

IN-VITRO MORPHOGENIC RESPONSE OF *IXORA PARVIFLORA* VAHL.

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KEY WORDS

callus, bud culture, node culture, root- proliferation, Rubiaceae

ABBREVIATIONS

2, 4-D - 2, 4- dichloro-phenoxy acetic acid,
KIN – 6-furfuryl amino purine (kinetin),
MS -Murashige and Skoog,
NAA – α -naphthaleneacetic acid

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ABSTRACT

Ixora parviflora Vahl. is an ornamental shrub which is used in whooping cough and anaemia. Its tissue culture study was undertaken to search out morphogenic responses of explants taken from different parts of the plant. 6-furfuryl amino purine (KIN) with α -naphthalene acetic acid (NAA) produced normal growth in the cultured shoot apex. Shoot apex, leaf, internode and node explants were found suitable for callus production. Murashige and Skoog medium with KIN and NAA induced normal growth in cultured floral bud. Internode was found most suitable for production of roots when cultured on NAA (1.5 mg/ L).

INTRODUCTION

The genus *Ixora* (Rubiaceae) comprises 160 species out of which 30 are found in India. *Ixora parviflora* Vahl. is one of the Indian species. It is a shrub cultivated in gardens for its beautiful white crown of flowers and evergreen leaves. Its flower is used in whooping cough and decoction of bark is used in anaemia (Santapan and Henry, 1973, Thacker et al., 1959). Its propagation through cuttings takes about six months in rooting. Thus, conventional method of propagation is time consuming and labour intensive. Micropropagation method through tissue culture has not been exploited and there has been no report of any tissue culture studies of *I. parviflora*. However, micropropagation has been reported in two other species, *Ixora coccinea* (Lakshmanan et al., 1997) and *Ixora singaporensis* (Malathy and Pai, 1998).

The objective of the present work was to investigate *in-vitro* morphogenic response of different parts of *I. parviflora* to establish foundation for biotechnological study and micropropagation of this important plant.

MATERIALS AND METHODS

Shoot apex, leaf, node, internode and floral bud, all in their early stage of development, were taken as explants. These explants were washed with 0.1% (v/v) Tween 80 for 30 min. After washing they were surface sterilized with 0.2% (w/v) HgCl₂ for 8 min and finally rinsed with sterile distilled water 3-4 times. About 1-1.5 cm segments of these explants and whole floral bud were dried on sterile filter paper and cultured on Murashige and Skoog (MS 1962) medium with 30g / L sucrose and 8g /L agar, KIN, NAA and 2,4-D (Hi-media, Bombay) at pH 5.6 (Table 1). The cultures were grown under 24-h

photoperiod with florescent light at $26 \pm 2^\circ\text{C}$. Calluses produced were subcultured on previously described media under similar physical condition. Ten replicates were maintained for each treatment. The cultures were evaluated after six weeks for morphogenic responses.

RESULTS AND DISCUSSION

Establishment of explant

Very high rate of contamination and browning of medium due to phenolics exuded were observed in all the explants during establishment to the culture medium. However, leaf and floral bud explants exuded less phenolics. Both the problems were solved by repeated subculturing of the explants and surface sterilization methods described earlier.

Success of tissue culture experiments is highly dependent on surface sterilization of the explants (Cramer, 1994). Process of surface sterilization is specific to species; same process does not work for different sources of explants even of the same species. Phenolics present inside explants gradually exhaust on repeated subculturing and thus help in establishment of the explants to the culture medium.

Shoot apex culture

Lower concentration of KIN and NAA induced normal growth in shoot apex without callus formation. However, increase in the concentration of KIN to 2.0-3.0 mg/L induced callus formation and restricted normal growth of shoot apex (Table 1). Similar to our finding Malathy and Pai (1998) reported that higher concentration of cytokinin restricts growths of shoots in *I. singaporensis*. The reason for non proliferation of axillary shoots might be the strong apical dominance as observed in *I.*

coccinea (Lakshmanan et al., 1997) and *I. singaporensis* (Malathy and Pai, 1998).

Apical dominance is caused by the action of basipetally transported auxin from the apex and the consequent inhibiting of axillary bud growth (Clime, 1994).

Leaf culture

Lower concentration of KIN and NAA favoured callus formation in cultured leaf explants from their cut ends especially at the regions of mid-veins and veinlets. As found in the present work, Panda et al. (1991) also found cut ends of leaf responsive for callusing. Combination of cytokinin and auxin in the media are known to give better response than only auxin as reported in *Sideritis* (Sanches-Gras and Segura, 1997).

Node culture

2, 4-D with KIN favoured development of callus in case of node culture (Table 1). Similar to our finding it was reported that for callus formation, 2, 4-D is the most potent auxin (Zagorska et al., 1997). It strongly antagonises organised development.

