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MORPHOGENIC STUDIES ON *ANNONA RETICULATA* (L.)

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ABSTRACT
Different concentration of phytohormones affected formation of callus of Annona reticulata. Leaf of *Annona reticulata* was cultured on "murashige and skoog's" (MS) medium supplemented with different concentrations of IAA, IBA, 2,4-D, NAA, KN, BAP, Coconut - water in isolation or in combination. Then white Callus was observed. Cytokinins was also used alone or in combination for callus induction. Most suitable medium for induction of Callus was 2, 4-D.

INTRODUCTION

Annona reticulata is deciduous, semideciduous or semievergreen small tree belonging to the family "Annonaceae". The species is best known for its fruits called custard Apple. Which is almost identical to the fruits of other allied species viz Annonachecimola and Annona Squamosa. Morphogenesis and plant developmental studies on molecular, physiological and bio-chemical aspects of cells in culture have been encountered Amin and Akhatar (1993); Amin *et al.,* (1992). Hutchinson (1981); Jaiswal and Mehta, (1987), Mehta *et al.,* (1998), Mehta *et al.,* (2005) have made substantial contributions for better understanding of cytodifferentiation and organogenesis in different plant species. Plant tissue- culture is an empirical process, therefore the selection of suitable explants, manipulation of salts, growth regulators and additives in the nutrient medium are very essential to establish plant culture system.

Effects of phytohormones on *in vitro* shootmultiplication and rooting in *Citrus- aurantifolia*. Micropropagation of *Annonasquamosa*, using shoot tips and node of explants in field grown mature plants has reported by Amin *et al.,* (1992).

In the present study, efforts have been made to study callus formation in *Annona reticulata*.

MATERIALS AND METHODS

The seeds of *Annona reticulata* were collected from Birsa Agricultural University, Kanke (Ranchi) for tissue culture studies. In certain cases, various plant parts are taken from the plant grown from seeds. The explants were kept under running tap water for 15-20 minutes and washed with 1-2 drops of Savlon for 2 minutes. After that, they were surface sterilized in 70% ethanol for 30 seconds and washed for 3-4 times in autoclaved distilled water, later on, they were immersed in 0.1% $HgCl_2$ for ½ minute and rinsed 3-4 times in autoclaved distilled water. Leaves were inoculated in test tubes containing MS basal medium. Calli having different morphology were also used as culture materials.

Culture media

Semi solid MS medium (Murashige and Skoog, 1962) containing 3% sucrose with varying concentration of phytohormones was employed for callus formation and roots and shoot regenerations. Combination of auxin (IAA, IBA, NAA, 2, 4-D) was also employed for root and shoot regeneration. Coconut milk with a high level of sugar and other salts were also used as growth supplements in plant tissue culture.

Culture condition

Cultures were inoculated at 25 ± 2ºC under cool fluorescent light (1500-2000 Lux) with a 16 hr/ 8hr light- dark cycle. Each treatment consisted of minimum 15 explants and all experiments were conducted under sterile conditions. The inoculation tools like forceps, needles, scissors, blades, scalpels and others were thoroughly autoclaved. Culture areas, glasswares and all other tools used were properly sterilized with UV Light and floor surfaces were swabble with 95% ethyl alcohol, inoculation tools were flamed from time to time.

RESULTS AND DISCUSSION

In the present analysis, shoot tips and young leafed plants were cultured on MS medium supllimented with varied phytohormones in different concentrations of 2, 4 –D, IAA, IBA KN and BAP. These growth regulators were employed either singly or in combinations.

