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BRIEF COMMUNICATION

Effect of auxins on *in vitro* rooting of *Plumbago zeylanica*: peroxidase activity as a marker for root induction

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Abstract

Induction of rooting in the microshoots of *Plumbago zeylanica* was achieved on halfstrength basal Murashige and Skoog's medium supplemented with 0.25 mg dm^{-3} indole-3-butyric acid. Rooting was totally inhibited when the microshoots were cultured *in vitro* under continuous light, however, maximum percentage of microshoots rooted when incubated in continuous light for 4 weeks before transfer to the rooting media. Peroxidase activity increased markedly during root induction indicating a key role of peroxidase in rooting of microshoots of *Plumbago zeylanica in vitro*.

Additional key words: medicinal plant, root initiation, tissue culture.

Plumbago zeylanica L. (*Plumbaginaceae*) is an important medicinal plant in the tropical regions of India. The roots of this plant are the main source of an alkaloid, plumbagin used as anticancer drug (Jayaraman 1987, Krishnaswamy and Purushothaman 1980). Pharmaceutical companies largely depend upon materials procured from naturally occurring stands which are being depleted rapidly, raising concern about possible extinction and providing justification for development of *in vitro* techniques for perpetuation of this species.

Rooting of microshoots is critical in plant production systems *in vitro*. Induction of rooting depends on a series of interdependent phases (induction, initiation and expression) (Moncousin *et al.* 1988, Gaspar *et al.* 1992, 1994). Various studies on adventitious root formation have shown the fundamental role played by peroxidase in rooting of plants cultured *in vitro* (Quoirin *et al.* 1974, Moncousin and Gaspar 1983, Berthon *et al.* 1989, Rival *et al.* 1997). The role of auxins in relation to the peroxidase activity in rooting of various plant species was also reported by Hausman *et al.* (1997) and Kevers *et al.* (1997). The present investigation was to determine the effect of auxins and photoperiod on rooting and record the peroxidase activity during root induction in the microshoots of *Plumbago zeylanica*.

Internodal segments (3 - 4 cm) of *Plumbago zeylanica* L. were collected from Chandaka Reserve Forest, Bhubaneswar, Orissa, washed with 2 % (v/v) detergent *Teepol* (*Qualingen*, Bombay, India) and rinsed with running tap water. The explants were surface sterilised in 0.1 % (m/v) aqueous mercuric chloride solution for 15 min followed by washings with sterile distilled water. Then the segments (0.5 - 1.0 cm) were placed on semi-solid basal MS (Murashige and Skoog 1962) medium supplemented with different concentrations and combinations of 6-benzyladenine (BA: 0, 0.25, 0.5, 1.0, and 1.5 mg dm^3), kinetin (Kn: 0, 0.25, 0.5, 1.0, and 1.5 mg dm^3), IAA (0, 0.01, 0.1, and 0.25 mg dm^3) for bud proliferation and multiplication. The pH of the media was adjusted to 5.8 before autoclaving. The cultures were maintained at 25 ± 2 °C, either under continuous light or 16-h photoperiod (cool, white fluorescent tubes, irradiance of 55 μmol m^{-2} s^{-1}).

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Abbreviations: BA - 6-benzyladenine, Kn - kinetin, 2,4-D - 2,4-dichlorophenoxyacetic acid, IAA - indole-3-acetic acid, IBA - indole-3-butyric acid, NAA - 1-naphthaleneacetic acid, PVP - polyvinyl pyrrolidone, MS medium - Murashige and Skoog's (1962) medium.

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For root induction, excised microshoots (1 - 2 cm) were cultured on half strength MS basal salts supplemented with different concentrations of IBA, IAA, NAA, or 2,4-D (0.05, 0.1, 0.25 and 0.5 mg dm^{-3}) and 2 % (m/v) saccharose. All the cultures were incubated at 25 ± 2 °C under 16-h photoperiod. Percentage of rooting was estimated at 2 d intervals upto 10 d. Rooted micropropagules were thoroughly washed to remove the adhering gel and planted in 2.5 cm earthen pots containing a sterile mixture of sand, soil and cow-dung manure in the ratio of 1:1:1 (v/v/v) and kept in the greenhouse for acclimatization. Fifteen cultures were used per treatment and the experiment was repeated at least three times. The data were statistically analysed by the Post-Hoc Multiple Comparison test (Marascuilo and McSweeney 1977).

