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MICROPROPAGATION OF *PEDILANTHUS TITHYMALOIDES* VAR. *GREEN* - A HYDROCARBON YIELDING PLANT

ANSHU RANI and **ASHWANI KUMAR**

Department of Botany, University of Rajasthan, Jaipur-302004, India.

Apical buds, stems and leaf explants of *Pedilanthus tithymaloides* var. *green* were cultured on Murashige and Skoog's basal medium supplemented with different concentrations of auxins and cytokinins. Shoot development was recorded from apical and axillary bud explants. Kinetin and BAP favoured multiple shooting from internodal and nodal stem explants respectively. Organogenesis in callus was observed with BAP. The shoots thus formed gave rooting on IAA containing medium. The plantlets were transferred to the pots and then to the garden soil.

Keywords: Explant culture; Multiple shoots; *Pedilanthus tithymaloides*; Plantlets.

Introduction

Pedilanthus tithymaloides var. *green* yields low molecular weight hydrocarbons which can be converted into petroleum like substances[1,2]. The seed formation in *P. tithymaloides var. green* is improper and hence vegetative propagation is the only means of propagation. However, this is a slow process and causes heterogeneity. Although some tissue culture work has been done on *Asclepias erosa*[3], *Euphorbia* sp[3-6], *Hevea* sp.[7] and *Parthenium argentatum*[8] but investigations on *Pedilanthus tithymaloides* are lacking. Attempts were made to develop a method of micropropagation through *in vitro* techniques using explants from different plant parts. Induction of organogenesis in callus cultures was also observed.

Material and Methods

Explants were taken from six months old plants of *Pedilanthus tithymaloides* var. *green* raised in the Department of Botany. Nodal and internodal portions of stems, shoot apices and leaves were transferred aseptically on Murashige and Skoog's medium[9] after sterilization with 0.1 per cent mercuric chloride. Various concentrations of auxins; IAA (1.0 - 15.0 mg/l), NAA (5.0 - 15.0 mg/l), IBA (5.0 - 15.0 mg/l), 2, 4-D (0.5 -2.0 mg/l) and cytokinins; kinetin (0.004-2.0 mg/l), BAP (0.5-8.0 mg/l) and their combinations, NAA (10.0 mg/l) + kinetin (0.04 mg/l) + kinetin (0.004, 0.04 and 0.4 mg/l). Out of all these treatments only significant results are being given in the table 1 and text. The medium was adjusted to pH 5.6. The cultures were maintained in a culture room at 25 ± 2°C temperature, 50-60 per cent relative humidity and under continuous flourescent light (ca.400 lux).

Results and Discussion

Regeneration from explants as well as callus cultures was observed.

1. Regeneration from explants:

(a) *Shoot apices* : Maximum root and shoot initiation was observed in shoot explants on a combination MS+IAA (5.0 mg/l) (Fig.1). The apices elongated when transferred to MS+IAA (1.0 mg/l) + Kn (0.4 mg/l) (Table 1). Roots

appeared at the base of the explants after about six weeks in most of the cases. Rooting was suppressed and compact brownish callus developed at the base of the explants in IAA/NAA (15.0 mg/l) supplemented medium (Table 1). In NAA (5.0 mg/l) callusing could be observed at the base of the explant and from this callus, roots proliferated.

(b) *Stem explants* : Both stem nodes and internodes were placed horizontally as well as vertically in separate flasks. The response of the horizontally placed explants was better.

Table 1. Organogenic responses in cultures derived from apical shoot, stem nodes and internodes of *Pedilanthus tithymaloides* var. *green* on MS-medium supplemented with phytohormones.

Phytohormones	Apical shoot			Stem					
(mg/l)				Node			Internode		
	Shoot	Root	Callus	Shoot	Root	Callus	Shoot	Root	Callus
IAA (5.0)	++++	+++	++	+++	+++	-	-	++	-
IAA (15.0)	+++	++	+++	++	-	+++	-	-	+++
NAA (5.0)	++	+++	+	++	+++	+	++	++	-
NAA (15.0)	+++	++	+++	++	++	+++	-	-	+++
Kn (0.04)	++	++	-	+++	++	-	++*#	+	-
Kn (0.4)	++	++	+	++	++	-	-	++	-
Kn (1.2)	+++	++	-	+	+	-	-	-	-
BAP (4.0)	+++	-	++	++++	+	+++	-	-	+++
BAP (6.0)	+++	+	++	++++*	+	+++	-	-	+++
IAA (1.0)+Kn(0.04)	+++	++	-	++	+++	—	—	++	++
IAA (1.0)+Kn(0.4)	+++#	++	-	+++	+++	—	—	++	++

+Upto 10 percent; ++ upto 50 percent; +++ upto 75 percent; ++++ upto 100 percent; *-multiple shooting; #-shoot elongation.

2. Regeneration from callus: In all the treatments shoot proliferation was greater from nodal explants than from internodal explants (Table 1). Lowering of the auxin level or their total absence alongwith higher concentration of cytokinin tended to increase in shoot formation. MS+kinetin (0.04 mg/l) highly favoured multiple shooting from horizontally placed internodal segements of stem (Table 1), while rooting was observed at the base of the vertically placed explants. In vertically placed explants, callusing was observed at higher auxin concentrations (15.0 mg/l) (Table 1). Direct shoot multiplication was observed when BAP (6.0 mg/l) was supplied to the stem segments (Fig.2). These shootlets elongated on subculturing on the same medium. Transfer

Figure 1-5: 1-Shoot explant on MS+IAA (5.0 mg/l); 2 - Nodal stem explant showing multiple shooting on MS + BAP (6.0 mg/l); 3 - Shoot Primordia arising from callus on MS + 4.0 mg/l; 4. Proliferated shoot primordia after subculture; 5. Plantlet with roots on MS+ IAA (5.0 mg/l).

of these shoots on medium containing IAA (5.0 mg/l) induced rooting. Medium containing BAP (4.0 mg/l) favoured profuse callus formation in the stem explants at the basal portion. This callus gradually exhibited yellowing and resulted in 40-50 shoot primordia (Fig.3). These shoot primordia proliferated after subculturing on the same medium within ten days. Further shoot multiplicatin was observed during prolonged incubation (Fig.4). These shootlets when transferred to MS medium supplemented with IAA (5.0 mg/l) gave rise to roots (Fig.5). These regernerated plantlets were washed with sterilized water and transferred to the pots aseptically having sterilized soil mixed with vermiculite in the ratio of 3 : 1. The plants were kept in continuous light and after their establishment, they were transferred to garden soil in pots after one month.In the present study BAP induced shoot bud formation from callus cultures and from nodal explants. Kinetin is considered to be a trigger for induction of mitotic activities whereby it activates enzymes related with mitosis[10]. Multiple shoot formation, in this way provides a way of rapid multiplication of selected clones. Auxin alone has been reported to be important in the induction of root primordia[11]. The effect of auxins on root proliferation was prominent in *Pedilanthus tithymaloides* cultures. Best rooting response was observed using IAA. These plants could survive under pot conditions.

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