IN VITRO MICROPROPAGATION OF *ABUTILON RANADEI* WOODR. & STAPF. CRITICALLY ENDANGERED PLANT SPECIES IN WESTERN GHATS

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Abstract: The present investigation was conducted to develop a protocol for rapid callus induction and plant regeneration from explant of *Abutilon ranadei* Woodr. & Stapf. Callus induction and plantlet regeneration at various frequencies were observed on MS using different concentrations of NAA alone or in combination with BAP and Kn. The highest percentage of callus induction was observed with 2.5 mg/l NAA 80 % and with combination of 0.5 mg/l Kn (75). Optimum shoot formation was observed on same medium but supplemented with 2.0 mg/l Kn and 1.0 mg/l NAA (8.2). Rooting experiments with half strength of MS revealed that NAA was more suitable for root induction compared to IBA and IAA. The rooted plantlets were hardened and successfully established in pots. More than 200 hardened plantlets in one successive year were transferred to their natural field of Botanical Garden.

Key words: *Abutilon ranadei,* Callus Induction, Micropropagation.

Introduction: *Abutilon ranadei* Woodr. & Stapf. (Son-Ghanta) belongs to Malvaceae. It is an critically endangered plants in Western Ghats (Mishra and Singh, 2001 & Walter and Gillett, 1997). It is grown as an erect branched shrub or small tree in the Western Ghats of India specially Maharashtra. It can grow up to 5-10 m in height and flowers are orange-yellow in colour (Almeida, 1996; Singh, 2000; & Yadav and Sardesai 2002).

Plant cell and tissue culture has become a major tool in the study of an increasing number of fundamental and applied programs in plant science. Tissue culture techniques are being used globally for the exist conservation of plants. Conservation of critically endangered plants has also been achieved through tissue culture (Gupta *et al.* 1997; Verma and Kant 1996; Hogue *et al.* 2000; Nichol *et al.* 1991 & Palai *et al.* 2000). Other two reports are genus *Abutilon indicum* in *in vitro* study (Jyoti, *et al.* 2009 & Nataraja and Patil, 1984.) However, there is no report on *in vitro* regeneration of *Abutilon ranadei* Woodr. & Stapf. In this study, the aim was to establish an efficient protocol for regenerating large number of plantlets *in vitro* from the explant derived callus cultures.

Materials and Methods: The young healthy immature fruit of *Abutilon ranadei* Woodr. & Stapf. were collected from Western Ghats of Maharashtra (Torna fort, Pune District). Freshly collected immature fruits zip lock polythene bags. They were washed first under running tap water (15 - 20 min) to remove surface adhered particles and then with 5% liquid hand wash dettole for 5 min followed by 70% ethanol for 5 Min. The inoculum was rinsed in distilled water (three - four times) and transferred to Laminarair flow cabinet. The inoculum was then surface sterilized by 0.1% (w/v) $HgCl_2$ for 5 minutes. Finally, the explants were washed in sterile distilled water for three - five times to remove the residual $HgCl_2$ and then cut into appropriate sizes for inoculation on to the sterile medium. Excised cotyledons were then aseptically incubated in flasks containing agar gelled MS medium (Murashige and Skoog, 1962). After 30 to 40 days root tip and shoot tip of seedlings were dissected out and cultured on MS medium. The callus induction medium composed of MS containing 3% (w/v) sucrose, 2% (w/v) clarigel with different concentrations of NAA alone or in combination with BAP and Kn for callus induction. The calli were transferred to the fresh medium for further proliferation and maintenance. The well developed calli were selected and subcultured on regeneration media. MS was supplemented with different concentrations of Kn and BAP alone or in combinations with NAA for shoot regeneration. Individual regenerated shoots were excised and used for rooting. Root induction was carried out on full strength of MS supplemented with NAA, IBA and IAA at different concentrations. Medium without plant growth regulators was used as a control. The pH of the medium was adjusted to 5.8 before autoclaving for 15 min at 121ºC. All the cultures were incubated at 25 ± 2ºC with a 16 hr photoperiod (40 $\mu E/cm^2/min/sec$) provided by cool white fluorescent tubes. Well developed rooted shoots were removed from the culture vessels, washed gently under running tap water and planted in pots containing 50 % soil and 50 % cocopith (1: 1). The plantlets were kept in the greenhouse for acclimation (two -three weeks) before their subsequent transfer to the field. Humidity was maintained by sprinkling water regularly (Jasrai *et al.* 1999). Plants were gradually exposed to the normal conditions and finally transferred to the Botanical Garden of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

