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Callus induction in *Simarouba glauca* D.C.

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Simarouba glauca is an oil yielding tree with a very high productivity and its rapid multiplication is essential for mitigating the edible/ industrial oil demand. For developing *in vitro* regeneration protocol, optimization of surface sterilization of explant and study of effect of auxins and cytokinins is essential. The explant was exposed to mercuric chloride (0.1 %) and Sodium hypochloride (1 %) for a period of 2-12 minutes. Both the surface sterilizing agents gave near about same results with no contamination even at lowest contact time, however, as the contact time was increased intense browning was observed. The callus induction was observed at all the concentration of 2,4-D and BAP. More callusing was observed with higher concentration of both 2,4 D and BAP.

Key words : Simarouba glauca, Callus, Auxins, Cytokinins.

INTRODUCTION

Growth in population and the improvement in general living standards, the demand for edible/industrial oils has frequently exceeded that of supply. As a result, India even with its enormous wealth of natural resources and being a country with strong agricultural base is compelled to import millions of tones of edible oil in recent years. Since the further horizontal expansion in oilseed cultivation in arable land is feared to adversely affect the production of essential food crops, as a progressive step towards self sufficiency, there is need to plan for vertical improvement preferably by growing varieties of oilseed plants/trees with high productivity. The introduction of Simarouba plant with the oil productivity of 2000-2500 kg/ha/year (Krishnamurthy, 1998; Satpathi, 1984) and with the ability to establish well even in the marginal/ wastelands with degraded soils has given new hope for alleviating the shortage of edible oil/fat (Munde, 2001).

Simarouba glauca DC is an exotic specy belonging to the family Simarubaceae. It was introduced in India long back in 1966 at NBPGR, Amravati (Joshi and Hiremath, 2000) and in 1970 at Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola as a fast growing soil binding tree species. The plant is valuable for its multiple uses like edible oil, industrial oil, furniture industry and medicinal properties (Engler and Prantl, 1872, Polito and Negrito, 1981). Mass multiplication techniques for this species are not yet standardized, as evident from the available literature. However, attempts have been made for its *in-vitro* shoot multiplication (Rout and Das 1994, Rout *et al.* 1999). A standard protocol is not available for rapid multiplication of these plants. The experiments were designed to study various initial aspects required to standardize the *in vitro* regeneration, such as standardization of surface sterilization and callus induction using nodal explants.

MATERIALS AND METHODS

The present study was carried out in 2007 at Biotechnology Centre, Department of Agricultural Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The nodal explants of Simarouba were collected from the College of Forestry, Dr.P.D.K.V., Akola. For incubation all the cultures were kept in culture room at temperature of 25 ± 2^0C with relative humidity at 55 ± 5 per cent and in dark condition.

Standardization of sterilization of explant :

For successful *in vitro* regeneration, an effective yet safe sterilization protocol for explant is essential. The explant were exposed to different surface sterilizing agents like mercuric chloride (0.1 %) and sodium hypochloride (1 %) for different contact time from 2 to 12 minutes. MS media was used as basic media for explant establishment. The observations were recorded after 10 days of inoculation. Nodal explants were washed thoroughly in running tap water for 1 hour to remove all adhering dirt and phenols. The explants were then treated with dettol (5ml/l) for 1 minute followed by washing with water. The surface sterilization of explant was carried out by keeping

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the explant with mercuric chloride (0.1%) or sodium hypo chloride (1%) for different contact time from 2 to 12 minutes. Then explants were given 3-4 wash with double distilled water and inoculated (Tule, 2004).

Callus induction :

After sterilization, the nodal explants were inoculated in test tube on media. The explants were inoculated separately on MS medium with different concentrations and combinations of 2, 4-D and BAP for callus induction. For testing each treatment combination, ten test tubes were inoculated with the explant. Observations were recorded after 25 days of inoculation.

