'CALLUS INDUCTION AND CALLOGENIC RESPONSE OF *RAUVOLFIA SERPENTINA* AND *CATHARANTHUS ROSEUS* BY USING VARIOUS GROWTH HORMONE CONCENTRATIONS SINGLY AND IN COMBINATION.'

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Abstract

Rauvolfia serpentina and *Catharanthus roseus, Linn.* (Apocynaceae) are an important medicinal, ornamental and evergreen shrubs. Alkaloids of these plants have a great medicinal importance to treat cardiovascular diseases, hypertension, diabetes, malaria, menorrhagia, cancer, etc. The members are wild as well as cultivated with milky saps that contain secondary metabolites which have medicinal value. In view of these facts the study was conducted for micropropagation of *Rauvolfia serpentina* and *Catharanthus roseus*. MS media supplemented with different concentrations (0.5–10.0 mg/l) of NAA, 2, 4-D, BAP and KIN were used singly and in combination. Among all the growth hormones 2, 4-D was the best for callus induction in *Rauvolfia serpentina* (93% in stem and 97% in leaf) and in *Catharanthus roseus* (85% in stem and 87% in leaf). In combination 2, 4-D and BAP in *Rauvolfia serpentina* (85% in stem and 95% in leaf) and in *Catharanthus roseus* (65% in stem and 71% in leaf). Day of callus induction started from 13th to 37th day. This variation is due to the differences in culture conditions and the age of explants. The fresh and dry weight and moisture content showed good growth of callus, which may be used for further studies in alkaloid production. Micropropagation of these plants allows production of clones at fast rate and in continuous manner. This work can lead to development of an efficient protocol for callus induction and other issues.

Key words: Callogenic response, Callus induction, *Catharanthus roseus*, Growth hormone, *Rauvolfia serpentina*.

RUNNING TITLE- CALLUS INDUCTION IN *CATHARANTHUS ROSEUS* AND *RAUVOLFIA SERPENTINA*

INTRODUCTION

Almost every civilization has a history of medicinal plant use. Few important perennial, evergreen, medicinal, shrubs of Apocynaceae family are-

Rauvolfia serpentina and *Catharanthus roseus.*

Rauvolfia serpentina

Rauvolfia serpentina. *L* is indigenous to India, Bangladesh and other regions of Asia and found to grow in the wild in many places around the country (Ghani, 1998). Alkaloids of this plant have a great medicinal importance to treat hypertension (Von Poser *et al*., 1990), breast cancer (Stanford *et al*., 1986) etc. Explants of an alkaloid producing plant cultured *in vitro* and has been found to retain the capacity to synthesis alkaloids identical to that in the intact plant (Yoshimatsu and Shimomura, 1991). Sometimes, high yield of secondary metabolites is observed in tissue grown callus masses produced during differentiation (Benavides and Caso, 1993). *Rauvolfia* is threatened in India due to exploitation of natural resources for commercial purposes to meet the requirements of pharmaceutical industry, coupled with limited cultivation (Nayar and Sastry, 1987). IUCN has kept this plant under endangered status.

As propagation by means of seeds is very much difficult due to low germination percentage (Salma *et al*., 2008). Low seed germination is due to the presence of Cinnamic acid and derivatives in the seed (Mitra, 1976). *Rauvolfia* has *in vitro* cultured to make it available in large amount by various scientists (Mitra and Kaul, 1964, Mathur et al., 1993).

Catharanthus roseus

The genus *Catharanthus* is well reported for producing biologically active terpenoid indole alkaloids (TIAs) with over 130 compounds isolated and identified (Samuelsson 1999). Alkaloids of this plant have a great medicinal importance to treat diabetes, malaria, anticancerous (Junaid *et al.*, 2006) etc. This plant is grown commercially for its medicinal and ornamental uses in India. The low yield of dimeric indole alkaloids from the plant (approximately 0.0005%) and their consequent high price have stimulated numerous efforts to develop alternative strategies for their production. As a promising alternative plant tissue culture technology has many advantages over

traditional field cultivation and chemical synthesis, particularly for many natural compounds that are either derived from slow growing plants or difficult to be synthesized with chemical methods (Zarate and Verpoorte, 2007). *Catharanthus* has *in vitro* cultured to make it available in large amount by various scientists (Yuan Y J *et al.*, 1994; Ramavat et *al.*, 1978).

