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IN VITRO MICROPROPAGATION OF *DATURA METEL* L. THROUGH CALLUS INDUCTION FROM LEAF AND ANTHER CULTURE

MAHAKANT JHA* AND RAMESH KUMAR PANDEY
Department of Botany,
Ranchi University, Ranchi - 834 008, Jharkhand
E-mail: jhamahakant@gmail.com

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***Corresponding author**

ABSTRACT
The present study was carried out to analyse the effects of growth regulators on Callus formation of *Datura metel* L. in different explants. *Datura metel* was cultured in MS medium supplemented with different concentration of 2-4 D, NAA, Kinetin, BAP and IAA (from 1mg/L to 5 mg/L). Medium supplemented with various phytohormone for callus and shoot regeneration. Culture was kept on 25 ± 2 degree temperature and 16h photoperiod while callus was observed on different concentration of auxin or cytokinin alone or in combinations. Most suitable medium for callus induction from leaf and anther were 2-4D (1.5mg/L) in alone and 2-4 D (1.5mg/L) + Kinetin (2mg/L) + IAA (1mg/L) in combinations.

INTRODUCTION

Datura metel Linn *(*Thorn-apple, Devil trumpet, Solanaceae) is a Nigerian medicinal plant widely used in phyto medicine to cure diseases such as asthma, cough, convulsion and insanity (Duke and Ayensu, 1985; Dabur *et al.,* 2004). Various parts of the plant (leaves, seeds, roots and fruits) are used for different purposes in medicine. Tissue culture protocols have been developed for several plants but there are many other species, having tremendous potential to be used in pharmaceutical industries and wide range of medicinally active compounded can be extracted needs conservation. Micropropagation holds tremendous potential as a tool for the production of high quality plant-based medicines. Callus-mediated organogenesis and regeneration through somatic embryogenesis are the usual mode of regeneration by tissue culture. The induction of callus growth and subsequent differentiation, organogenesis and somatic embryogenesis are accomplished by the differential application of growth regulators and the control of conditions in the culture medium. Callus forms naturally on plants in response to wounding, infestations, or at graft unions (Bottino, 1981). The purpose of anther and pollen culture is the production of haploid plants by the induction of embryogenesis from repeated divisions of microspores or immature pollen grains (Dodds and Roberts, 1985). Limited *In vitro* studies of plant regeneration was achieved in *Datura* sp. *in vitro* embryo cultures of *Datura stramonium* plant regeneration enhancement of callus induction and regeneration efficiency by adjusting carbon sources and concentrations has been studied (Amiri *et al.*, 2011). *D.metel L.* were propagated from nodal explants collected from both *in vitro* germinated seedlings and field grown plants(Muthukumar *et al.,* 2004) and from internodal segments of *Datura metel* L. (Arockiasamy *et al.*, 1999). Callus was initiated from leaf discs of *Datura innoxia* derived from plants with different capacities for alkaloid biosynthesis (Herouart *et al.*, 1991). The conventional method of propagation of these species is through seeds. However, poor germination potential restricts their multiplication. Micropropagation technique offers an alternative method for cloning these plants. The limited micropropagation studies of *D.metel* L. emphasized its need of propagation as well as conservation because, plant is of high ethnobotanical importance, Used extensively at various occasions, plant contains tropane alkaloids having high medicinal values and only propagated through seeds, having long germination time. The present investigation describes the micro-propagation of *D.metel L.* from leaf discs and anther and successful establishment of calli and regenerate cultures from different explants of *Datura metel* and evaluation plus determination of callus growth giving rise to organogenesis.

MATERIALS AND METHODS

Datura metel L. were collected from the campus of the Ranchi University. Leaf and anther were used as explants for callus cultures. MS media with suitable modifications were prepared according to the explants type. The explants were kept under running tap water to 25 to 30 minutes. They were washed with 2% soap solution for 5 minutes. They were surface sterilized in 70 % alcohol for 30 seconds and washed 5-6 times with autoclaved distilled water. They were immersed in

0.1% Hg Cl_2 for 30 seconds. They were rinsed with 4-5 times with autoclaved distilled water. Explants were inoculated in test tubes containing MS basal media and Ms with phytohormone for callus induction. MS media (Murashige and Skoog, 1962) containing 3% sucrose and 0.8% agar with varying concentration of phytohormones was used for callus formation and shoot regeneration. Combination of auxin 2-4D, NAA, Kinetin, BAP and IAA were used for callus induction. The pH of media was adjusted to 5.8 by adding 0.1% HCl and 0.1 % NaOH solution. Media were autoclaved for 15 to 20 minutes at 15 psi and 121degree. Surface sterilized leaves and anther were inoculated into the culture medium in culture room. After aseptic inoculation, the culture vials were incubated at 25 ± 2 degree centigrade and were exposed to 16h photoperiod. The callus tissues were maintained by serial subculturing at suitable time.