Each experiment was repeated more than two times. Data were recorded on the percentage of response,

number of shoots per explants, number of roots and root length per shoot. Means and standard errors were estimated for each treatment.

Results and Discussion: Explants segments were cultured on MS with various levels of growth regulators, namely NAA alone (0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) or in combination with BAP or Kn (0.25, 0.5, 1.0, 1.5 2.0 and 2.5 mg/l) for callus induction. Morphogenic potentialities of the explants were found to differ depending upon growth regulator supplements (Table 1). After one week of inoculation with NAA (2.5 mg/l) the tissue started swelling and induction of callus (Fig. 1A). Among different concentrations of auxins, 2.5 mg/l NAA alone was found to be most effective (80%) for callus

Table 1. Effect of different concentrations of NAA alone or in combination with BAP or Kn in MS for callus induction from leaf explants of *A. ranadei*.

Growth regulators (mg/l)	Response (%)	Callus colour	Degree of callus formation
Hormone free MS	-	-	-
NAA			
0.5	50	C	*
1.0	55	CG	*
1.5	65	YG	**
2.0	75	YG	**
2.5	80	CG	***
NAA + BAP			
2.5 + 0.5	30	C	*
2.5 + 1.0	55	CG	*
2.5 + 1.5	70	C	**
2.5 + 2.0	50	YG	*
2.5 + 2.5	40	YG	*
NAA + Kn			
2.5 + 0.5	60	YG	**
2.5 + 1.0	70	CG	***
2.5 + 1.5	65	YG	**
2.5 + 2.0	50	C	*
2.5 + 2.5	50	C	*

*Slight callusing. **Considerable callusing and ***Profuse callusing. C = Creamy. CG = Creamy green. YG = Yellow green. Data were taken after eight weeks of culture and each treatment consisted of 20 Jam bottles.

induction (Fig. 1A). Callus induction gradually increased up to 2.5 mg/l NAA and then declined. Where a combination of NAA and Kn were applied, the highest callusing rate of 70% was observed for the explants in the medium containing 2.5 mg/l NAA + 0.5 mg/l Kn. When different concentrations of NAA with BAP were tried, 2.5 mg/l NAA + 1.0 mg/l BAP produced 70% of callus. Several researchers observed that NAA was the best auxin for callus induction for monocot and dicot plants (Chee 1990, Malamug *et al.* 1991). In the present study NAA alone showed better effect for callus induction in *A. ranadei*. Similar results were also observed in leaf explants of *Abutilon indicum* L. (Jyoti *et al.* 2009). Calli produced under light conditions and transferred to MS supplemented with various concentrations and combinations of BAP/ Kn (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l)] along with NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) for shoot regeneration (Fig. 1B). Hormone free MS was used as a control, which induced shoots at a rate of 2.1 shoots per explant. Shoot formation was noted to be highly influenced by concentrations and type of the growth regulators. Comparisons between the best individual concentrations for each growth regulator of cytokinins (Kn and BAP) revealed an average of 4.2 (2.0 mg/l Kn) and 3.5 (2.0 mg/ l BAP) shoots,

Table 2: Effect of different concentrations of Kn/ BAP alone or in combination with NAA on shoot formation from leaf callus of *A. ranadei*. There were 20 explants in each treatment and data (mean ± SEM) were recorded after 6 weeks of culture

Growth regulator (mg/l)			Response (%)	No. of shoots/culture
Kn	BAP	NAA		(Mean ± SEM)
Hormone free MS			60	2.1 ± 0.2