There are lots of works done separately on these plants but still there is no any common protocol. This work can lead to development of an efficient protocol for callus induction and other issues.

MATERIALS AND METHODS

1. Collection of Explant

Explants {Stem (nodal), Leaf} of *Rauvolfia serpentina* and *Catharanthus roseus* were collected from the departmental garden of Botany, Patna University, Patna-5.

2. Surface Sterilization

Explants– leaf and stem (nodal) were washed thoroughly with running tap water for 30 minutes and then dipped for 15 seconds in 70% ethanol. Later on they were submerged in a disinfectant calcium hypochlorite (0.5%) for 25 minutes. Tween 80 was added to the above solution to improve contact between tissue and disinfectant. Explants were removed from disinfectant and were washed 5 times in sterile distilled water. Explants were blotted on filter paper in 5 replicates in Laminar Air Flow before placing it on MS media.

3. Explant Implantation and Culture Conditions

Standard procedure was followed for the preparation of media (Murashige and Skoog, 1962). The pH of the media was adjusted to 5.8 and heat resistant growth regulators (NAA, 2, 4-D, BAP and KIN) were added to the media prior to sterilization done at 15 lbs/in for 15 min. All media were solidified with 8g/l agar. After autoclaving further work was done under Laminar Air Flow. Stem and leaf about 5 mm in length were aseptically prepared and were implanted on MS medium prepared with specific concentrations of hormones. Stock culture, stem and leaf explants were incubated in dark in a culture chamber at 25°C.

4. Determination of Callus's Fresh Weight

The callus was collected from tissue culture lab and its media were washed with sterile distilled water. They were placed under a fan (on a blotting paper) to remove water and weighed.

5. Determination of Callus's Dry weight

After fresh weight determination, the materials were placed on Petridishes and kept in an oven for 48 hours at 65^{o}C for drying. Dry weight was weighed with an electronic balance.

6. Determination of Callus's Moisture Content

The moisture content was determined using the fresh and dry weight of callus by following way-.

A = weight of empty Petridish

B = weight of Petridish with fresh cell material

C = weight of Petridish with dried cell material

Moisture content percentage = (B-A)-(C-A) / (B-A) x 100.

RESULTS

All the experiments were carried in triplicates and the mean value was recorded, figure-1.

1 Effects of different concentrations of auxins and cytokinins singly on callus induction-

MS media supplemented with different concentrations (0.5–10.0 mg/l) of 1-naphthaleneacetic acid (NAA) showed stimulatory effects on callus induction (Table 1). Maximum callusing response Rauvolfia *serpentina* (70%- in stem and 74% in leaf) and *Catharanthus roseus (*75% in stem and 79% in leaf) was recorded at 1.5 mg/l of NAA. At 0.5mg/l the callusing response was recorded less and it increased up to 2mg/l. At 2.5mg/l on ward callusing response was reduced and found minimum at

5mg/l. At 10mg/l no callusing or growth was observed. It was observed that the higher concentration of NAA in media had an inhibitory effect on callus proliferation.

2, 4-Dichlorophenoxyacetic acid (2, 4-D) with different concentration (0.5-10 mg/l) showed stimulatory effects on callus induction (Table2 and figure-2). Maximum callusing response in *Rauvolfia serpentina (*93% in stem and 97% in leaf) and *Catharanthus roseus (*85% in stem and 87% in leaf) was noted at 2.5mg/l.

No callus formation was observed on stem and leaf explants inoculated on MS media supplemented with 0.5 mg/l to 10 mg/l of Kinetin (KIN) (Table 2).