For determination of peroxidase activity samples (100 mg) were collected at 2-d intervals and homogenised in cold 0.1 M phosphate buffer (pH 6.1) containing 30 mg of insoluble PVP and 15 mg sodium ascorbate. The homogenate was filtered through four layers of miracloth and centrifuged at 12 000 *g* for 10 min at 4 °C. The supernatant was used for the peroxidase assay. The assay mixture contained 0.1 M phosphate buffer (pH 6.1), 4 mM guaiacol, 3 mM H_2O_2, and crude enzyme extract. The absorbance at 420 nm was measured using a double beam UV-spectrophoto-meter (*UVIDEC-650*, *Jasco*, Tokyo, Japan). The enzyme activity was expressed as μmol(H_2O_2 destroyed) mg^{-1}(protein) s^{-1} (Bergmeyer *et al.* 1974). Protein content was determined according to the method of Bradford (1976) using bovine serum albumin as a standard.

Table 1. Effect of IAA, IBA, NAA and 2,4-D on rooting from excised shoots of *Plumbago zeylanica* cultured on MS basal salts supplemented with 2 % (m/v) saccharose. Means ± SE of 15 cultures per treatment in three repeated experiments (a - basal callusing at the cut end).

Auxin	[mg dm^{-3}]	Rooted shoots [%]	Time to rooting [d]	Auxin	[mg dm^{-3}]	Rooted shoots [%]	Time to rooting [d]
IAA	0.05	0	0	NAA	0.05	0	0
	0.10	42.6 ± 0.6	10		0.10	32.8 ± 0.4	12
	0.25	30.8 ± 0.4	12a		0.25	24.6 ± 0.3	12a
	0.50	24.6 ± 0.2	14a		0.50	18.5 ± 0.6	13a
IBA	0.05	0	0	2,4-D	0.05	33.7 ± 0.3	10
	0.10	72.8 ± 0.7	8 - 9		0.10	30.5 ± 0.4	12
	0.25	94.5 ± 0.5	7 - 8		0.25	a	a
	0.50	50.6 ± 0.3	10a		0.50	a	a

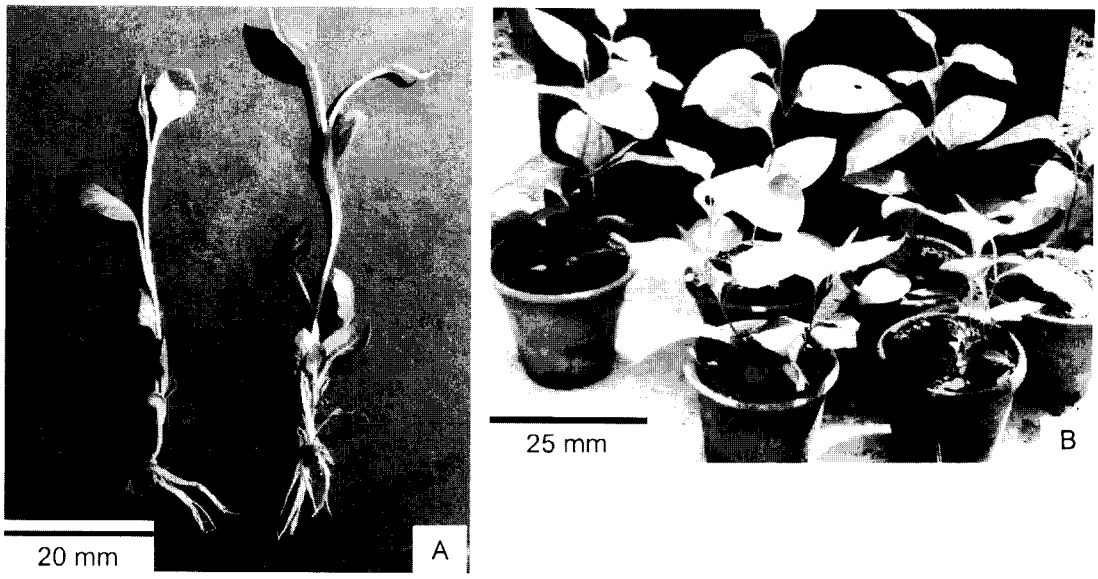

Fig. 1. Rooting of the *in vitro* derived shoots after 7 d of culture on MS basal medium + 0.25 mg dm^{-3} IBA + 2 % (m/v) saccharose (*A*) and plants established in soil after 6 weeks (*B*).

