Indian J.Sci.Res.2(4) : 45-49, 2011

REDUCTION OF PHENOLICS AND ENDOPHYTIC FUNGUS IN THE NODAL AND INTERNODAL EXPLANTS OF *Jatropha curcas* L.

KUMARI ANJALI[a1], RANJANA[b] AND L.N. SHUKLA[c]

[a]Department of Botany, B.R. Ambedkar Bihar University, Muzaffarpur, Bihar, India
E-mail: k.anjali.bot@gmail.com
[b]Department of Botany, J.P. University, Chhapra, Bihar, India
E-mail: ranjana.brabu@rediffmail.com
[c]Department of Botany, B.R. Ambedkar Bihar University, Muzaffarpur, Bihar, India
E-mail: lnshukla.botany@gmail.com

ABSTRACT

***Jatropha curcus* is being planted for the production of oil which in being used as biodisel. Plants generated from seeds are not superior. Therefore, Planting material regenerated through micropropagation is getting more importance. During tissue culture of *Jatropha* exudation of Phenolics and endophytic fungal growth cause great hindrance in the multiple shoot regeneration. In the present work attempt has been made to reduce, phenolics exudation and reduction in the growth of endophytic fungus. Three different antioxidant at different concentration have been used for the reduction of phenolics exudation where the concentration at 50mg/l each of ascorbic acid and citric acid have been found more suitable, which reduced exudation of phenolics to the greater extents. Here, 90% explants revealed inhibition of the exudation of phenolics. Among the fungicides used Bavistin at 1% concentration and 3 hours of duration of treatment was found more suitable than the rest of the fungicides either at the same concentration or at different concentrations. Fungicides at higher concentrations and even at 2 hours duration of treatment damage the explants severely which was perhaps due to toxic effects of the fungicides.**

KEYWORDS : Fungicides, antioxidants, phenolics, jatropha, micropropagation

Jatropha curcas L. of the family Euphorbiaceae is now getting much importance because the oil extracted from the seed is being used as a biodiesel. Normally the plants are found growing as a wild species on the marginal lands of railways and roadways. The normal mode of reproduction is through the seed. However, the seed production is generally observed when the plant becomes 3-4 years old. The fruits are produced in winter when plant becomes leafless. Seeds are produced in bunch and they are released from the fruit on maturity.

Once a good germplasm of *Jatropha* is selected its reproduction through seeds creates cytoplasmic uniformity in plants making them more susceptible to disease and insect infestation. If the propagation of *Jatropha* is done through tissue culture then, the clones shall be produced true to its mother plant. Therefore, Micropropagation is considered to be the best method for the production of large number of uniform planting material.

Clonal propagation is achieved by culturing the nodal segment as an explant. In the present investigation Micropropagation of *Jatropha* was done in *vitro* during which it was observed that the explant exudes phenolics as well as there were severe infection by the endophytic fungus. Both the above hindered the growth and induction of axillary buds. Therefore, it became essential to reduce the phenolics as well as the growth of endophytic fungus for successful induction of multiple shoots.

From the survey of literature, it is observed that we do not get detailed information regarding the methods for reduction of phenolics and the growth of endophytic fungus. Here we report the complete reduction of phenolics as well as the reduction in the growth of the endophytic fungus.

MATERIALS AND METHODS

The nodal explants of Jatropha after sterilization were treated with antioxidants that was ascorbic acid and citric acid 50 mg each in 1 litre of solution. In the MS basal medium (Murashige and Skoog, 1962) different concentration of ascorbic acid such as 10, 20, 30 and 50 mg/l was added. In other experiments similar concentrations of citric acid was added. In another experiments 30 mg & 50 mg each of ascorbic acid and citric acid were added in the culture medium. Similarly 50 mg, 100 mg and 250 mg of polyvinyl pyrrolidone were added. In the all above culture medium 20 nodal explants of *Jatropha* were inoculated and cultured in the culture room at 26 $\pm$ 1 ^{0}C temperature and artificial light generated by the white fluorescent tube.

[1]**Corresponding author**

For the reduction of endophytic fungus four different fungicides at different concentrations were used for the treatment of the explant. The fungicides were Indophil, Captan, Dithan-Z, and Bavistin at 0.5, 1.0, 2.0 and 3.0% concentration. The explant were raised in the above solution for different duration such as 1, 2 and 3 hours. After treating the explant with the above fungicides, the explants were rinsed with distilled water four times for 3-5 minutes of duration. The explants were then inoculated in above culture medium and the cultures were incubated in culture room. Observation were made upto 3 weeks on alternate day and growth of the endophytic fungus were observed in the different fungicides at their different concentrations in the culture medium. The experiments were performed in triplicate and the data were collected after taking the mean of the result.

