56.8 ± 2.14	12.2 ± 0.76
1.0		0.2	26.6 ± 1.66	6.2 ± 0.65	47.6 ± 2.10	10.2 ± 0.51
1.5		0.5	21.0 ± 1.14	5.4 ± 0.45	32.6 ± 1.63	8.4 ± 0.91
2.0		0.5	16.2 ± 0.86	3.4 ± 0.45	18.4 ± 0.93	7.4 ± 0.66

mature tree of *F. religiosa* L. sprouted on MS medium supplemented with 5.0 mg/l BAP and 0.2 mg/l IBA within four days and multiple shoots were obtained when these explants were transferred to MS containing 1.5 mg/l BAP and 1.5 mg/l AdS. A similar phenomenon was observed in *F. bengalensis* L. by Rahman *et al.* (2004) and Munshi *et al.* (2004).

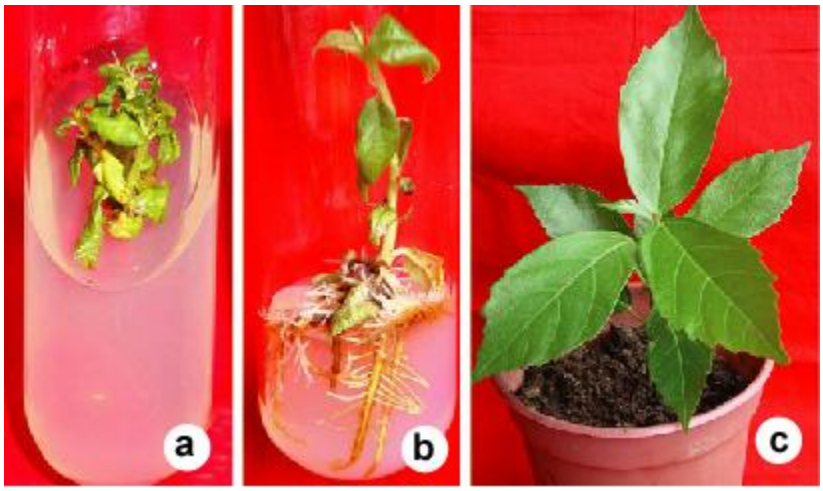

Fig. 1a-c: *In vitro* regeneration of *Ficus glomerata* from shoot tip and nodal explants. (a) Induction of shoots in four-week-old culture on MS + 0.5 mg/l BAP. (b) Rooting of regenerated shoots cultured on half strength MS + 2.0 mg/l IBA + 0.1 mg/l NAA after three weeks. (c) Four months old acclimatized plant.

After four weeks the rooted shoots were transferred to pots. None of the plantlets survived when they were directly transferred from rooting medium to the pot under natural conditions. About 82 per cent of the transplanted plantlets of *F. glomerata* survived when the plantlets in the rooting culture

Table 2. Effects of auxins in half strength MS medium on root induction in four weeks old regenerated shoots of *Ficus glomerata*. n = 15, ± = Standar error.

IAA	IBA	NAA	% of shoots producing roots	Number of roots/shoot
0.5			-	-
1.0			-	-
1.5			-	-
2.0			-	-
	0.5		47.2 ± 0.66	3.2 ± 0.20
	1.0		54.8 ± 0.86	3.4 ± 0.24
	1.5		67.2 ±1.07	3.6 ± 0.40
	2.0		74.4 ± 0.75	3.8 ± 0.37
		0.5	24.4 ± 0.51	2.2 ± 0.20
		1.0	28.2 ±1.56	2.4 ± 0.24
		2.0	33.8 ± 0.86	2.6 ± 0.40
1.0		1.0	37.4 ±1.60	2.4 ± 0.40
2.0		2.0	24.4 ± 0.51	2.2 ± 0.20
	1.0	0.1	52.4 ± 0.75	3.4 ± 0.514
	1.0	0.5	49.2 ± 1.53	3.2 ± 0.37
	1.0	1.0	37.4 ± 1.60	2.4 ± 0.40
	2.0	**0.1**	**83.2 ± 1.65**	**6.2 ± 0.73**
	2.0	0.5	72.4 ± 0.98	4.8 ± 0.73
	2.0	2.0	68.8 ± 0.66	4.4 ± 0.51
1.0	1.0	1.0	46.6 ± 0.68	3.0 ± 0.32
2.0	2.0	2.0	64.2 ± 1.11	3.6 ± 0.51

tubes were kept in normal room temperature for seven days before transplantation in pots. The plantlets were reared for three weeks at 30 ± 2º C and light (2000 lux) in a chamber with 80 per cent relative humidity. During this period shoots elongated, their leaves expanded and turned deep green and healthier (Fig. 1c).

Among different concentrations and combinations of IBA, NAA and IAA, 2.0 mg/l IBA + 0.1 mg/l NAA in half strength MS medium resulted in highest per cent (83.2) of rooting from regenerated shoots (Fig. 1b, Table 2). Use of auxins singly or in combination for rooting was also reported by different authors in *F. religiosa* (Deshpande *et al.* 1998, Munshi *et al.* 2004 and Hassan *et al.* 2008) and in *F. benghalensis* (Rahman *et al.* 2004).

After three weeks, plants were transferred to an open place and gradually acclimatized in the outdoor conditions, where 82% plants survived. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation for sustainable use.

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