RESULTS AND DISCUSSION

Callus cultures were established from different explants of *Datura metel* L. in MS media. Young leaf and anthers were cultured on MS medium supplemented with various phytohormones with different concentration of 2-4 D, Kinetin, BAP, NAA and IAA. These phytohormones were used singly or in combinations. Three groups A, B, C and ten replicates of each group with each phytohormone in alone and in combination were selected. In all groups growth of good callus was obtained in light conditions with different phytohormone combinations. Percent culture showing callus induction was recorded.

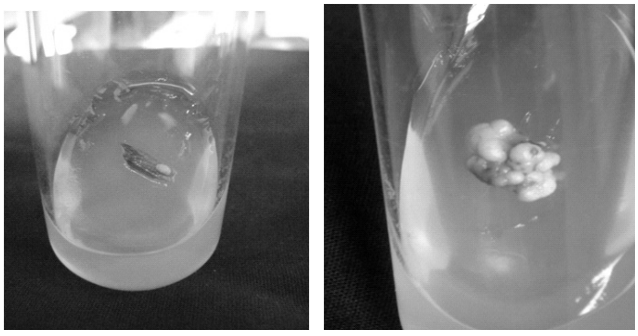

Figure1 and 2: (1): Three weeks old culture of Leaf explants on MS basal media; (2): Three weeks old callus of Leaf explants on 2-4D (1.5mg/L) + Kinetin (2mg/L) + IAA (1mg/L)

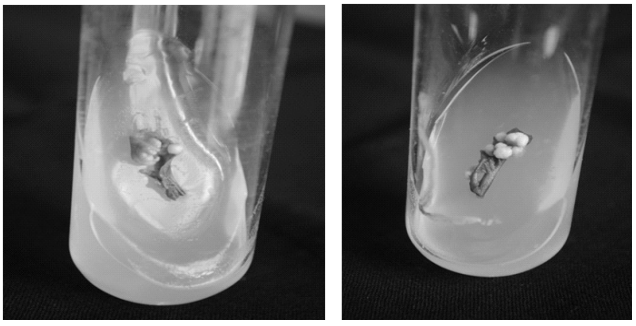

Figure 3 and 4: (3):Three weeks old culture of callus induction of Anther explants on 2-4D (1.5mg/L) + Kinetin (2mg/L) + IAA (1mg/L.); (4): Three weeks old culture of callus production from Anther explants on MS medium supplemented with 2-4D (1.5mg/L) + Kinetin (2mg/L) + IAA (1mg/L).

The effect of phytohormones from 1mg/L to 5 mg/L was studied alone and in combinations. The growth of callus induction was recorded till 3 weeks from the beginning and % response after (1, 2, and 3) weeks in all groups (A,B,C) with all growth regulators in alone and in combinations were recorded. All auxins were investigated (2-4 D, NAA, IAA) from 1mg/L to 5 mg/L in leaf and anther culture in alone as well as in combinations. In all growth hormones investigated on different explants of *D.metel* L. IAA showed the least average culture percentage at concentration of 1mg/L (37.33%) which was best response in itself among all ranges of IAA applied (1mg/L – 5mg/L) and 2,4-D showed the maximum average culture percentage at concentration of 1.5mg/L (78%) callus induction capacity after three weeks of culture which was again best among all ranges of 2,4-D were investigated(1mg/L-5mg/L). These findings showed disagreement from other investigation where addition of 1mg/L of each BA and NAA was more suitable for callus production from *D. metel* and *D. stramonium* (Torres, 1988). Maximum response of Kinetin was observed at 2mg/L (72.33%) out of all ranges investigated in alone and least response was observed at 5mg/L (5.66%). The other similar study which support the present findings about the role of KN and NAA in micropropagation of *Catharanthus roseus* L.G. Don plant. MS medium supplemented with NAA (0.2 mg/L) and KN (2mg/L) multiple shoot proliferation and shoot elongation was observed on MS medium supplemented with NAA (0.5mg/L) and KN 2mg/L(Akcam *et al.*, 1995). BAP in alone showed maximum response at 1.5mg/L (69.66%) and least response at 4.5 mg/L (12.66%) out of all ranges

Table1: Effects of various concentrations of auxins and cytokinins on Leaf and Anther culture of *Datura metel* L. (% culture showing callus induction and shoot formation)

MS + phytohormone	2-4 D			Kinetin			BAP			NAA			IAA		
(mg/L)	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1.0	8	11	16	20	18	27	30	45	35	19	24	45	30	34	48
1.5	77	88	69	35	46	51	64	67	78	32	27	56	24	31	40
2.0	10	33	40	69	70	78	35	47	53	40	64	61	19	25	33
2.5	9	19	25	11	21	30	11	27	33	23	30	35	20	22	37
3.0	6	21	27	6	19	25	13	24	29	17	25	31	16	18	27
3.5	7	11	19	11	17	23	10	27	30	15	28	23	23	11	15
4.0	3	10	12	10	13	18	16	9	13	6	13	16	12	7	11
4.5	NR	6	8	5	4	9	16	9	13	6	13	16	12	7	11
5.0	NR	2	5	6	8	3	5	19	17	7	10	18	NR	4	6