Internode culture

NAA alone was found suitable for proliferation of roots from internode (Table 1). Best rhizogenesis from internode was achieved when cultured on medium with 1.5 mg/L NAA (Figure 1). Similar to our finding, Lakshmanan et al. (1997) reported NAA to be the most effective auxin for initiation and growth of root in *I. coccinea*.

Floral bud culture

KIN together with NAA induced growth in floral bud and produced normal flower (Figure 2). However, Lakshmanan et al. (1997) reported requirement of another cytokinin (BA) for production of normal flowers in *I. coccinea*.

We found that different explants responded differently on the culture medium. The

variation in responses of explants may be attributed to altered levels of endogenous hormones, variation in degree of differentiation and finally their response to exogenous hormones present in the medium (Sudha Vani and Reddy, 1996).

In order to develop micropropagation protocol we undertook tissue culture study of different parts of *I. parviflora*. Findings of the study as reported by us will make foundation for future biotechnological studies.

Table 1. *In-vitro* response of *Ixora parviflora* cultured on MS (1962) medium

MS medium supplemented with growth hormones (Mg/L)	Cultured Explant	% of Cultures producing callus	Cultures producing roots		Other response
			No. of roots produced per explant	Length (cm) of roots	
KIN (0.5-1.0)+NAA(1.0-2.0)	Shoot apex	-	-	-	NG
KIN(2.0-3.0)+NAA(1.0-2.0)	Shoot apex	86.6	-	-	-
KIN(0.5-3.0)+NAA(0.5-2.0)	Leaf	70.7	-	-	-
KIN(0.0)+NAA(0.5)	Internode	25.0	2.0±0.7	1.5±0.2	-
KIN (0.0) + NAA (1.0)	Internode	32.0	2.5±0.6	2.0±0.3	-
KIN (0.0) + NAA (1.5)	Internode	35.0	2.8±0.4	1.8±0.4	-
KIN (0.0) + NAA (2.0)	Internode	25.0	1.9±0.6	1.2±0.5	-
KIN (1.0-3.0)+ NAA (1.0-3.0)	Internode	47.3	-	-	-
KIN (1.0-3.0) + 2,4-D(3.0-5.0)	Internode	39.3	-	-	-
KIN (1.0-2.0) +2,4-D(4.0-5.0)	Node	47.0	-	-	-
KIN(0.5-2.0) + NAA(1.0-2.0)	Floral bud	-	-	-	NG
KIN (1.0-2.0) + NAA (1.0-2.0)	Callus	60.2	-	-	-
KIN (1.0-2.0) + 2,4-D(4.0-5.0)	Callus	70.8	-	-	-

Results noted after six weeks of culture, Mean and standard error based on ten replications.

NG = normal growth.

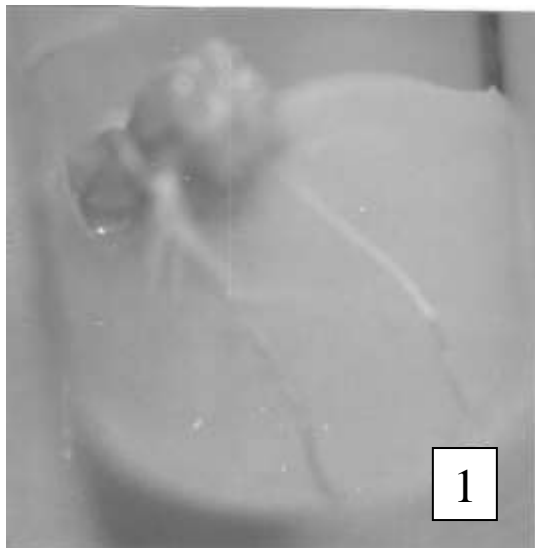


Figure 1. Differentiation of roots from internode cultured on MS medium with NAA (1.5 mg/ L). (X 2.3)

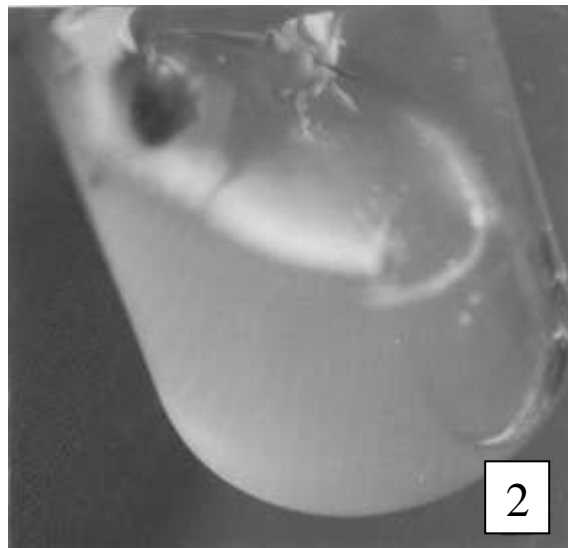


Figure 2. Development of normal flower from floral bud cultured on MS medium with KIN (2.0 mg/ L) and NAA (1.5 mg/ L) (X 2.1)

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