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Effect of the explants from different aged mother plant on callus induction and direct regeneration in jatropha(*Jatropha curcas* L.)

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Abstract: *Explants from one year and five year old mother plants of Jatropha (Jatropha curcas L.) have been evaluated for their response to callus induction and direct regeneration. Leaf disc, petiole and nodal explants were cultured on a range of concentration of Kinetin, BAP and NAA in combination with BAP for callus induction. Shoot tip was cultured on various concentration of GA_3 in combination with IAA for direct regeneration. Leaf discs from one year old mother plant cultured on BAP showed maximum callus induction frequency (56.15%) as compared to leaf disc from five year old mother plant (37.76%). The nodal explants from one year old mother plant cultured on kinetin is more effective in callus initiation compared to nodal explants from five year old mother plant cultured on BAP. Similarly, the age of mother plant had more effect on days to initiation of callus and callus induction frequency in petiole explants. Callus induction frequency was the maximum in petiole cultured in NAA (1.0 mg/l) + BAP (0.5 mg/l) (51.95%) and it was the minimum in petiole explants from five year old plant (31.56%). Shoot tip from one year old plant cultured on GA_3 (3.0mg/l) + NAA (5.0 mg/l) resulted in maximum shoot length and direct regeneration frequency as compared with shoot tip from five year old mother plant. Results are indicative to juvenility factor of mother plant having an effect on response to callus induction and direct regeneration in Jatropha.*

Key words: Jatropha, Callus, Direct regeneration, IAA, GA_3.

INTRODUCTION

Techniques for the regeneration of *Jatropha curcas* L. from various explants have been developed. Not much work has been reported on protoplast, cell, tissue and organ cultures of *Jatropha spp* compared to other crop species. But the accumulated literature on plant regeneration through tissue culture, especially during this decade demonstrates the potential use of *in vitro* techniques for Jatropha improvement. In recent years, plantlet regenerations from different *J. curcas* explants have been reported (Sujatha and Mukta, 1996; Sardana *et al*., 2000; Rajore and Batra, 2005).

MATERIALS AND METHOD

Different explants (nodes, petioles and leaves) of *Jatropha curcas* L. were collected from the one year and five year old mother plants maintained at K- block, GKVK, University of Agricultural Science, Bengaluru. The leaves at $3^{rd} – 4^{th}$ node from the apex were collected from 5- year and 1 year old plant were first thoroughly washed with tap water, then dipped in 0.5 per cent Bavistin (BASF India Ltd.) solution for 5 min and washed two to three times with sterile distilled water. The leaves were then surface sterilized with 0.1 per cent $HgCl_2$ (w/v) solution for 5–8 min and washed four to five times with sterile distilled water to remove any traces of the $HgCl_2$. Leaves were cut into 1.0 cm^2 segments and placed with the abaxial side in contact with the medium. Nodal explants (3 cm) collected from 5- year and 1 year old plant were first thoroughly washed with tap water for 20 mins, then dipped in 0.5 per cent Bavistin (BASF India Ltd.) solution for 5 min. The explants were surface disinfested with 0.1% mercuric chloride for 12 minutes, followed by rinses in sterile distilled water. The nodal explants were trimmed (1–1.5 cm) at the base and cultured with the cut surface in contact with the medium surface. Similar procedure was followed for petiole.

Shoot tips excised from 1- year and 5- year old plants were thoroughly washed with tap water for 20 mins, then dipped in 0.5% Bavistin (BASF India Ltd.). The explants were surface disinfested with 0.1% mercuric chloride for 2- 3 minutes, followed by washing three times with sterile distilled water. These explants were then introduced vertically into semisolid MS medium supplemented with various concentrations and combinations of growth hormones.

All the experiments were conducted in the laboratory, under well defined conditions of the culture room maintained at 25^{o} $\pm 2^{o}$c. Uniform light (Ca.1000lux) was provided by fluorescent tubes (7200K) over a light/dark cycle (18/6 hours). Experiments were set up in Completely Randomized Design with 3 replicates per treatment, which includes 2-3 explants per treatment. Observations on the number of explants forming callus, number of shoot tip responding for direct regeneration were recorded by visual observations.

RESULTS AND DISCUSSION

Use of leaf disc explant

Leaf disc from one year old plant cultured on kinetin and BAP showed more or less similar response towards initiation of callus (Tables1 and 2) but the callus induction frequency varied being with maximum in explants cultured on medium supplement with BAP (56.15 %) and minimum in explants cultured on medium supplement with kinetin (53.63%). On contrary to this Jha *et al.* (2007) recorded maximum frequency in explants cultured on medium supplement with in Kinetin.