RESULTS AND DISCUSSION

Standardization of surface sterilization method :

Nodal explants of Simarouba were collected from field grown one year old plants, so their surface sterilization was necessary. From the perusal of Table 1, it is observed that out of various treatments tried, the treatment involving 0.1% mercuric chloride and 1 % sodium hypchloride gave the same results for all the contact time tested. No contamination was observed with even the lowest contact time (2 min) of both the sterilizing agents and the explants were established. However, as the contact time of explant with the surface sterilizing agent was increased there was intense secretion of phenolics and browning of the media. Thus even with the lowest contact time of two minutes the explants were successfully established with no contamination. Since the surface sterilizing agents are known to cause damage to the tissues, their minimum concentration and minimum contact time yet effective prevention of contamination is very effective in establishing the tissue culture protocol. With increase in the contact time between the explant and surface sterilizing agents, the secretion of phenolics was increased as evident from the browning of the media (Plate I). Mercuric chloride (0.1%) for a contact time of four minutes was selected as surface sterilizing agent for all the further experiments. To prevent the necrotic effects of these phenolics charcoal and ascorbic acid was added to the media which helped in better establishment of was added

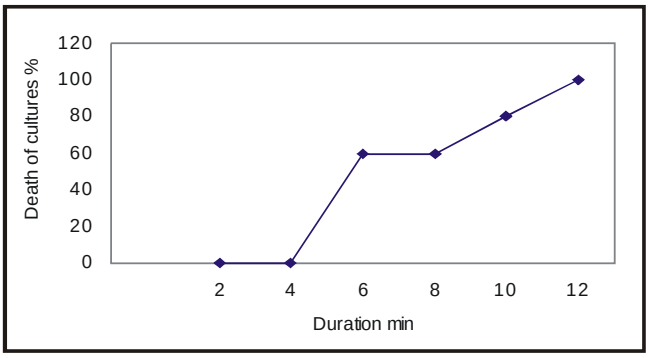


Fig. 1 Effect of 0.1% Merculic chloride on explnt establishment

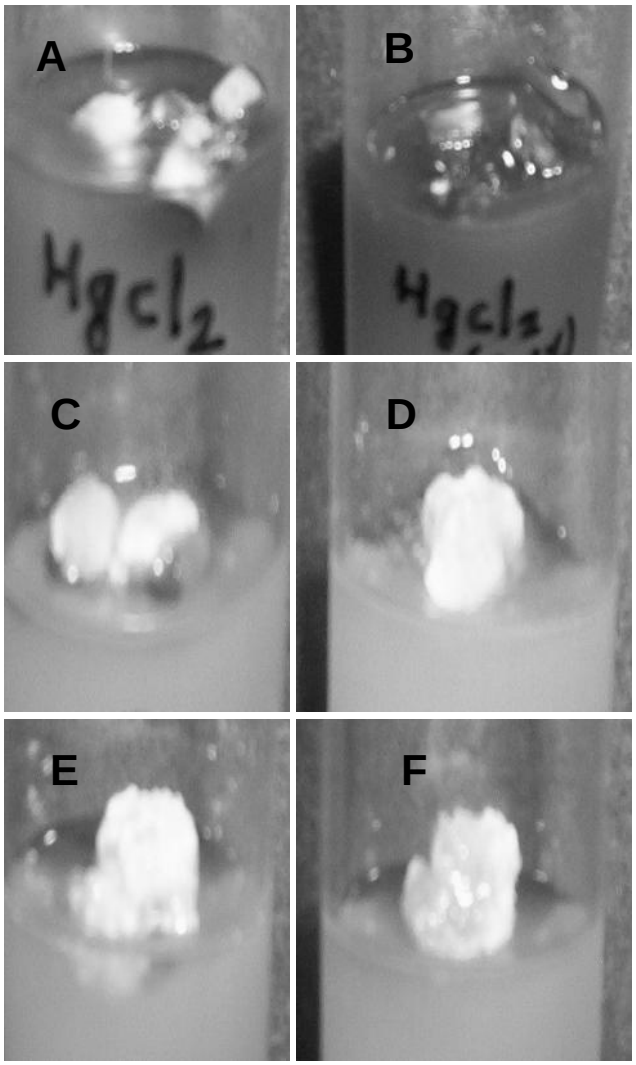
Table 1: Effect of different treatments on surface sterilization of nodal explants in Simarouba.