0.5			60	2.2 ± 0.1
1.0			80	4.2 ± 0.1
1.5			80	3.4 ± 0.2
2.0			70	3.5 ± 0.2
2.5			70	2.0 ± 0.1
0.5			55	2.2 ± 0.1
1.0			75	3.5 ± 0.2
1.5			70	3.1 ± 0.1
2.0			60	2.5 ± 0.3
2.5			50	1.0 ± 0.1
2.0		0.5	80	5.2 ± 0.2
2.0		1.0	85	8.2 ± 0.2
2.0		1.5	70	6.6 ± 0.2
2.0		2.0	60	2.2 ± 0.2
2.0		2.5	60	2.5 ± 0.2
	2.0	0.5	80	3.4 ± 0.3
	2.0	1.0	70	2.1 ± 0.2
	2.0	1.5	60	2.1 ± 0.1
	2.0	2.0	55	2.2 ± 0.1
	2.0	2.5	60	3.3 ± 0.3

A lower number of shoot multiplication and elongation was observed at higher concentration of Kn and BAP (Table 2). The highest regeneration was achieved on MS medium supplemented with 2.0 mg/l Kn in combination with 1.0 mg/l NAA after six weeks of inoculation (Fig. 1C). On this combination, shoot organogenesis was 80% and the number of shoots per culture was 8.2 (data not shown). The synergistic effect of cytokinins in combination with a low concentration of auxin has been reported earlier for several medicinal and endangered plants plant species like *Azadirachta indcia* (Shahin-uz-zaman, *et. al.* 2008), *Vanasushava pedata* (Karuppusamy, *et al.* 2006), *Exacum travancoricum* (Kannan, 2007), and *Ceropegia fantastica* (Chandore *et. al.* 2010). The present study also exemplified the positive modification of shoot multiplication efficacy by low concentrations of auxin (NAA) in combination with Kn. There were significant differences among treatments for root induction. Among different concentrations of NAA (0.5, 1.0, 1.5 and 2.0 mg/l) was found to give comparatively better response than IBA and IAA (Table 3). The highest number of roots (4.2 per explant) was observed with half strength of MS supplemented with 0.2 mg/l NAA

Table 3: Effect of different concentrations of auxins on root formation from the *in vitro* grown shoots on half strength of MS medium. Data represents the mean of 20 cultures. Data were recorded after four to six weeks of culture (mean ± SEM).

Growth regulators (mg/l)	Response (%)	No. of roots (Mean ± SEM)	Root length (cm) (Mean ± SEM)
Hormone free ½ MS	40	-	-
NAA			
0.1	65	3.0 ± 0.2	3.6 ± 0.1
0.2	75	5.2 ± 0.3	4.5 ± 0.1
0.5	60	2.9 ± 0.2	3.1 ± 0.2
1.0	60	2.1 ± 0.2	2.3 ± 0.1
IBA			
0.1	60	1.7 ± 0.2	2.3 ± 0.1
0.2	60	2.2 ± 0.8	3.0 ± 0.2
0.5	55	3.0 ± 0.2	3.7 ± 0.2
1.0	55	2.3 ± 0.2	4.3 ± 0.2
IAA			
0.1	50	1.4 ± 0.1	0.8 ± 0.2
0.2	60	3.6 ± 0.3	1.6 ± 0.2
0.5	60	3.1 ± 0.3	2.5 ± 0.2
1.0	50	2.2 ± 0.1	2.4 ± 0.1

(Fig. 1D). The findings agreed with those observed in other plant species such as *Cephaelis ipecacuanha, Plantago Ovata* (Jha and Jha 1989, Wakhlu and Barna 1989). Rooting did not occur in auxin free control. The *in vitro* developed plantlets were transferred to small pot containing sterilised mixture of soil and cocopith (1 : 1) for acclimation. The hardened plants were transferred to the field after 45 days of good growth (Fig. 1E & F). The survival rate was 85% in the garden.

The present study describes an efficient and easy to handle protocol for *in vitro* regeneration of *A. ranadei* which could be considered for large scale multiplication and propagation of this critically endangered plant in Western Ghats.

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