Leaf disc from five year old plant cultured on kinetin had taken minimum days for initiation of callus while explants cultured on medium supplement with BAP took maximum days for initiation of callus. However, it showed maximum callus induction frequency (37.76%) compared to kinetin (28.38%). But this result recorded minimum callus induction frequency as compared to the result reported by Deore *et al.* (2008) in explants cultured on medium supplement with BAP. It may be due to the interaction effect of age of plant and treatment. Irrespective of explant and treatment, the explant from one year old plant showed minimum days for callus induction frequency than five year old plant. Similar observations were made by Sujatha and Mukta (1995), when they used leaf disc from third and fourth expanding leaf.

Use of petiole explant

The petiole explant from one year old plant cultured with the combination of NAA (5.5 m/l) + BAP (1.3 m/l) showed minimum days for callus initiation. However the maximum callus induction frequency was observed in petiole explant cultured on NAA (5.5 m/l) + BAP (1.7 m/l) with minimum days for initiation of callus induction (Table 3). Hence this treatment is better for the petiole explant compared to all other treatment combinations. The petiole explant from five year old plant has recorded maximum frequency of callus induction (42.53 %) in combination of NAA (5.5m/l) + BAP (1.7 m/l).

The age of plant had more effect on callus induction frequency as the callus induction frequency was maximum in explant from one year old plant (51.95%) and minimum in explants from five year old plant (31.56%). Comparable result was observed by Verma *et al*. (2008). Discoloration of media was more in callus from explants of five year old plant than callus from one year old plant. It appears that phenolic secretion is more from matured plant than early aged group; however there was no much difference in nature of callus from explants of different age group plant. Callus induction responses observed in this study reflects the degree of age of the plants as has been documented by George and Sherington (1984).

Use of nodal explant

Nodal explant collected from one year old plant cultured on MS medium supplemented with kinetin had taken minimum days for initiation of callus where as nodal explant cultured in medium supplemented with BAP had taken maximum days for initiation of callus. The maximum frequency of callus induction was observed in nodal explant from one year plant cultured in medium supplemented with kinetin (31.96%) whereas nodal explant cultured on medium supplemented with BAP recorded 19.78% (Table 4 and 5). Nodal explant from five year old plant cultured on kinetin took minimum days for initiation of callus (10.33 days) compared to that cultured on BAP (12.11days). Nodal explant from five year old plant cultured on kinetin showed maximum callus induction frequency (23.40 %) compared to explant cultured on BAP (16.91%). This indicates that explant from early age of plant with kinetin is more effective in callus initiation compared to explant from old age of plant with BAP. Datta *et al.* (2007) and Banerjee *et al*. (2007) recorded maximum frequency of callus induction as they used explant from *in vitro* grown plants. Probably this indicates the effect of juvenility of the mother plant on callus induction.

Use of shoot tip

In both the age groups, the explant cultured on medium supplemented with combination of GA_3 (3.0 mg/l) + IAA (5.0mg/l) treatment recorded maximum length of shoot and maximum callus induction frequency for direct regeneration from shoot tip (Table 6). But when compared between age of the plant, the explant from one year old plant showed the maximum length of the shoot (3.46cm) and maximum direct regeneration frequency (59.15%), where as direct regeneration from shoot tip of five year old plant recorded minimum length of shoot (1.82cm) and minimum direct regeneration frequency (28.59%). Jyoti *et al*. (1998) observed maximum results as they excised shoot tip from *in vitro* grown seedling. Probably, here also the juvenility of the mother plant played an important role in direct regeneration.

Table 1. Effect of kinetin on callus induction from leaf disc explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	13.22	53.63	Soft friable, white
5 Year plant	13.66	28.38	Compact, light brown
SEm±	0.117	0.54	
CD at 5%	0.34	1.59	
Kinetin(mg/l)			
0.5	11.16	17.98	Soft, mushy, white
1.0	12.33	41.01	Soft friable, white
2.5	12.16	25.58	Soft friable, white
3.0	13.66	44.41	Soft friable, light white
4.0	14.50	57.83	Compact, white
4.5	16.33	59.23	Compact, brown
SEm±	O.20	0.94	
CD at 5%	0.59	2.76	
Interaction			
1 year + Kn(mg/l)			
0.5	10.66	25.83	Soft, mushy, white
1.0	12.66	51.83	Soft friable, light white
2.5	12.00	31.96	Soft friable, white
3.0	12.66	58.06	Soft friable, white
4.0	15.33	82.20	Compact, light brown
4.5	16.00	71.90	Compact, white
5 year + Kn(mg/l)			
0.5	11.66	10.13	Soft friable, light white
1.0	13.00	30.20	Soft friable, white
2.5	12.33	19.20	Compact, light brown
3.0	14.66	30.76	Compact, white
4.0	13.66	32.80	Compact, brown
4.5	16.66	47.23	Compact dark brown
CD at 5%	0.84	3.93	
CV %	3.92	5.70	
SEm±	0.28	2.33	
GM	13.44	41.01	

