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Effect of growth regulators on callus and multiple shoot formation of *Datura metel* L

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Abstract
Effect of growth regulators on callus and multiple shoot formation was studied in *Datura metel* L. The terminal bud and nodal segments were cultured in MS basal medium containing four different growth regulators such as 2,4-D, Kinetin, IAA and BA. MS medium supplemented with combinations of 2mg/l kinetin and 1mg/l IAA showed profuse callus formation. Nodal segments also showed profuse callus formation when MS medium was supplemented with combinations of 1.5mg/l Kinetin and 2mg/l BA. When combinations of 1mg/l Kinetin and 2mg/l BA were given only nodal segments showed multiple shoot formation.

Keywords: Datura, callus, multiple shoot, 2, 4-D, Kinetin, IAA, BA

1. Introduction
Datura metel L. locally called 'nila-Ummattu' is an important medicinal plant belongs to the family Solanaceae. This plant is distributed in India, common as a weed, growing in waste places and roadsides. Indians have long been familiar with the narcotic and intoxicating properties of this drug plant. The entire plant is used as a medicine. Leaves are simple, alternate, dark green, broadly ovate, shallowly lobed and glabrous. Flowers are large, solitary, and trumpet-shaped with a sweet fragrance usually appreciated in the mornings and evenings, with a wide range of colors, ranging from white to yellow and light to dark purple. The flowers are hermaphrodite and are pollinated by insects. The fruit is in the form of a capsule covered with short spines. A variety of phytochemicals have been found to occur in *D. metel*. These phyto constituents comprise alkaloids, flavonoids, phenols, tannins, saponins and sterols. The phyto-constituents of *Datura* were analyzed from various parts of the plant like the leaf (Donatus and Ephraim 2009) [6] root (Jamdhadel *et al*., 2010) and shoot (John De Britto *et al*., 2011) [8]. The main active constituents of the plant are the medicinally important tropane alkaloids- hyoscyamine and scopolamine. The plant finds application in the treatment of diarrhoea and skin diseases. It is also used in the treatment of burns. In traditional medicine this is a reputed drug in the treatment of bites from rabid dogs and is also used to cure insanity. The drugs give good complexion, improves digestion and it is also useful in respiratory ailments, ear ache, eye diseases, rheumatism and elephantiasis. Roots are also used for sores in mouth, pain in chest, epilepsy, convulsions and small pox. Leaves are used for dandruff, blisters and hydrocele. Seeds are used for decaying teeth, leprosy and wounds (Anonymous). The important formulations using the drug are kanakasavam, dhurdhuradi tailam, dhurdhurapatradi coconut oil, mritasanjivini.

Due to its medicinal use the plant has been used for various research studies. There are various reports of tissue culture studies in different species of *Datura*. Arockiasamy *et al*., (1999. 2007) [4, 5] reported green and compact calli from intermodal explants of *D. metel* on MS medium supplemented with different concentrations of BAP. Shoot buds were proliferated from the calli when transferred to the same medium with BAP. Fast shoot elongation and root growth in MS containing BAP 2.0 mg/l, GA3 1.0 mg/l and IBA 1.0 mg/l. (Muthukumar *et al*., 2004) [4] cultured the explants on MS medium with BAP (0.5-3.0 mg/l) and NAA (0.5 Mg/l). The nodal explants isolated from *in vivo* source exhibited a greater number of healthy multiple shoots than *in vitro*. (Akharaiyi, 2011) [2] assayed the antibacterial efficacy of crude aqueous and ethanol extracts leaf, stem bark and roots of *D. metel* at 20mg/ml against eight clinical bacterial isolates.

Among the tested bacterial isolates *Staphylococcus aureus* was the most inhibited majorly with the ethanol extract. (Varahalarao and Kaladhar, 2012) [11] evaluated the antimicrobial activity of aerial parts of *D. metel* against the resistant pathogens belong to aquatic, human and plant origin. Among all microorganisms studied *Erwinia caratovara* and *Pseudomonas syringae* showed the considerable growth inhibition with chloroform and methanolic extracts. (Ashwin, 2016) [3] investigated the effects of increasing concentrations of heavy metals in soil, on species *in vitro* and results showed that *D. innoxia* can tolerate higher concentrations of four toxic heavy metals like Pb, Cd, Cr and Ni indicating its phyto-remediation potential. Micropropagation of *D. metel* is very important considering its potential application in the field of medicine and pharmacology. Though various tissue culture studies have been reported in many species of *Datura*, not much work has been attempted in *D. metel*. The aim of this study was to evaluate the effect of growth regulators on callus and multiple shoot formation of *D.metel* L.