With 6- benzylaminopurine (BAP) maximum callusing response in *Rauvolfia serpentina (*60% in stem and 64% in leaf) and *Catharanthus roseus (*59% in stem and 63% in leaf) was noted at 2.5mg/l (Table3). Lower concentration of BAP (0.5mg/l to 1.5mg/l) was unable to induce callusing and higher concentration of BAP (10 mg/l) in media had an inhibitory effect on callus induction.

2. Effects of different concentration and combination of growth hormones on leaf and stem callus induction-

2, 4-Dichlorophenoxyacetic acid (2, 4-D) and 6-benzylaminopurine (BAP) with different concentration (0.5-10mg/l) showed stimulatory effects on callus induction (Table 4 and figure 3). Maximum callusing response in *Rauvolfia serpentina (*85% in stem and 95% in leaf) was recorded at BAP 1mg/l and 2, 4-D 2mg/l and in *Catharanthus roseus (*59% in stem and 64% in leaf) was noted at 1mg/l and 1.5mg/l for BAP and 2, 4-D respectively. At 3mg/l of BAP and 2, 4-D 1 mg/l swelling of callus was observed. At 5mg/l to 10mg/l of BAP and 2, 4-D no callusing or growth was observed.

MS media supplemented with different concentrations (0.5–10.0 mg/l) of 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) showed stimulatory effects on callus induction (Table 5). Maximum callusing response (75% in stem and 77% in leaf) was recorded at 0.5mg/l and 1 mg/l for BAP and NAA respectively.

Different concentrations (0. 5–10.0 mg/l) of 1-naphthaleneacetic acid (NAA) and KIN showed stimulatory effects on callus induction (Table 6). Maximum callusing response in Rauvolfia *serpentina* (63% in stem and 73% in leaf) was recorded at KIN1mg/l and NAA 2mg/l and *Catharanthus roseus (*69% in stem and 70% in leaf) was recorded at 1mg/l and 1.5 mg/l for KIN and NAA respectively. At 2. 5mg/l to 10mg/l of KIN and NAA no callusing or growth was observed.

3. Effects of different concentrations of growth hormones singly and in combination on nature and moisture content of callus-

The moisture content varied in the callus derived from different explants {leaf and stem (nodal)} of different plants - *Rauvolfia serpentina* and *Catharanthus roseus* under influence of various growth hormones. It was observed that moisture content varied from 70% to 80% which supports good growth of callus (Table 7).

DISCUSSION AND CONCLUSION

In the present study, two explants leaf and nodal stem were used in which leaf explants appear the best for callus induction which is in accordance with the earlier findings (Mathur *et. al.*, 1987). MS media without any growth hormone was unable to induce callus (Shah et. al., 2003). Among all the growth hormones, 2, 4-D was the best for callus induction which is similar to the earlier finding (Bhaskaran and Smith, 1990; Chaudhury and Qu, 2000).

In the present work KIN alone could not induce callus as according to earlier findings (Murashige *et al*. 1974 and Murashige and. Skoog, 1962). In further experiments KIN was supplemented to the MS media in combination with auxins (2, 4-D and NAA). It was observed that KIN had enhanced callus growth in the presence of auxins.

MS media fortified with 2, 4-D and BAP was found best for callus induction as reported earlier (Roja et al. 1996; Shahrear et al. 2002; Tiwari et al 2003; Mahmooda and Riffat 1994). Day of callus induction was indigenous which started from 13^{th} to 37^{th} day(Schrawat et al2002). . This variation observed in the present investigation may be attributed due to the differences in culture conditions and the age of explants. The fresh and dry weight and moisture content showed good growth of callus.