Table 2: Effects of various phytohormones showing maximum callus induction and shoot regeneration % from Leaf and Anther culture of *Datura metel* L

MS + phytohormone (mg/L)	Group	1st Week	2nd Week	3rd Week
MS Basal media	A	4	7	12
	B	NR	5	6
	C	8	11	15
2-4 D (1.5mg/L)	A	12	21	77
	B	18	32	88
	C	9	28	69
Kinetin (2mg/L)	A	11	41	69
	B	8	38	70
	C	17	33	78
BAP (1.5 mg/L)	A	7	23	64
	B	14	37	67
	C	19	48	78
NAA (2 mg/L)	A	12	21	40
	B	15	41	64
	C	7	39	61
IAA (1mg/ L)	A	6	19	30
	B	10	23	34
	C	17	33	48
2-4 D (1.5 mg/L)+ Kinetin (2mg/L)	A	16	31	72
	B	19	42	90
	C	14	37	81
NAA (1mg/L) + Kinetin (2mg/L)	A	11	28	58
	B	17	22	49
	C	9	37	53
BAP (1.5mg/L) + Kinetin	A	19	42	91
(1 mg/L) + IAA (1mg/L)	B	23	33	79
	C	17	38	88
2-4 D (1.5mg/L)+ Kinetin	A	18	44	86
(2mg/L)+ IAA (1mg/L)	B	20	53	94
	C	15	49	89

investigated. NAA in alone showed maximum response at 2mg/L (55%) and least response at 4.5/5 mg/L (11.66%). Kinetin 1.5 mg/L showed better response then BAP in callus induction process. Coconut milk (10%) gave the same response as BAP (1.5%). BAP showed better response then NAA which were better than IAA in callus induction process. Cytokinins such as BAP, Kinetin were used from 1mg/L to 5mg/L in alone and combinations with auxins with leaf and anther culture. When phytohormones were used in combinations good friable callus was obtained in the following media combinations.

(Average performance after 3 weeks)
MS + 2,4D (1.5mg/L) + Kinetin (2mg/L): 81%
MS + NAA (1 mg/L) + Kinetin (2 mg/L): 53%
MS + BAP (1.5 mg/L) + kinetin (1mg/L) + IAA (1mg/L): 86%
MS + 2-4 D (1.5mg/L) + Kinetin (2mg/L) + IAA (1mg/L): 90%
Addition of IAA to MS + BA + Kin and MS + 2,4D + K in media increased callusing. Slightly greenish friable callus was formed in light while cream coloured callus in dark. The similar studies were conducted about the role of BAP and NAA about callus induction and shoot regeneration from nodal explants of *D.metel* L. Explants were cultured on MS medium suuplimented with BAP (0.5-3.0 mg/L) and NAA (0.5 mg/L). The nodal explantsts isolated from *in vivo* source exhibited greater number of healthy multiple shoots than *in vitro*. BAP at 3 mg/L with NAA at 0.5 mg/L was found to be optimal for regeneration of shootlets (Del *et al.,* 1987). The other similar studies investigated established undifferentiated callus from stem explants of *Datura innoxia* using MS medium supplied with BA at 1mg/L and IAA at 0.5mg/L for 6 weeks (Zayed *et al.*, 2006). But these results showed disagreement with *Datura innoxia* Mill calli cultures which formed shoots after 2-4 weeks on media containing; GA3, IAA, low concentrations of NAA, 2,4-D and BA or media without substances (Engvild, 1973). The growth of callus raised from microspore-derived embryos has been investigated (Babbar *et al.,* 1986a). They have also studied the effects of using activated charcoal on microspore embryogenesis in *Datura metel L.* A good mass of spongy, cream coloured callus was obtained for anther in MS + 2,4D (1.5mg/L).The anther explants inoculated onto MS media with higher concentration of 2,4D showed Somatic embryogenesis. Callusing in a media supplemented with both auxin and cytokinin was compact. It seemed to have increased with increase in Kinetin and TDZ (Bajaj and Petri, 1989). The absence of cytokinins like Kinetin decreased the compactness of callus. In the present study callus induction is greatly effected by type of explants, age of explants and phytohormones used. The above discussion concludes that in alone treatment IAA was least effective and 2,4-D was most effective phytohormones in terms of callus induction capacity after three weeks of culture and in combinations MS + NAA(1mg/L)+ KN (2mg/L) were less effective (53%) and MS+ 2-4 D(1.5mg/L) +KN(2mg/L) + IAA(1mg/L) were most effective among all combinations of phytohormone investigated in callus induction capacity in both leaf and anther culture. Very low and very high concentration of phytohormone investigated also showed poor response in

alone as well as in combinations in callus induction process.

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