RESEARCH PAPER | BOTANY | Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

Development of technique for efficient in vitro propagation of Jatropha curcas L.from nodal explants.

KEYWORDS: Micropropagation , Jatropha ,True genotype , cytokinin , Auxin

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ABSTRACT *Micropropagation technique is being applied for the production of large number of plants within a limited period and space. Therefore, this technique is being applied to generate large number of planting material with uniform genotype. In the present work multiple shoots on the nodal explants have been raised in MS basal medium supplemented with seven different concentrations viz., (0.5- 3.5) mg/l of BAP and 1.0 mg/l kinetin +0.5 mg/l IBA . Out of the above concentration , BAP 3.0 mg/l + KN 1.0 mg/l + 0.5 mg/l IBA was found more suitable than any other concentration of the similar combination or any other concentration of BAP alone or BAP + IBA. Induction of shoots were noted after 18th day of inoculation. These shoots were excised and sub cultured on the same medium. Denovo initiation of multiple shoots was observed after three weeks of the inoculation in sub culture. Half strength MS medium supplemented with IBA 3.0 mg/l + IAA 0.5 mg/l was found more suitable in which the average number (6-8) of roots were found. Well developed and rooted plants were used for hardening in artificial polychamber. They were planted in polybag and pots containing 1:1:1(Soil +Sand+Vremiculite) and the survival rate was 72%.*

INTRODUCTION

The micro propagation techniques is being applied for the production of plants at commercial scale. Micro propagation of *J.curcas* L. family Euphorbiaceae needs special attention as the plant has got importance in present scenario. Its seeds are being used for the production of biodiesel so farmers are attracted towards its cultivation at large scale. Traditional method of propagation of *Jatropha* is through the seeds. This practice is not beneficial here because one hand we get low percentage of seed germination on the other hand the quantity of seeds to be used for biodiesel shall be reduced. Secondly the heterogeneous characters shall segregate through it, and therefore, uniform plants shall not be available to the farmers. The micro propagation technique therefore, appears to be an alternative for the production of large number of uniform plants which may be used as planting materials. It has been reported by Pant *et al* ;(2006) and Jha *et al;* (2007) that there is significant variations in seed yield and oil content among the plants of *Jatropha* raised through seeds. Due to low seed germination and short viability of seeds, propagation through seed may not provide high quality material for sustainable agriculture (Heller 1996). Thus micro propagation is an alternative means of propagation and the technique would not only help in raising high quality planting material but also will permit the seed to be used for biodiesel production. Micro propagation through nodal explants will also conserve the genetic material which is diluted among the progeny produced through seed. Development of an efficient technique for micro propagation of *Jatropha* therefore, shall be of great importance because the method will facilitate the large scale production of true to type plants and one would get uniform planting material that shall be authentic for a particular genotype.

Attempts have been made to regenerate *Jatropha curcas* using different explants (Sujatha & Mukta 1996; Kalimuthu et al;2007, Reddy et al; 2008, Deore and Johanson; 2008, Warakgoda and Subhasinghe,2009,Khurana et al; 2010, N. Kumar and Reddy; 2010,Siang et al; 2012, Wei et al 2004; Sujatha et al 2005; Jha et al 2007; Rajore and Batra 2007). The above workers regenerated plants either through callus or direct shoot morphogenesis with interspersed callus. As we know that the callus mediated regeneration is not suitable for the production of true- to- type plants therefore, in true sense this technique may not become appropriate for micro propagation. The nodal explants contains pre- existing meristematic buds. So, production of multiple shoots may be true to type of its mother plant and there shall be no genetic variation among the progeny produced through it. Therefore, in the present research work experiments have been done to produce multiple shoots on the nodal explants of Jatropha curcas L. *in vitro*.

Materials and Method :

Plant materials for nodal explant were collected from two years old *Jatropha* plant grown in the garden of University Department of Botany, B.R.Ambedkar Bihar Universirty Muzaffarpur.The twig were washed with running tap water for one hour followed by treatment with 0.1% $HgCl_2$ for 4-5 minutes. The flasks containing the material was shaken vigorously for uniform contact of the chemicals with the explants .The materials were taken out and rinsed with sterile distilled water thrice. Because in earlier experiments exudation of phenolics and systemic infection were noted so the explants were treated with systemic fungicides (bavistin 0.1%) and the medium was supplemented with antioxidants such as 50 mg/l each of ascorbic acid and citric acid to reduce exudation of phenolics. From the above branches of *Jatropha* single node about 0.8 cm long were used for inoculation as initial explant .