Table 2. Effect of BAP on callus induction from leaf disc explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	13.27	56.15	Soft friable, Light white
5 Year plant	15.27	37.76	Compact, light brown

SEm±	0.14	1.05	
CD at 5%	041	3.07	
BAP(mg/l)			
0.5	13.50	25.90	Soft friable, light white
1.0	13.16	38.00	Soft friable, white
2.5	14.00	46.76	Compact, light brown
3.0	13.83	51.58	Compact, white
4.0	15.50	58.41	Compact, brown
4.5	15.66	61.06	Compact dark brown
SEm±	0.24	1.82	
CD at 5%	0.71	5.32	
Interaction			
1 year + BAP(mg/l)			
0.5	13.66	30.53	Soft, mushy, white
1.0	13.33	42.50	Soft friable, white
2.5	11.66	56.66	Soft friable, light white
3.0	12.00	70.86	Soft friable, white
4.0	14.33	75.80	Compact, white
4.5	14.66	60.53	Compact, brown
5 year + BAP(mg/l)			
0.5	13.33	21.26	Soft, mushy, white
1.0	13.00	19.16	Soft friable, white
2.5	15.66	32.20	Soft friable, white
3.0	16.33	51.30	Soft friable, light white
4.0	16.66	41.03	Compact, white
4.5	16.66	61.60	Compact, brown
CD at 5%	1.01	5.32	
CV %	4.20	9.37	
SEm±	0.34	1.82	
GM	14.27	46.95	

Table 3: Effect of NAA and BAP on Callus induction from petiole explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	11.88	51.05	Soft mushy light yellow
5 Year plant	14.55	31.56	Compact, white
SEm±	0.26	0.61	
CD at 5%	0.78	1.80	
MS + GR (mg/l)			
N1.0 +B0.1	10.50	53.88	Soft friable, white
N1.0 +B0.5	11.16	56.48	Compact, light yellow
N1.0 + B1.0	12.33	51.21	Compact, white
N1.5+ B0.1	14.00	35.15	Soft, mushy, white
N1.5 + B0.5	15.33	29.56	Compact, light brown
N1.5 + B1.0	16.00	21.55	Compact, brown
SEm±	0.46	1.07	
CD at 5%	0.78	3.13	
Interaction			
1 year + NB(mg/l)			

N1.0 +B0.1	9.33	68.16	Soft friable, white
N1.0 +B0.5	9.66	70.43	Soft, mushy, white
N1.0 + B1.0	10.00	69.53	Compact, white
N1.5+ B0.1	12.66	40.66	Compact, white
N1.5 + B0.5	14.33	32.20	Compact, light brown
N1.5 + B1.0	15.33	25.30	Compact, brown
5 year + NB(mg/l)			
N1.0 +B0.1	11.66	39.60	Soft, mushy, white
N1.0 +B0.5	12.66	42.53	Soft friable, white
N1.0 + B1.0	14.66	32.90	Soft friable, white
N1.5+ B0.1	15.33	29.63	Compact, light brown
N1.5 + B0.5	16.33	26.93	Compact, white
N1.5 + B1.0	16.66	17.80	Compact, white
CD at 5%	1.92	4.42	
CV %	8.64	6.36	
SE±	0.65	1.51	
GM	13.22	41.30	

Note:- N- NAA, B- BAP

Table 4. Effect of Kinetin on Callus induction from nodal explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	10.16	31.96	Compact, light white
5 Year plant	10.33	23.40	Compact, Mushy, light brown
SEm±	0.124	0.30	
CD at 5%	0.362	0.898	
Kinetin (mg/l)			
00	00	00	Nil
0.5	10.16	18.4	Soft fleshy, light white
1.0	12.00	37.68	Soft friable, white
2.5	11.00	24.50	Compact, light yellow
3.0	13.33	44.23	Compact , light white
4.0	15.0	41.28	Compact , light white
SEm±	0.21	0.53	
CD at 5%	0.62	1.55	
Interaction			
1 year + Kinetin(mg/l)			
00	0.00	0.00	Nil
0.5	9.66	20.10	Soft friable, white
1.0	12.66	29.96	Soft fleshy, light white
2.5	11.66	45.90	Compact, light yellow
3.0	13.33	50.30	Compact , light white
4.0	13.66	45.53	Compact , light white
5 year + Kinetin (mg/l)			
00	0.00	0.00	Nil
0.5	10.66	16.70	Soft friable, white
1.0	11.33	18.90	Compact , light white
2.5	10.33	29.46	Soft friable, white
3.0	13.33	38.16	Compact, brown

