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RESPONSE OF LEAF EXPLANTS OF *DATURA METEL* L. TO DIFFERENT TYPES OF AUXINS AND CYTOKININS IN LS AND MS MEDIUM

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The choice of nutrient components and growth regulators are crucial in tissue culture studies especially in propagating valuable economic and medicinal plants. *Datura metel* L. is a rare and important medicinal plant useful in treating chronic lung diseases. The leaf explants were cultured on both MS and LS media with different concentrations and combinations of auxins (2,4 - D and IBA) and cytokinins (BAP and Kinetin). The callus induction frequency was higher in 2,4-D + BAP combination than IBA + Kinetin combination . Of the two media, MS medium was found to be suitable for regeneration of shoots.

Keywords : Auxins; Cytokinins; Morphology; MS medium.

Introduction

Tissue culture is a unique technique to propagate the valuable economic and medicinal plants. The application includes cloning of plants which implies large scale *in vitro* propagation of plants of uniform or differing quality produced more rapidly and abundantly than are possible by conventional methods of classical breeding[1]. The degree of success in any technology employing plant cell, tissue or organ culture is related to relatively a few factors. The significant factor is the choice of nutritional components and growth regulators. The Murashige and Skoog (MS)[2] and Linsmaier and Skoog (LS)[3] are the most widely used media especially in procedures where plant regeneration is the objective[4] . Another important factor is the hormonal content of the culture medium which is crucial to any sustained growth of the culture. Auxins are required for induction of cell division, cell expansion and frequency for rooting. Auxins are often used in combination with cytokinins. They have essential role in induction and plant regeneration. Recent emergence of a new branch of science Ethnobotany is aimed at exploring and bringing about more and more species into modern therapeutics. The general demand for herbal medicine is increasing in spite of a steady supply of synthetic drug during recent times. Collection of plant parts from the wild population leads to depletion and finally to extinction. Thus measures are required to promote the cultivation of medicinal plants on large amount to conserve them[5]. *Datura metel* L. is one of the very important medicinal plants useful in asthma, cough and skin diseases[6,7]. The present study is aimed at understanding the response of leaf explant of *D.metel* to different hormone combinations and culture media.

Materials and Methods

Uniform and healthy seeds of *D.metel* L. were collected from the field grown plants near the research station. The seeds were treated with 0.1% (w/v) Bavistin for 5 minutes and dried in the sunlight. As the rate of seed germination is very slow in *D.metel*, the seeds were scarified to improve the rate of germination before inoculation. The scarified seeds were sterilized with 0.1% $HgCl_2$ for 3 minutes and washed thrice with sterile double distilled water. The sterilized seeds were inoculated on sterile wet cotton in the culture tube. The seed germinated within 15 days. The explants were collected from the aseptically germinated seedlings. The leaf explants (1 cm in size) were inoculated on MS and LS media containing different concentrations and combinations of auxins (2,4-D and IBA) and cytokinins (BAP and Kinetin). Sterilization, inoculation procedures

were done inside the Laminar air flow chamber to avoid any contamination. The pH of the medium was adjusted to 5.8 , agar (0.8%) was added and autoclaving was done at 121°C for 15 minutes. The cultures were incubated under cool - white fluorescent light (1500 lux) with a 16 hours photoperiod.

Results and Discussion

Morphologically different calli were obtained from leaf explants of *D. metel* within 15 days of inoculation on LS medium supplemented with different concentrations of 2,4-D (1-3mg/1) and BAP (1and 2 mg/1) . The relative callus growth was very high at 2 mg/l 2,4-D alone and the rate of callus induction was decreasing with other concentrations and combinations of 2,4-D and BAP (Table 1). The effect of 2,4-D on callus induction was remarkable when it was given separately than in combination with BAP . The nature of the calli range from friable to hard and the colours of the calli were whitish to greenish. Though in all concentrations and combinations, the callus induction was triggered, 2mg/1 2,4-D alone was found to be the best concentration to induce friable callus and 1mg/1 2, 4-D + 2mg/1 BAP was the best combination for green hard callus induction. In still higher concentrations of 2,4-D (3mg/1) and BAP (1and2 mg/1) the explants were necrosed.

When the calli were subcultured on the same medium with the same combinations of 2,4-D and BAP, the green hard calli responded positively. Shoots initiated from the calli within 7 days of subculture and maximum number of shoots regenerated at 1 mg/1 2,4-D + 2mg/1 BAP combination followed by 1 mg/1 2,4-D + 1mg/1 BAP where 1or 2 shoots produced.

The leaf explants responded differently in IBA (1mg/1) and Kinetin (1 and 2mg/1) combination. The frequency of callus induction was high at 2 mg/1 IBA + 1 mg/1 kinetin and the callus was greenish and hard. While in all other concentrations the calli were brownish hard to friable. Brownish nodular callus was obtained at 1 mg/1 IBA + 1 mg/1 kinetin. When all the calli were subcultured on the same medium with the same concentrations of growth regulators, shoots were regenerated within 15 days of subculture from the green hard calli. In certain combination the calli become rhizogenic (Table 1). The nodular callus produced shoots and roots simulaneously.