It can be concluded that Common media that can be used for callus induction of both the explants is MS media supplemented with 2, 4-D (2.5 mg/l) alone. In combination MS media with BAP (1 mg/l) and 2, 4-D (2 mg/l) is the most suitable to *Rauvolfia serpentina* and BAP (0.5 mg/l) and 2, 4-D (2 mg/l) is the most favorable to *Catharanthus roseus.* Though on an average MS media fortified with BAP (0.5 mg/l) and 2, 4-D (2mg/l) can be used for both the plants which give 73 % and 71% callus

induction in *Rauvolfia serpentina* and *Catharanthus roseus* respectively. Day of callus induction varied from 17th to 37th in *Rauvolfia serpentina* and 13th to 23th in *Catharanthus roseus.*The shortest day of callus induction is 13th in stem of *Catharanthus roseus.*

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TABLE CAPTIONS-

Table 1: Callus induction on stem (nodal) and leaf explants on MS medium under the influence of different concentration of NAA mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

Table 2: Callus induction on stem (nodal) and leaf explants on MS medium under the influence of different concentration of 2, 4-D mg/l and KIN mg/l separately (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

Table 3: Callus induction on stem (nodal) and leaf explants on MS medium under the influence of different concentration of BAP mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

Table 4: Callus induction on stem (nodal) and leaf explants on MS medium supplemented with different concentration and combination of BAP and 2, 4-D mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

Table 5: Callus induction on stem (nodal) and leaf explants on MS medium supplemented with different concentration and combination of BAP and NAA mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

Table 6: Callus induction on stem (nodal) and leaf explants on MS medium supplemented with different concentration and combination of KIN and NAA mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

Table 7: Callus growth observation by measuring callus fresh and dry weight and moisture content of callus of randomly selected samples from different concentration (mg/l) of growth hormones of *Rauvolfia serpentina* and *Catharanthus roseus.*

LEGENDS OF THE FIGURES-

Figure 1: Explants in triplicates on MS media with growth hormones for callus induction.

Figure 2: Callus induction of MS fortified with 2, 4-D (2.5 mg/l).

Figure 3 Callus on MS supplemented with 2, 4-D (1.5 mg/l) and BAP (1 mg/l).

Table 1. Callus induction on stem (nodal) and leaf explants on MS medium under the influence of different concentration of NAA mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

medium composition NAA mg/l	*Rauvolfia serpentina*						*Catharanthus roseus*					
	Stem			Leaf			stem			Leaf		
	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction
0.5	23	+	29	41	+	27	28	+	21	45	+	22
1	73	+++	21	75	+++	20	63	++	15	69	+++	16
1.5	70	+++	20	74	+++	19	75	+++	13	79	+++	14
2	65	++	22	69	++	21	60	++	15	63	++	15
2.5	45	+	27	55	++	25	42	+	17	49	++	19
3	37	+	29	43	+	27	35	+	18	41	+	18
4	29	+	31	35	+	29	28	+	19	37	+	20
5	22	+	33	27	+	31	21	+	21	29	+	22
10	No callusing	-	-	No callusing	-	-	No callusing	-	-	No callusing	-	-

(-) indicates no regeneration and (+) indicates status of callus induction.

+ = poor, ++ = good, +++ = excellent.

Table 2. Callus induction on stem (nodal) and leaf explants on MS medium under the influence of different concentration of 2, 4-D mg/l and KIN mg/l separately (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

medium composition 2,4-D mg/l	*Rauvolfia serpentina*						*Catharanthus roseus*					
	stem			leaf			stem			Leaf		
	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction
0.5	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-
1.5	-	-	-	-	-	-	-	-	-	-	-	-
2	76	+++	21	80	+++	20	-	-	-	73	+++	15
2.5	93	++++	20	97	++++	19	85	+++	13	87	+++	14
3	25	+	31	21	+	29	35	+	17	47	+	17
4	19	+	33	23	+	27	23	+	19	25	+	19
5	15	+	35	17	+	31	17	+	21	21	+	23
10	No callusing	-	-	No callusing	-	-	No callusing	-	-	No callusing	-	-
KIN mg/l												
0.5	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-
1.5	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
2.5	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-

(-) indicates no regeneration and (+) indicates status of callus induction.