Of the two cytokinins used, BA was more effective for shoot proliferation than kinetin. Rapid multiplication of shoots was achieved on medium containing 1.0 mg dm^{-3} BA with 0.01 mg dm^{-3} IAA within 4 weeks of culture. Among four auxins used, IBA was more effective for root induction than NAA, IAA and 2,4-D. Rooting was totally inhibited on the medium devoid of auxins. The root initiation took place within 7 - 8 d of culture on MS medium supplemented with 0.25 mg dm^{-3} IBA with 2 % saccharose (Table 1). The percentage of rooting was the maximum (94.5 %) on this medium (Fig. 1*A*). The percentage of shoots forming roots and the number of roots/shoot significantly varied with different concentrations of IBA, IAA, NAA and 2,4-D induced rooting with intervening callus. The medium having 2,4-D promoted rooting at the lower concentration but callusing at the cut ends was noted at higher concentrations (0.25 - 0.5 mg dm^{-3}). The effects of auxins on rooting in different plant species were studied by various researchers (Blakesley *et al.* 1991, Rout and Das 1994, Gaspar *et al.* 1997, Kevers *et al.* 1997, Saxena *et al.* 1998). The irradiance had significant effects on induction of rooting (Seibert and Kadkade 1980). The rooting was inhibited in continuous light. However, when the multiple shoots were incubated in the continuous light for 4 weeks before transfer to the rooting medium, the maximum percentage of rooting was recorded. The rate of rooting dependent on growth regulators and photoperiod was also reported (Murashige 1974, Seibert and Kadkade 1980, Baraldi *et al.* 1988, Samantaray *et al.* 1995).

In case of control, there was no change in the peroxidase activity during course of the experiment (Fig. 2). Auxin treatments induced a sharp increase in the peroxidase activity. The peroxidase activity was minimum in the inductive phase (0 d and 3 d) and maximum in the initiative phase (7^{th} to 8^{th} day) in microshoots grown on medium containing 0.25 mg dm^{-3} IBA (Fig. 2*A*). Similar trend was found in *Sequoiadendron giganteum* (Berthon *et al.* 1990), poplar (Hausman 1993) and oil palm (Rival *et al.* 1997). When the rooted plantlets were transferred to pots in greenhouse about 95 % of the plantlets established well within 6 weeks of transfer (Fig. 1*B*).

The present study confirmed the role of auxin and photoperiod on the rooting of *Plumbago zeylanica* and increased peroxidase activity during root induction.

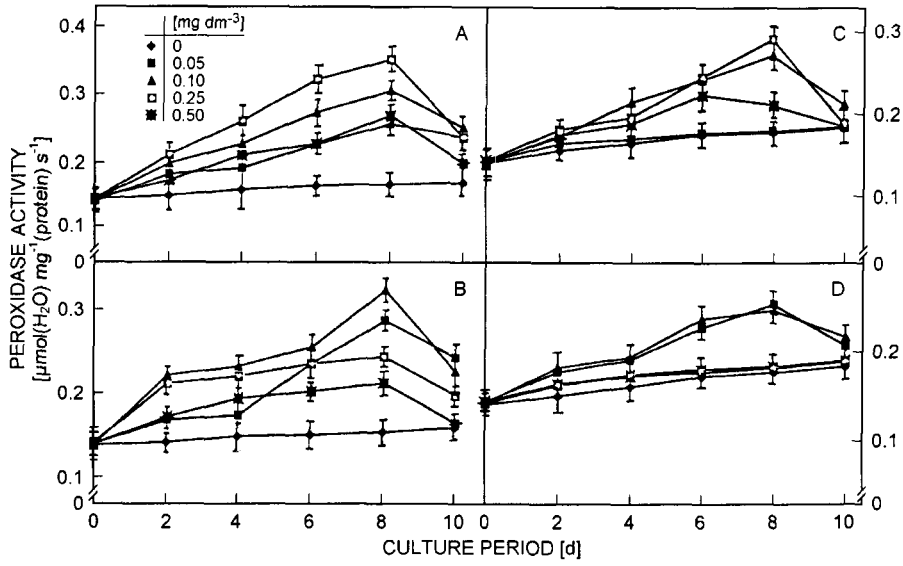

Fig. 2. Peroxidase activity in microshoots of *Plumbago zeylanica* grown *in vitro* at different concentrations of IBA (*A*), IAA (*B*), NAA (*C*), and 2,4-D (*D*). Activity was measured prior to inoculation on rooting media (0 d) and 2, 4, 6, 8 and 10 d after inoculation. Bars represent SE of the mean of the three independent experiments, 10 samples per treatment.

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