S. No.	Sterilant	Duration (min)	Contamination (%)	Death of culture (%)	Culture Establishment (%)	Browning intensity
1	$HgCl_2$(0.1%)	2	-	00	100	+
2	$HgCl_2$ (0.1%)	4	-	00	100	+
3	$HgCl_2$ (0.1%)	6	-	60	40	++
4	$HgCl_2$ (0.1%)	8	-	60	40	++
5	$HgCl_2$ (0.1%)	10	-	80	20	+++
6	$HgCl_2$ (0.1%)	12	-	100	00	++++
7	NaOCl (1%)	2	-	00	100	+
8	NaOCl (1%)	4	-	00	100	+
9	NaOCl (1%)	6	-	60	40	++
10	NaOCl (1%)	8	-	60	40	++
11	NaOCl (1%)	10	-	80	20	+++
12	NaOCl (1%)	12	-	80	20	+++

+ : Less ++ : medium +++ : High ++++ : Very high

Table 2 : Fresh and dry weight of callus.

S. No.	Concentration mg/L 2,4 D : BAP	Mean Weight of callus in gm	
		Fresh weight	Dry weight
1	0.5:0.5	0.109	0.015
2	1:1	0.226	0.020
3	1.5:1.5	0.230	0.021
4	2.0:2.0	0.297	0.023
5	2.5:2.5	0.361	0.031
6	3.0:3.0	0.464	0.032
7	4 : 4	0.418	0.031
8	5 : 5	0.468	0.034



A: Surface sterilization with 0.1% Mercuric chloride (2min
B: Surface sterilization with 0.1% Mercuric chloride (12mi
C: Callus Induction with 0.5:0.5 mg/L 2,4D:BAP
D: Callus Induction with 1.5:1.5 mg/L 2,4D:BAP
E: Callus Induction with 3:3 mg/L 2,4D:BAP
F: Callus Induction with 5:5 mg/L 2,4D:BAP

the explants (Yeoman and Evans,1967,Yeoman and Macleod, 1977).

Callus induction :

MS basal medium was supplemented with different concentrations of 2,4-D and BAP in combination to study the response of explants for their establishment. Observations were recorded on 25th day of inoculation and are presented in Table 2

It is observed that maximum callus induction and proliferation (0.464 and 0.468 gm. Callus) in Simarouba was found on MS + 3 mg/ L 2, 4-D : 3 mg /L BAP and on MS + 5 mg/ L 2, 4-D : 5 mg /L BAP on fresh weight basis (Table2 and Fig.2). The maximum dry weight of callus (0.034 gm) in medium MS + 5 mg/ L 2, 4-D : 5 mg /L BAP followed by (0.032 gm) in medium MS + 3 mg/ L 2, 4-D : 3 mg /L BAP and the minimum dry weight of callus (0.015 gm) observed on MS + 0.5 mg/ L 2, 4-D : 0.5 mg /L BAP on dry weight basis. The present results indicated increasing trend of callus induction and proliferation for

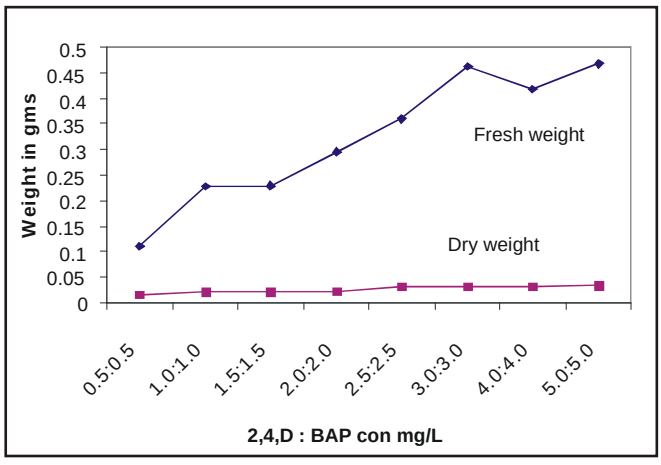


Fig.2 : Fresh weight and dry weight of callus

lower concentration to higher concentration of 2, 4-D and BAP on both fresh weight and dry weight basis. This trend is quite evident from the literature (Bhojwani and Razdan , 1996) and well established, also holds true for Simarouba. Since 2,4 D is well known for callus induction, increasing concentration has shown to induce more callus both on fresh and dry weight basis. The quality callus (embryogenic, whitesh green, friable) however, was observed in MS + 3.0 mg/L : 3.0 mg/L BAP and relatively hard and compact callus was observed in lower dozes of 2, 4-D and (white and soft).

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