+ = poor, ++ = good, +++ = excellent.

Table 3. Callus induction on stem (nodal) and leaf explants on MS medium under the influence of different concentration of BAP mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

medium composition BAP mg/l	*Rauvolfia serpentina*						*Catharanthus roseus*					
	stem			Leaf			Stem			Leaf		
	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction
0.5	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-
1.5	-	-	-	-	-	-	-	-	-	-	-	-
2	45	+	27	51	+	25	53	+	16	57	++	17
2.5	60	++	23	64	++	23	59	++	15	63	+++	15
3	51	+	27	57	++	25	43	+	16	47	+	17
4	37	+	29	39	+	27	35	+	17	41	+	17
5	21	+	33	25	+	31	19	+	19	21	+	20
10	No callusing	-	-	No callusing	-	-	No callusing	-	-	No callusing	-	-

(-) indicates no regeneration and (+) indicates status of callus induction.

+ = poor, ++ = good, +++ = excellent.

Table 4. Callus induction on stem (nodal) and leaf explants on MS medium supplemented with different concentration and combination of BAP and 2,4-D mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

medium composition mg/l		*Rauvolfia serpentina*						*Catharanthus roseus*					
		leaf			stem			leaf			Stem		
		% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction
MS		-	-	-	-	-	-	-	-	-	-	-	-
BAP	2,4-D												
0.1	2	67	++	23	47	+	25	27	+	21	23	+	23
0.5	2	73	+++	20	69	++	21	71	+++	17	65	++	18
1	1.5	21	+	31	20	+	33	64	++	18	59	++	18
1	2	95	+++ +	19	85	+++	19	61	++	18	55	++	19
1.5	1.5	23	+	31	20	+	33	21	+	19	19	+	20
1.5	2	42	+	25	45	+	27	35	+	19	40	+	19
1.5	2.5	39	+	26	35	+	29	37	+	20	33	+	20
2.5	1	25	+	30	23	+	31	29	+	22	25	+	22
2.5	2	17	+	33	21	+	33	21	+	21	18	+	23
3	1	swelling	-	-	swelling	-	-	swelling	-	-	swelling	-	-

(-) indicates no regeneration and (+) indicates status of callus induction.

+ = poor, ++ = good, +++ = excellent.

Table 5. Callus induction on stem (nodal) and leaf explants on MS medium supplemented with different concentration and combination of BAP and NAA mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

medium composition mg/l		*Rauvolfia serpentina*						*Catharanthus roseus*					
		leaf			stem			Leaf			Stem		
		% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus Induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction
MS		-	-	-	-	-	-	-	-	-	-	-	-
BAP	NAA												
0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-
0.5	0.5	17	+	31	-	-	-	21	+	20	-	-	-
1	0.1	21	+	29	15	+	33	25	+	20	15	+	23
1.5	0.5	70	+++	21	65	++	23	65	++	17	20	+	19
2	0.1	77	+++	21	71	+++	23	69	+++	14	60	++	17
0.1	1	85	++++	19	80	+++	20	75	+++	14	67	++	17
0.5	1	83	+++	19	79	+++	20	81	+++	15	78	+++	15
1	1	67	++	25	61	++	23	77	+++	15	71	+++	15
1.5	1	75	+++	21	71	+++	23	73	+++	15	65	++	17
2	1	65	++	25	63	++	27	70	+++	14	85	++++	13
1	2	87	++++	20	80	+++	20	75	+++	14	70	+++	15
3	2	61	--	27	60	++	29	91	++++	14	60	++	17
5	4	No growth	-	-	No growth	-	-	67	++	-	No growth	-	-
10	5	swelling	-	-	swelling	-	-	swelling	-	-	swelling	-	-

(-) indicates no regeneration and (+) indicates status of callus induction.

+ = poor, ++ = good, +++ = excellent.