4.0	16.33	37.03	Compact, dark brown
CD at 5%	0.888	2.20	
CV %	4.69	4.25	
SEm±	0.304	0.75	
GM	12.30	33.20	

Table 5. Effect of BAP on Callus induction from nodal explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	11.61	19.78	Compact friable, light white
5 Year plant	12.11	16.91	Compact friable, light white
SEm±	0.13	0.33	
CD at 5%	0.38	0.96	
BAP(mg/l)			
00	0.0	0.0	
0.5	12.0	11.38	Soft fleshy, light white
1.0	13.50	23.20	Soft friable, white
2.5	14.16	23.43	Compact, light yellow
3.0	15.00	26.98	Compact , light white
4.0	16.50	25.10	Compact , light white
SEm±	0.22	0.57	
CD at 5%	0.65	1.67	
Interaction			
1 year + BAP(mg/l)			
00	0.0	0.0	
0.5	11.66	12.16	Soft friable, white
1.0	13.66	31.60	Compact , light white
2.5	13.00	19.70	Soft friable, white
3.0	14.66	26.46	Compact, brown
4.0	16.66	28.80	Compact, dark brown
5 year + BAP(mg/l)			
00	0.0	0.0	
0.5	12.33	10.60	Soft friable, white
1.0	13.66	14.80	Soft fleshy, light white
2.5	15.00	27.16	Compact, light yellow
3.0	15.33	27.23	Compact , light white
4.0	16.33	21.40	Compact , light white
CD at 5%	1.07	2.37	
CV %	4.66	6.86	
SEm±	0.81	1.51	
GM	14.23	21.98	

Table 6: Effect of GA_3 and IAA direct regeneration plantlets from shoot tip of different age grouped plants

Treatment	Days for initiation of bud	Length of shoot	Survival %
1 Year plant	15.50	3.46	59.15
5 Year plant	15.20	1.82	28.59
SEm±	0.15	0.035	0.32
CD at 5%	0.43	0.10	0.92
MS+ GI(mg/l)			

$G_{1.0} + I_{3.0}$	12.83	1.45	33.71
$G_{3.0} + I_{3.0}$	13.16	1.73	37.23
$G_{5.0} + I_{3.0}$	13.66	2.08	37.73
$G_{8.0} + I_{3.0}$	14.66	2.31	38.80
$G_{1.0} + I_{5.0}$	15.83	2.70	47.21
$G_{3.0} + I_{5.0}$	17.83	4.05	54.83
$G_{5.0} + I_{5.0}$	17.83	3.83	53.63
$G_{8.0} + I_{5.0}$	17.00	3.21	49.33
SEm±	0.30	0.07	0.64
CD at 5%	0.86	0.20	1.84
Interaction			
1 year + GI(mg/l)			
$G_{1.0} + I_{3.0}$	12.33	2.16	41.20
$G_{3.0} + I_{3.0}$	13.66	2.93	52.93
$G_{5.0} + I_{3.0}$	12.66	2.60	47.26
$G_{8.0} + I_{3.0}$	15.33	3.06	58.90
$G_{1.0} + I_{5.0}$	16.00	3.46	63.76
$G_{3.0} + I_{5.0}$	18.33	4.83	72.53
$G_{5.0} + I_{5.0}$	18.33	4.56	74.13
$G_{8.0} + I_{5.0}$	17.33	4.10	62.53
5 year + GI(mg/l)			
$G_{1.0} + I_{3.0}$	13.33	0.73	26.56
$G_{3.0} + I_{3.0}$	12.66	0.53	21.20
$G_{5.0} + I_{3.0}$	14.66	1.56	27.53
$G_{8.0} + I_{3.0}$	14.00	1.10	18.36
$G_{1.0} + I_{5.0}$	15.66	1.93	30.66
$G_{3.0} + I_{5.0}$	17.33	3.26	35.13
$G_{5.0} + I_{5.0}$	1733	3.10	33.13
$G_{8.0} + I_{5.0}$	16.66	2.33	35.46
CD at 5%	1.22	0.28	0.92
CV %	4.70	6.75	3.59
SEm±	0.42	0.10	0.90
GM	15.35	2.64	43.87

Note:- G- GA_3, I- IAA

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