RESULTS AND DISCUSSION

Perusal of the table1 indicates that exudation of phenolics were maximum when the explants were cultured in the medium supplemented with 10mg/l either ascorbic acid or in citric acid. The intensity of the exudation of Phenolics was such that the explants became black and the medium brown after second day of the inoculation. It is further noted that the exudation of phenolics was gradually reduced in ascorbic acid and in citric acid containing medium where the concentrations were increased gradually. In case of ascorbic acid at 50 mg/l concentration the percentage of response was 42 where the rest revealed browning of the medium. At this concentration in case of citric acid, there was only 38% response and on 4th day the medium became brown.

Table 1 : Impact of antioxidant on exudation of Phenolics in different explants of *Jatropha* in *invitro* condition

No. of explants	Antioxidant ascorbic acid mg/l + Citric acid mg/l	Percent response	Response Days after	Remark
20	-- --	Zero	2nd Days	Browning blacking
20	10 --	00	3rd Days	,,
20	20 --	20	3rd Days	rest browning
20	30 --	28	4th Days	browning
20	50 --	42	5th Days	Medium browning only
20	-- 10	00	2nd Days	browning
20	-- 20	15	3rd Days	browning
20	-- 30	26	4th Days	browning
20	-- 50	38	4th Days	Medium browning
20	30 30	46	5th Days	Medium browning
20	50 50	98	5th Days	Browning of medium in 2%
20	Polyvinyl Pyrrolidone (PVP)			
	50 mg	00	5th Day	All black
	100 mg	26	5th Day	Rest Black
	250	40	5th Day	Rest Black

(---) absence of antioxidants.

When ascorbic acid and citric acid were used together at 30 mg/l concentration the percent response was 46% and browning of the medium was noted at 5th day of inoculation. When these two chemicals were used together at the concentrations of 50 mg/l, 98% explants responded it and browning of the medium was observed in 2% explant only. Therefore, in rest of the experiments this concentration was followed for micropropagation through nodal explant. Use of PVP at different concentrations were not found so suitable because even at 250 mg/l concentration only 40% explant responded and rest became black due to excessive phenolics.It was further noted that explants treated with 50mg/l ascorbic acid solution for 3-5 minutes and then inoculated in MS medium supplemented with 50mg/l ascorbic acid and 50mg/l citric acid gave better result in comparison to the explants which were not treated before inoculation. It was further noted that if explants were left in running water for 24 hour then treated with 50 mg/l solution of ascorbic acid and then inoculated in MS medium containing equal amount of ascorbic acid and citric acid (50 mg/l each) gave better result in figure 1 & 2.

To reduce the growth of endophytic fungus the explants were treated with four different concentrations (0.5, 1.0, 2.0, 3.0%) of four different fungicides such as Indophil, Captane, Dithane-Z and Bavistin. The data obtained were presented in the table 2 and figure 3, 4 & 5.

From the table it is noted that no suppression of endophytic fungal growth were noted at 0.5% concentration in all the above fungicides when the treatment was given upto 2 hours. However, when the duration of treatment was enhanced upto 3 hours 22% explants revealed reduction in the growth of endophytic fungus. At 1% concentration Bavistin reduced growth of the fungus in 42% of the explants even after 2 hours of treatment. At the 3 hours duration of treatment the reduction was 100% in this fungicides.

All the fungicides at 2 and 3% concentration revealed suppression of growth of endophytes even after 1 hour of duration of treatment. However the percentage was poor. Bavistin at 2% concentration and 3 hours of duration reduced growth but the explants became black at this concentration.

It may therefore, be concluded that among the fungicides Bavistin was the best at the concentration of 1% and 3 hours duration was found to be more suitable. Indophil was less effective than Captane or Dithane-Z. Thus, the experiments was carried on 1% concentration of Bavistin in which the explants were treated for 3 hours with manual shaking.

Browning of the medium is due to oxidation of Phenolic substance leached out from the cut surface of the explants. This turn the medium dark brown and is often toxic to the tissue. Therefore, its reduction is essential. Antioxidants used to reduce the phenolics includes ascorbic acid, citric acid, PVP etc. Several different methods have to be used to overcome this problem. (Press and compton 1991). Keeping the cultures in dark and quick transfer of explants in the fresh medium sometimes may reduce browning problem (Adam's et al., 1979; George and Sherrington 1984). Use of Ascorbic acid at the rate of 50-100 mg/l or citric acid 150 mg/l to the culture medium is recommended by Sondahl and Sharp, 1977; Skirvin and Chu, 1979. Similarly emmersion of shoot pieces for different duration in the solution of antioxidants before inoculation may help in the reduction of browning of the medium of the tissue. Deore and Johanson (2008) also reported treatment of the explants of *Jatropha curcas* L. However, they used leaf for culture. Vershney and Johanson (2010) reported regeneration from embryo cultures of *J. curcas* L. They also did not mention about phenolics and endophytic fungus.