Materials and Methods

The study material *D. metel* was collected from the nearby areas of college campus. Terminal buds and nodal segments of the plant were used as explants for the present study.

Culture medium

MS was used as basic medium for experimental purposes. The medium consisted of macro elements, microelements, vitamins, amino acids, sugar, iron source, solidifying agents like agar and growth regulators. The macro elements and micro elements and other accessories required for the preparation of MS media are given in the following Tables. (Tables1-3)

Table 1: Macro elements required for the preparation of MS medium in mg/l

Sl. No	Macronutrients	Quantity
1	$MgSO_4 7H_2O$	370
2	KH_2PO_4	170
3	KNO_3	1900
4	NH_4NO_3	1650
5	$CaCl_2.2H_2O$	440

Table 2: Micro nutrients required for the preparation of MS medium in mg/l

Sl. No	Micronutrients	Quantity
1	$MnSO_4.4H_2O$	22.30
2	$ZnSO_4.7H_2O$	8.60
3	$Na_2M0O_4.2H_2O$	0.25
4	$CuSO_4.5H_2O$	0.025
5	$CaCl_2.6H_2O$	0.025
6	KI	0.83
7	H_3BO_3	6.20

Table 3: Supplementary nutrients of MS media in mg/l

Sl. No.	Supplementary nutrients	Quantity
	Iron source	
1	$FeSO_4.7H_20$	27.85
2	Na_2.EDTA	37.25
	Carbon source	
3	Sugar	30,000
4	Agar	9,000
	Vitamins	
5	Thiamine HCl	0.10
6	Pyridoxine HCl	0.50
7	Nicotinic acid	0.50
8	Myo-inosotol	100.00

$P^{H=5.8}$

Preparation of stock solution

Stock solutions of macronutrients, vitamins and hormones were prepared. Approximate quantities of the stock solution were taken, mixed and diluted to prepare the media.

One litre of MS medium was prepared and used as the stock solution (Table 4). The media with 30% sucrose with no addition of hormone was taken as the control. The $_P{}^H$ of the medium was adjusted to 5.8. Basal medium supplemented with different concentrations of 2,4-D, kinetin, IAA and BA were taken in different test tubes. The concentrations of different growth regulators used are given in tables5 and 6. The test tubes were closed tightly with cotton plugs and autoclaved.

Table: 4 Ingredients for the preparation of 1 litre MS medium

Sl. No	Ingredients	Quantity
1	Macronutrients	200ml
2	Micronutrients	1ml
3	Iron source	10ml
4	Vitamins	1ml
5	Sucrose	30gm
6	Agar	9gm
7	Water	upto 1000ml

$P^{H=5.8}$

Sterilization of non-living articles

As the medium containing sugar supports the growth of micro organisms, the surface of plant tissue and non-living articles including medium were sterilized. Glass metal equipments and cotton plugs were wrapped in aluminium foil and were autoclaved at 15lb pressure at a temperature of 120 ºC for 25-30 minutes.

Sterilization of plant material

The plant material was thoroughly washed with soap solution and running water. This was again washed in distilled water to remove all the traces of the soap. The terminal bud and nodal segments were treated with 20% Bavistine for 2-5 minutes and washed thoroughly in distilled water. Then the explants were transferred to 0.1% mercuric chloride solution and washed for about 1-2minutes. Then the explants were transferred to culture room and inoculated in the culture medium. After aseptic inoculation, the culture vials were incubated at 25±2 degree centigrade and were exposed to 16h photoperiod.