Effects of auxin

Table 1: Effects of various concentrations of Phytohormones for induction of callus and shoot differentiation from shoot tip of *Annona reticulata*

Conc. of phytohormones on MS Media mg L^{-1}	2,4-D					IAA					IBA					NAA				
0.5	8	7	8	6	9	1	8	10	9	8	5	6	8	7	9	NR	NR	NR	NR	NR
1.0	21	20	19	17	16	19	22	18	20	17	11	8	5	8	9	11	8	11	9	9
1.5	36	33	37	35	33	23	28	34	39	36	26	22	23	25	29	16	23	22	17	21
2.0	72	71	75	79	70	41	49	57	50	48	34	31	29	20	27	20	34	34	38	35
2.5	96	97	91	93	95	61	69	69	69	65	38	34	31	32	33	39	48	47	46	46
3.0	81	83	79	75	76	82	80	83	86	85	36	42	42	42	35	43	56	55	54	54
3.5	66	62	64	63	65	79	78	75	74	73	47	39	45	48	40	63	62	61	60	62
4.0	57	54	55	56	57	80	72	71	70	73	46	47	76	78	75	76	77	79	75	74
4.5	56	57	53	51	51	72	70	65	60	63	40	42	83	80	79	83	82	83	83	83
5.0	48	44	45	42	43	61	60	53	51	52	37	39	89	80	88	89	85	84	85	85
5.5	36	33	38	34	36	46	48	44	42	42	33	35	87	80	80	87	85	83	80	82
6.0	20	24	27	25	24	33	34	39	35	38	35	31	78	75	70	78	78	72	72	72
6.5	17	18	20	25	23	23	27	23	25	22	28	24	68	65	64	69	64	62	60	63
7.0	16	19	22	20	20	19	20	18	19	19	21	20	36	30	54	57	60	55	52	53
7.5	12	12	11	11	13	19	17	20	22	23	20	21	40	46	46	45	47	47	48	49
8.0	10	9	9	8	9	10	10	16	16	17	19	15	18	30	33	32	36	33	33	34
8.5	7	5	7	6	5	8	9	12	13	13	13	11	26	25	24	34	31	32	32	33
9.0	5	4	4	4	4	6	7	9	7	7	11	6	26	20	20	26	29	27	27	27
9.5	3	1.5	1.5	1.5	1.5	7	6	5	4	3	7	6	17	15	15	18	15	11	12	13
10.0	NR	NR	1.5	NR	1.5	1	1	3	4	3	3	3	9	6	3	9	9	6	5	4

The effect of auxins on the induction collusing from shoot tips and leaves were different. The callus developed was white in colour in nature. Only callusing was evident but no symptoms of organogenesis, was seen in medium containing 2, 4-D (Fig. 1) 2, 4 –D at 2.25 μm gave maximum shoot initiation shoot number and mode number, identical results were obtained by Sen and Sharma (1991) in *Withania – Somnifera* callus induction and organogensis in Sesbania spp. were obtained by Khattar and Mohan Ram (1982, 1983) and Subhan *et al.,* (1998) using B_5. The best result was encountered at 2.5mg/L concentration of auxins in the present work. Hundred percent cultures exhibited callusing at this concentration. IAA was proved to be more effective than IBA in inducing callus from shoot tip culture. In the shoot tip culture on MS medium containing IAA (0.5 mg/L) the callusing from its anterior end was found. Incorporation of NAA on MS medium at concentration raging from 0.5 to 10 mg/L yielded callusing from cut end of shoot tips. 5 mg /L was recorded as the best concentration for callus induction and 100% culture exhibited callusing (Fig. 2; Table 1).

Table 2: Effect of various concentration of cytokinins and combination of Cytokinin and auxin on callus induction and shoot differentiation from shoot tip Culture of *Annona reticulata*