In another experiment the leaf explants of *D. metel* were inoculated on MS medium fortified with different concentrations of same auxins and cytokinins. In the first combination (2-4 D and BAP) the relative callus growth was very high at 2mg/1 2,4-D alone. The response coincides with the observation in LS medium. The callus induction frequency was high with 2,4-D than in combination with BAP. In higher BAP and lower 2,4-D combination all the calli were green and hard natured (Table 2). In higher concentration of 2,4-D the induction of callus was very poor. The green hard calli responded positively for shoot regeneration when subcultured on the same medium with the same concentrations of growth regulators. The shoot induction was high at 1 mg/1 2, 4-D + 2mg/1 BAP followed by 1.5 mg/1 2, 4-D + 2mg/1 BAP and 2 mg/1 2, 4-D +2mg/1 BAP.

In IBA and kinetin combination the callus inductin was high at 2mg/1 IBA but comparatively lower than the 2,4-D combination. The colour ranges from white to brown. The relative callus growth was poor in this combination than in other combinations and the regeneration of shoots was also not impressive. 2,4-D was ideal for friable callus induction in both (MS and LS) media when compared to IBA. The relative callus growth was high in 2,4-D + BAP combination than IBA + kinetin combination. Green hard callus induction and regeneration of shoots were high in MS medium than LS medium, Similar results were observed in *D. insignis*[8] and in *D. innoxia*[9] when 2,4-D and IBA were used separately and in combination. Kinetin was not always effective in promoting bud formation.

Table 1. Response of leaf explants of *D. metel* L. to different concentration of Auxins and Cytokinins on LS medium.

Growth regulator		Relative callus growth	Nature of Callus	Regeneration
2, 4 - D mg/l	BAP mg/l			
1	0	++	White, friable	
1	1	+	Brownish green, hard	1 or 2 shoots
1	2	+	Greenish, hard	4 shoots
1.5	0	+++	White, friable	
1.5	1	*	Brownish	
1.5	2	*	Brownish	
2	0	++++	Brownish, friable	
2	1	*	Brownish green, hard	
2	2	*	Brownish, hard	
2.5	0	++	Brownish, friable	
2.5	1	*	Brownish, hard	
2.5	2	*	Brownish, hard	
3	0	*	Brownish, friable	
3	1	Explant necrosed		
3	2	Explant necrosed		

Growth regulator		Relative callus growth	Nature of Callus	Regeneration
IBA mg/l	Kinetin mg/l			
1	0	-	-	Roots from the explants
1	1	++	Brownish, nodular	2-3 shoots and roots from the callus
1	2	+	Brownish, hard	
1.5	0	*	Brownish, friable	Rhizogenetic
1.5	1	+	Brownish, friable	
1.5	2	++	Brownish, hard	
2	0	++	Brownish, friable	
2	1	+++	Greenish, hard	3 or 4 shoots
2	2	*	Brownish, friable	
2.5	0	*	Brownish, friable	
2.5	1	+	Brownish, friable	
2.5	2	+	Brownish, hard	
3	0	+	Brownish	Rhizogenic
3	1	+	Brownish	Rhizogenic
3	2	necrosed	-	

* : Very Low + : Low ++ : Moderate
+++ : High ++++ : Very High

Table 2. Response of leaf explants of *D. metel* L. to different concentration of Auxins and Cytokinins on MS medium.

Growth regulator		Relative callus growth	Nature of Callus	Regeneration	Growth regulator		Relative callus growth	Nature of Callus	Regeneration
2, 4 - D mg/l	BAP mg/l				IBA mg/l	Kinetin mg/l			
1	0	+++	White, friable		1	0	+	White, Friable	Rhizogenic
1	1	+	Brownish, Hard		1	1	+	Greenish, slightly Friable	4-5 shoots
1	2	++	Greenish, Hard	10-15 shoots	1	2	+	Greenish, hard	
1.5	0	+++	White, friable		1.5	0	++	White, friable	
1.5	1	++	White, friable		1.5	1	++	White, friable	
1.5	2	++	Greenish, hard	5-8 shoots	1.5	2	+	Greenish, hard	1 or 2 shoots
2	0	++++	White, friable		2	0	+++	White, friable	
2	1	++	White, friable		2	1	++	White, friable	
2	2	++	Greenish, hard	4-6 shoots	2	2	+	Brownish, hard	
2.5	0	++	White, friable		2.5	0	++	White, friable	
2.5	1	+	Brownish, friable		2.5	1	+	Brownish, friable	Rhizogenic
2.5	2	*	Brownish, hard		2.5	2	+	Brownish, hard	
3	0	*	White, friable		3	0	+	Brownish, friable	
3	1	*	Brownish, friable		3	1	*	Brownish, Hard	
3	2	*	Brownish, hard		3	2	*	Brownish, Hard	