Sometimes PVP is also much beneficial. However, in *Jatropha*, we observed that PVP was not suitable and citric acid and ascorbic acid 50 mg/l each when used together gave better result. *In vitro* regeneration technique offer a powerful tools for germplasm conservation, mass multiplication of true to type plants. However, sometimes the phenolics and endophytic fungus do create problems. Therefore, in the present work attempt has been made to reduce these two for clonal propagation in *Jatropha curcas* through nodal explant.

ACKNOWLEDGMENT

The authors are grateful to the Head of Department of Botany for providing laboratory facilities to perform the above experiments.

Table2: Impact of four different fungicides at four different concentrations and three different duration on the endophytic fungus of the explant (nodal) taken from *Jatropha curcas*

Fungicide	Concentration of fungicides	Duration of treatment		
		%age response in explant at		
Fungicide	.5%	1 hour	2 hour	3 hour
Indophil		-	-	10%
Captan		-	-	16%
Di-than-z		-	-	18%
Bavistin		-	-	22%
Fungicide	1%	1 hour	2 hour	3 hour
Indophil		-	12	18
Captan		-	16	26
Di-than-z		-	24	40
Bavistin		-	42	100
Fungicide	2%	1 hour	2 hour	3 hour
Indophil		8	18	12
Captan		10	24	34
Di-than-z		16	30	56
Bavistin		18	48	62
Fungicide	3%	1 hour	2 hour	3 hour
Indophil		12	22	30
Captan		14	28	44
Di-than-z		18	32	58
Bavistin		25	52	Black

(-) No response in explant.
Black – Colour of Explant after 3 hour treatment.

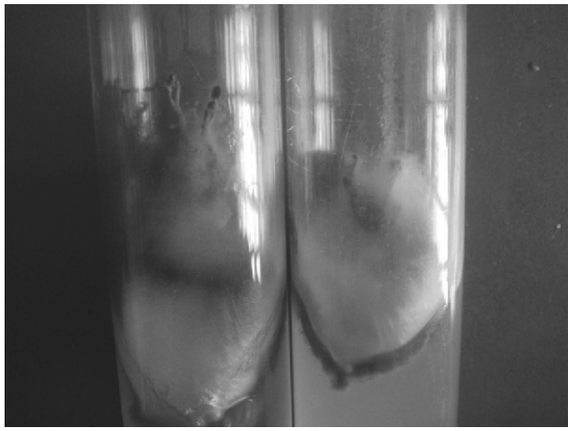

Fig.1 : Showing fungal growth in explants

Fig. 2 : Showing phenolics exudation in shoot tip

Fig. 3: Showing budding in shoot tip

Fig. 4 : Showing budding in shoot tips

Fig. 5 : Showing axillary shoots in nodal explants

REFERENCES

Adams II, R.M., Koenigsberg S.S., Langhans R.W., 1979. In vitro propagation of the butterwort *Pinguicula* moranensis. Hort. Science, **14**:512-513.

Deore A. C. and Johnson; 2008. High frequency plant regeneration from leaf-disc cultures of *Jatropha curcas* L. On important biodiesel plant. Plant Biotechnol Rep., **2**: 7-11.

George E.F. and Sherrington P.D.,1984. Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories, Exegetics, Hands, U.K.: 709.

Murasighe and Skoog, F.,1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures Physiol. Plants, **15** :473-497.

Press J.E. and Compton M.E., 1991. Problems with explants exudation in micropropagation. In: Biotechnology in Agriculture and Forestry, High-Tech and Micropropagation I. Vol. 17. Edited by Y.P.S. Bajaj (Springer), Berlin:169-189.

Skirvin R.M. and Chu M.C., 1979. In vitro propagation of forever yours rose. Hort Science, **14**:608-610.

Sondahl M.R. and Sharp W.R., 1977. High frequency induction of somatic embryos in cultured leaf explants of *Coffea arabica* L.Z. Pflanzenphysiol, **81**:395-408.

Vershney A. and Johnson, 2010. Efficient plant regeneration from immature embryo cultures of *Jatropha curcas*. Plant Biotechnol Rep., **4** : 139-148.