MS medium supplemented with	Group	% Culture showing shoot formation 1st week	2nd week	3rd week	4th week
KN(1mgL^{-1})	A	11	13	20	23
	B	10	14	19	24
	C	9	16	21	25
KN(1mgL^{-1})	A	8	16	21	29
	B	9	17	22	30
	C	10	18	23	28
BAP (1mgL^{-1})	A	14	23	35	38
	B	16	24	34	37
	C	15	22	36	39
BAP (2mgL^{-1})	A	8	15	19	22
	B	9	16	20	23
	C	7	14	18	21
KN(0.5mg / L) + NAA	A	42	60	73	85
	B	43	62	76	87
	C	44	58	75	88
KN(1mg / L) + IAA	A	22	46	54	64
(2mg/2)	B	25	47	56	65
	C	26	45	55	63
KN(2mgL^{-1}) + IAA	A	20	28	41	56
(1mgL^{-1})	B	21	29	42	56
	C	19	27	43	55
KN(2mgL^{-1})	A	20	28	41	56
	B	21	29	42	56
	C	19	27	43	55
KN(3mgL^{-1}) + IAA	A	43	61	74	95
(1.5 mgL^{-1})	B	41	63	75	94
	C	42	62	76	96
KN(5mgL^{-1}) + IAA(	A	14	29	37	52
2.5mgL^{-1})	B	16	30	36	53
	C	15	28	38	51
KN(10mgL^{-1}) + IAA	A	7	14	20	25
(5mgL^{-1})	B	8	15	21	26
	C	5	17	19	24

Effects of cytokinin

When MS medium was supplemented with 5mg/L concentration of Cytokinins(KN,BAP) callus formation was induced . It was observed that the callus was not fast growing and it did not grow further sub-culture in the medium even similar composition Root differentiation was never observed in any such cultures.

Differentiation of shoot bud from shoot tips was observed in 45% cultures on MS medium supplemented with 1.5 mg/L on concentration of BAP (Table 2). Kenetin was found most effective in differentiation of shoot buds from shoot tips in comparison to BAP (Table 2). However, Manickam *et al.,* (2000) recorded multiple shoot formation in BAP 4.44 mm. Khattar and Mohan Ram (1982) encountered regeneration in *Sesbania spp* directly and indirectly on B_5. However regeneration in MS medium is equally directed towards both indirect and direct organogenesis.

Effects of combination of auxins and cytokinins

Figure 1 and 2: (1) 14-days old callus from shoot tip culture on MS medium containing 2,4-D;(2) 3-weeks old callus from shoot tip on MS medium containing NAA

Figure 3 and 4: (3) 3-weeks old callus on MS + Kinetin + IAA; (4) 4-weeks old culture of callus on MS + Kinetin + NAA

The MS basal medium, supplemented with combination of cytokinin and auxin, shooting and rooting were observed. Kinetin was found to be the most effective cytokinins for shoot differentiation in combination with auxin. The most suitable medium found or shoot formation from shoot tips was a combination of Kinetin (3mg/L) + IAA (1.5MG/L). 90% of cultures exhibited shoot differentiation in above combination (Table 2; Fig. 3 and 4). Kinetin in combination with auxin was also found effective in stimulating shoot formation from shoot tips (Fig. 2 and 4) Kapoor and Gupta (1986) employed B_5 medium with 4.92 μm. IBA for regeneration in *Sesbania bispinosa* and Zhao *et al.,* (1993) recorded, root and shoot regeneration in medium without growth regulators. Organagenic responses had been recorded by various previous workers in different plant species. It was observed that in higher Cytokinin supplementation, shoot were recorded with stunted growth, short internodes and crowded leaves in the present investigations which are confirmatory with Rudra and Jewarkar (2002). Various authors in the past had reported that several plant species were highly responsive on MS + Auxin and Cytokinin combinations (Radhamani and Chandel, 1992).

In the present study, medium containing coconut water (20%V/V) showed shoot growth and multiple shoot production reported earlier in a number of species of Fabaceae. Effects of Cytokinin and auxin on micropropagation were recorded previously in *Cleoptera ternatea* by Zhao *et al.,* (1993) for plant regeneration from callus and explants of *Sesbania* sp. Callus formation was greatly affected by the type and the age of explants and growth regulators requirements (type concentrations of auxin to - Cytokinin ratio) for callus formation depending upon the genotype and endogenous harmone contents of the tissue. The present investigation lends support to the view that disease free plants of *Annona reticulata* are very likely to be produced in quite large scale in lesser time through tissue culture technique which would be beneficial to the farmers.

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