MS medium (Murashige & Skoog 1962) supplemented with BAP alone and with kinetin at various concentration were used as basal medium. This was supplemented with

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3% sucrose and the medium was gelled with 0.8gm agar .The pH was adjusted to 5.8 and autoclaved . 20ml of culture medium was taken in the culture tube and were properly plugged with cotton plug. These cotton plugs were wrapped with aluminium foil before autoclaving. Autoclaving was done at 15 lb pressure for 20 minutes. Inoculation was done in Laminar flow and the inoculated flasks were incubated in culture room at 26 $\pm$1 ^{O}C with 16 hour photoperiod where the light was provided by cool white fluorescent tube light. Observation was made on an alternate day and culture tubes showing any contamination or phenolics were discarded. Rooting was done in the half strength MS medium supplemented with various concentration of auxins either alone or in combination.

The data obtained were analysed statistically and the result were arranged in the tables 1 and 2 . Number of shoots, date of initiation and % response all were recorded.

RESULTS : The culture tubes inoculated with the nodal explants were incubated in the culture room with controlled temperature and light. After three weeks of primary culture, explants showed growth response on different culture medium. After perusal of the table -1, it is apparent that the shoots were induced from the nodal explants on MS basal medium supplemented with different concentrations of BAP and Kinetin(0.5-3.5 mg/l), + (0.5mg/l IAA), either BAP+KN only or various concentrations of BAP(0.5-3.5 mg/l)+0.5 mg/l or 1.0mg/l KN +0.5 mg/l IAA . From the table it may be noted that the most congenial condition for shoot induction was MS medium containing 3-0 mg/l BAP +1.0mg/l KN+0.5 mg/l IAA where the percent of response was 76.The mean number of shoots was 8.5 and the average length of the shoots was 2.8 cm. Nodal explants cultured on MS medium supplemented with higher concentration of BAP 3.5 mg/l with 1.0 mg/l KN + 0.5 mg/l IAA revealed lower response. Similarly at lower concentrations of BAP+KN revealed no response. Even BAP+KN at different concentrations had less stimulatory effect on nodal explants.

Rooting in the well grown plantlets raised through tissue culture was initiated in the MS basal medium supplemented with different concentrations of IAA and IBA, either alone or in combinations (table-2).Roots were induced in medium supplemented with different concentrations of IAA or IBA alone or in combination, but the best combination and concentration for root induction was MS+0.5 mg/l IAA $\pm$ 3.0 mg/l IBA where the percent response was 74, the mean number of roots was 5.8 and average length 2.6 cm after three weeks of incubation. In MS medium supplemented with either IAA or IBA alone at various concentration the response was less than the above.

DISCUSSION ;- Multiple shoots from the nodal explants were induced in MS medium supplemented with 3.0mg/l BAP+1.0 mg/l KN and 0.5 mg/l IAA. Sujatha and Mukta; (1996); Sujata et al;(2005); Ajay *et al* ;(2007); Kalimuthu *et al* ;(2007); Warakagoda & Subasinghe;(2009); also reported multiple shoots induction from the nodal explants of *Jatropha curcas* at higher concentration of BAP (3.0mg/l). Thus our finding corroborates with the finding of the above workers. Reddy and Kumar ;(1996) tried to develop shoots from leaf disc, Deore et al ;(2008) also reported regeneration of plantlets from the leaf disc of *Jatropha curcas.* Kumar and Reddy developed multiple shoots from petiole of *Jatropha curcas* Siang et al; (2012) reported induction of embryogenic calli of *Jatropha curcas.* Yi -L et al (2004) also used epicotyl for regeneration in *Jatropha curcas* .

However , nodal segments as explants and cytokinins are one of the most important hormones for shoot proliferation. (Lane; 1979, Bhojwani; 1980).From the survey of literature it is apparent that BAP is the most reliable and effective cytokinin for multiple shoot induction (Sudharsan *et al*; 2001, Mao *et al* ;1995) reported requirement of higher concentration of BAP to induce multiple shoots through nodal segment of *Clerodendrum ,* Khan *et al*; (1998) has also reported that BAP has caulogenic effect on nodal explants.

Based on the findings of the present work, it may be concluded that BAP is best hormones for the *in vitro* shoot regeneration from the nodal explants .This protocol may be exploited for the production of large number of planting materials that would be true to its parent with respect to its genotype.

Table - 1
Effect of growth regulators either alone or with IBA on nodal explants of Jatropha sps.

MS medium + Growth regulator mg/l	Frequency response %	No. of Shoots/ explants	Length in Cm.	Days after 30 days.