* : Very Low + : Low ++ : Moderate
+++ : High ++++ : Very High

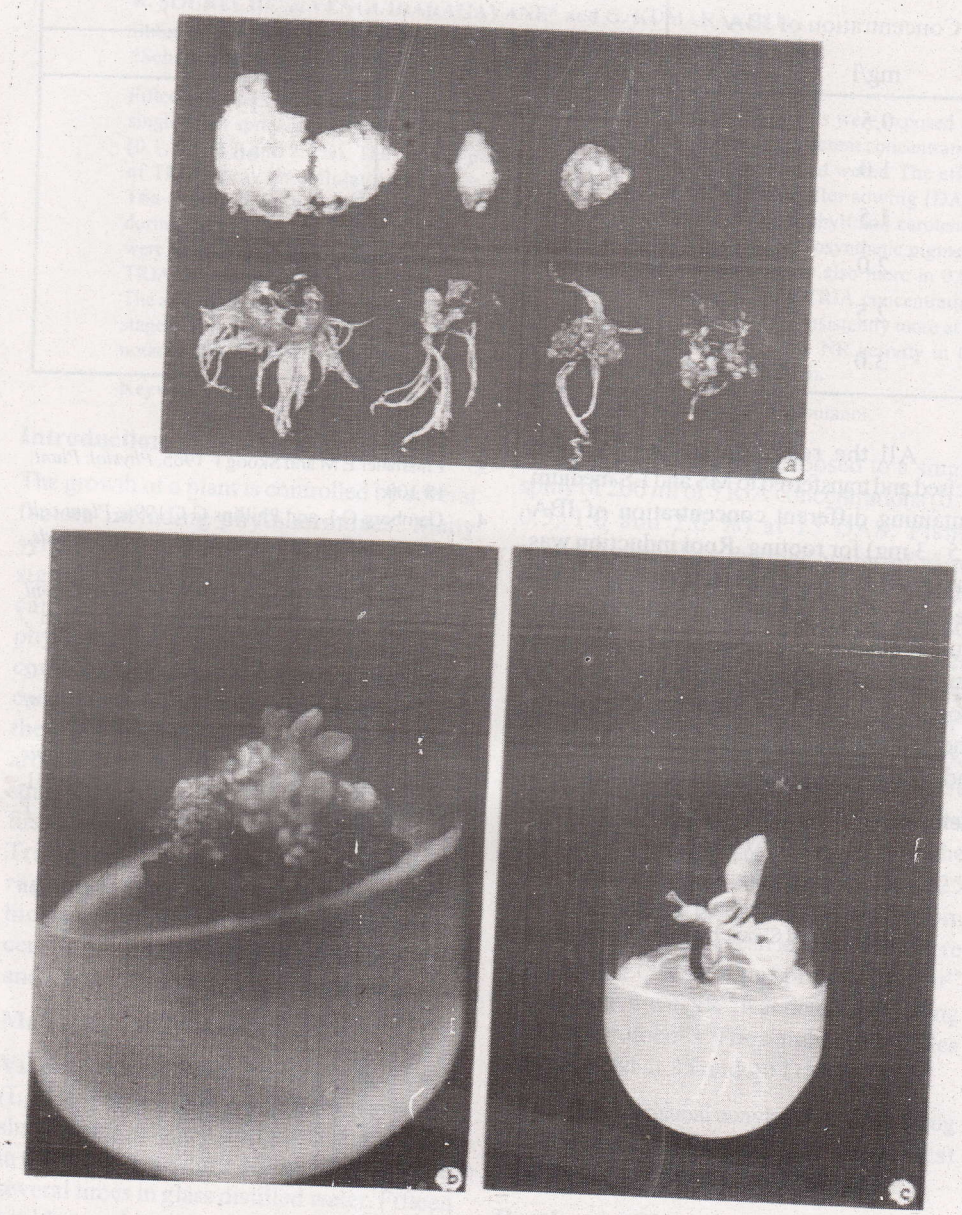

Fig.1 a : Induction of differnt types of calli. [LS medium supplemented with IBA (1-3 mg/l) and Kinetin (1 and 2 mg/l)].

b : *In vitro* shoot regeneration from callus. [MS medium supplemented with 1 mg/l 2,4-D + 2 mg/l BAP].

c : *In vitro* root induction in regenerated shoots. [Ms medium supplemented with 2 mg/l IBA].

Table 3. *In vitro* root induction of *D. metel.*

Concentration of IBA mg/l	% of Rooting	
	LS medium	MS medium
0.5	10.0	13.3
1.0	56.7	66.0
1.5	60.0	73.3
2.0	93.3	96.0
2.5	20.0	20.0
3.0	03.3	06.6

All the regenerated shoots were excised and transferred to MS and LS medium containing different concentration of IBA (0.5 - 3 mg) for rooting. Root induction was maximum at 2 mg/1IBA in both LS and MS medium (Table 3). This result coincides Muthukumar *et al.*[10] where 2mg/1 IBA was found to be ideal for root induction for shoots regenerated from internodal explants. The regenerated shootlets were hardened and transferred to soil.

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