Results

The terminal buds and nodal segments of *D. metel* were cultured in MS Medium containing four different growth regulators. Continuous observations were made for a period of three months and the growth responses of terminal bud and nodal segments were noted

Effect on callus formation

Callus was found to be initiated in medium containing combinations of 0.5mg/l kinetin and 1mg/l IAA. Profuse callus was formed by using terminal buds with combinations of 2mg/l kinetin and 1mg/l IAA (Fig:1). Callus formation was also observed in nodal segments with combinations of kinetin 1.5mg/l and BA1mg/l and also in kinetin 1.5mg/l and BA 2mg/l (Fig:2). But the response was more in higher concentrations of BA. The colour of the callus was white. No response occurred in other media. The results are summarized in Table 5**.**

Table 5: Effect of different growth regulators on callusing

Explant	MS medium+ various concentrations of growth regulators				No of days for callus formation	Type of callus	Colour
	2,4-D	Kinetin	IAAmg/l	BA mg/l			
	mg/l	mg/l					
Terminal	0.5	-	-	-	No response		
buds		-		-	No response		
	-		1				
		0.5		-	22		
	-		1				
		2		-	30	Small size	
	-		1				
		1		1	No response	Profuse	White
	-		-			Callus formation	
	-	-	1	-	No response		
	-	0.5	1	-	No response		
Nodal segment	-	1.5	-	1	25	Small size	
	-	1.5	-	2	32	Profuse	
						callus formation	Pale yellow

Effect on multiple shoot formation

Nodal segments also showed multiple shoot formation in tube with combinations of kinetin and BA. After inoculation active growth of multiple shoots were noted. Multiple shoot formation was slight in combinations of kinetin0.5mg/l and BA 0.5mg/l. Many shoots (5-10) were obtained in combinations of kinetin 1mg/l and BA 2mg/l (Fig 3:). It was found that the number of shoots increased with the increasing concentrations of BA. The multiple shoots were green in colour and healthy. There was no multiple shoot formation in terminal buds cultured on the other media. The results are summarized in Table 6.

Table 6: Effect of various concentrations of growth regulators on multiple shoot formation

Explant	MS medium+ various concentrations of growth regulators				Multiple shoot formation	Remarks
	2,4-D mg/l	Kinetin mg/l	IAA mg/l	BA mg/l		
Nodal segment	-	0.5	-	0.5	Small size	Slight response
	-	-	1	0.5	No response	
	1	-	1.5	-	No response	
	-	0.5	-	1	Many shoots	2-5
	-	1	-	2	Many shoots	5-10
Terminal bud	-	0.5	-	-	No response	
	0.5	1	-	-	No response	
	-	0.5	-	1	No response	
	-	1	-	1.5	No response	

Fig 1: Callus formation in terminal bud of *D. metel* When MS Medium supplemented with 2mg/l Kinetin and 1mg/l IAA

Fig 2: Callus formation in nodal segment of *D. metel* when MS medium supplemented with 1.5mg/l kinetin and 2mg/l BA

Fig 3: Multiple shoot formation in nodal segment of *D.metel* when MS medium supplemented with1mg/l kinetin and 2mg/l BA

Discussion

Datura metel Linn. is a member of the family Solanaceae. Solanaceae members are highly responding to the callusing media and also in regeneration. Most of the works in *Datura* were carried out in pollen grains.

The results obtained from the study show the effect of growth regulators on callus and multiple shoot formation of *D. metel*. Kinetin and IAA had the best effect on callus initiation. Combinations of BA and kinetin were tried in different concentrations for multiple shoot formation. Best results were obtained in MS basal media supplemented with higher concentrations of BA and kinetin.

The performance of different explants varied with different hormones and their concentrations. There were no callus formation and multiple shoot formation in the control medium. But the explants *viz*: terminal buds and nodal segments showed callus formation. Nodal segments alone showed shoot formation. Similar results were obtained in single and combination treatments of hormones in the MS basal medium.

Callus culture

For callus formation different explants such as terminal buds and nodal segments with single and combinations of hormones were used. In single hormone treatments the two different explants showed no response. Callus formation was observed when terminal buds were used as an explants. In MS basal medium supplemented with 2mg/l kinetin and 1mg/l IAA profuse callus formation occurred. When nodal segments were used as the explants the hormones 1.5mg/l kinetin and 2mg/l BA gave better results.

Multiple shoot formation

Multiple shoot formation was observed only in the nodal segments with different concentrations of hormones in the MS basal medium. The nodal segments showed slight response when 0.5mg/l kinetin and 0.5mg/l BA where used along with MS basal medium. The best results were obtained with the combination of 1mg/l kinetin and 2mg/l BA in the MS medium. The number of shoots increased with increasing concentrations of BA.

Conclusion

The present investigation revealed that by the manipulation of MS basal medium with the optimum concentrations of right growth regulators such as 2mg/l kinetin and 1mg/l IAA, 1.5mg/l kinetin and 2mg/l BA, it is possible to induce callus growth and multiple shoot.

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