Table 6. Callus induction on stem (nodal) and leaf explants on MS medium supplemented with different concentration and combination of KIN and NAA mg/l (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 10).

medium composition mg/l		*Rauvolfia serpentina*						*Catharanthus roseus*					
		Leaf			stem			leaf			Stem		
		% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction	% of callus induction	degree of callusing	day of callus induction
MS		-	-	-	-	-	-	-	-	-	-	-	-
KIN	NAA												
0.1	2	25	+	29	20	+	31	17	+	20	10	+	21
0.5	2	52	++	25	48	++	27	41	+	19	35	+	21
1	2.5	-	-	-	-	-	-	45	++	17	-	-	-
0.5	2.5	15	+	37	25	+	31	50	++	16	47	++	17
1	1.5	19	+	35	15	+	35	70	+++	15	69	+++	16
1	2	73	+++	20	63	++	23	65	++	16	60	++	16
1	2.5	33	+	27	35	+	27	40	+	20	33	+	19
1.5	1.5	20	+	29	20	+	31	33	+	21	25	+	20
1.5	2	18	+	33	15	+	35	27	+	22	21	+	22
1.5	2.5	10	+	36	19	+	35	17	+	23	15	+	22
2.5	2	No growth	-	-	No growth	-	-	No growth	-	-	No growth	-	-

(-) indicates no regeneration and (+) indicates status of callus induction.

+ = poor, ++ = good, +++ = excellent.

Table 7. Callus growth observation by measuring callus fresh and dry weight and moisture content of callus of randomly selected samples from different concentration (mg/l) of growth hormones of *Rauvolfia serpentina* and *Catharanthus roseus.*

medium composition mg/l	*Rauvolfia serpentina*						*Catharanthus roseus*					
	stem			leaf			stem			Leaf		
	fresh weight (mg)	dry weight (mg)	moisture content (%)	fresh weight (mg)	dry weight (mg)	moisture content (%)	fresh weight (mg)	dry weight (mg)	moisture content (%)	fresh weight (mg)	dry weight (mg)	moisture content (%)
MS	-	-	-	-	-	-	-	-	-	-	-	-
2,4-D (2.5)	2.330	0.713	70	2.112	0.623	71	1.653	0.394	76	1.533	0.371	76
2,4-D (5)	0.357	0.085	76	0.314	0.075	76	0.831	0.167	79	0.815	0.3171	79
NAA(1.5)	1.722	0.402	77	1.651	0.375	78	1.741	0.435	75	1.672	0.410	75
NAA(5)	0.931	0.197	78	0.917	0.193	79	0.931	0.197	78	0.917	0.193	79
BAP (2.5)	1.453	0.314	78	1.357	0.297	78	1.323	0.319	76	1.211	0.313	74
BAP (5)	0.853	0.175	79	0.803	0.169	78	0.659	0.161	76	0.633	0.151	74
KIN (0.5)	-	-	-	-	-	-	-	-	-	-	-	-
BAP (1)+ 2,4-D (2)	1.975	0.573	71	1.873	0.535	72	1.291	0.275	79	1.213	0.249	79
BAP (0.5)+ 2,4-D (2)	1.837	0.535	71	1.774	0.529	70	1.372	0.291	79	1.332	0.283	79
BAP (0.5) + NAA (1)	1.892	0.417	78	1.835	0.397	78	1.821	0.531	71	1.731	0.513	70
BAP (1) + NAA (2)	1.675	0.396	76	1.617	0.375	77	1.313	0.285	79	1.293	0.271	79
KIN (1) + NAA (2)	1.531	0.355	77	1.496	0.349	77	1.272	0.261	75	1.255	0.275	78
KIN (0.5) + NAAS (2)	1.210	0.273	78	1.193	0.317	73	0.912	0.197	79	0.901	0.173	73

Figure 1: Explants in triplicates on MS media with growth hormones for callus induction.

Figure 2: Callus induction of MS fortified with 2, 4-D (2.5 mg/l).

Figure 3 Callus on MS supplemented with 2, 4-D (1.5 mg/l) and BAP (1 mg/l).