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BAP+KN+IBA	---			
0.5 + 0.5 + ---	---	---	---	30 Days
1.0 + 1.0 + ---	7.5	---	---	30 Days
1.5 + 1.5 + ---	9.2	1.2 + 0.4	0.8	30 Days
2.0 + 2.0 + ---	10.5	1.8 + 0.7	0.8	30 Days
2.5 + 2.5 + ---	10.8	2.4 + 0.9	1.2	30 Days
3.0 + 3.0 + ---	11.4	2.8 + 1.12	1.6	30 Days
3.5 + 3.5 + ---	32.5	3.0 + 1.22	1.8	30 Days
0.5 + 0.5 + 0.5	38.6	3.4 + 1.26	1.2	30 Days
1.0 + 0.5 + 0.5	42.8	3.8 + 1.36	1.6	30 Days
1.5 + 0.5 + 0.5	44.6	4.6 + 1.45	1.8	30 Days
2.0 + 0.5 + 0.5	52.4	4.8 + 1.48	2.0	30 Days
2.5 + 0.5 + 0.5	60.8	5.4 + 1.62	2.2	30 Days
3.0 + 0.5 + 0.5	54.5	6.2 + 1.68	2.4	30 Days
3.5 + 0.5 + 0.5		4.8 + 1.38	1.8	
0.5 + 1.0 + 0.5	34.2	3.6 + 1.38	1.30	30 Days
1.0 + 1.0 + 0.5	41.5	3.8 + 1.42	1.60	30 Days
1.5 + 1.0 + 0.5	47.6	5.4 + 1.48	1.80	30 Days
2.0 + 1.0 + 0.5	54.4	5.8 + 1.52	2.0	30 Days
2.5 + 1.0 + 0.5	58.6	6.4 + 1.64	2.2	30 Days
3.0 + 1.0 + 0.5	76.5	8.5 + 1.74	2.8	30 Days
3.5 + 1.0 + 0.5	56.8	5.4 + 1.68	1.6	30 Days

Table No :- 2
Effect of IAA and IBA supplemented in ½ strength MS medium on in vitro rooting of shoots of Jatropha raised through tissue culture.

Growth Hormone mg/l	Percent re-sponse	No. of roots per plant	Root length	Callusing
IAA + IBA				
0.5 + 0.0	-----	------	------	+
1.0 + 0.0	25.0	1.5 ± 1.20	0.6 ± 1.26	+
1.5 + 0.0	38.0	1.8 ± 1.32	1.4 ± 1.32	------
2.0 + 0.0	42.0	2.0 ± 1.26	1.6 ± 1.30	------
2.5 + 0.0	48.0	2.2 ± 1.34	1.8 ± 1.24	------
3.0 + 0.0	58.0	2.8 ± 1.40	2.0 ± 1.38	------
3.5 + 0.0	52.0	2.0 ± 1.30	1.6 ± 1.25	------
0.0 + 0.5	----	-----	-----	-----
0.0 + 1.0	40.0	2.4 ± 1.44	1.0 ± 1.28	------
0.0 + 1.5	46.0	2.8 ± 1.52	1.6 ± 1.36	------
0.0 + 2.0	48.0	3.6 ± 1.46	2.2 ± 1.42	------
0.0 + 2.5	56.0	4.5 ± 1.36	2.4 ± 1.48	------
0.0 + 3.0	60.0	5.4 ± 1.38	2.8 ± 1.32	------
0.0 + 3.5	54.0	2.5 ± 1.40	1.2 ± 1.35	-----
0.5 + 0.5	-----	-----	-----	-----
0.5 + 1.0	48.0	2.8 ± 1.46	1.6 ± 1.42	------
0.5 + 1.5	62.0	3.2 ± 1.30	2.2 ± 1.36	------
0.5 + 2.0	66.0	4.8 ± 1.28	2.8 ± 1.40	------
0.5 + 2.5	68.0	5.0 ± 1.34	3.0 ± 1.32	------
0.5 + 3.0	74.0	5.8 ± 1.42	3.6 ± 1.24	------
0.5 + 3.5	70.0	2.8 ± 1.36	1.8 ± 1.26	------

Values are mean ± standard error of three replicate with ten cultures per replicate, data collected after 25th day of inoculation.

Fig (i) showing axillary buds and callus in shoot tip.

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Fig (ii) showing axillary shoots in nodal explants.

Fig (iii) showing growth of root in culture medium.

Fig (iv) showing formation of new plantlet before transfer to the soil.

ACKNOWLEDGMENT

Authors are highly obliged to the Head of the Department providing the research facilities in the University Department of Botany, B. R. Ambedkar Bihar University Muzaffarpur.

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