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Standardization of callus induction in *Saraca indica* Auct, Non Linn

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ABSTRACT

Saraca indica auct, non Linn commonly called as "Ashoka" is a vulnerable species, becoming rarer in its natural habitat. Callus culture was initiated from leaf, stem and flower bud explants of Saraca indica using Murashige and Skoog (1962) basal medium containing 3% sucrose and 0.8% agar supplemented with different concentrations (2, 3, 4 and 5 mgL^{-1}) of 2,4-Dichlorophenoxy acetic acid (2,4-D). Leaf and stem explants produced 100 percent callusing in all the concentrations tried, whereas poor callusing response was observed with flower bud explant. Leaf explant was chosen for a detailed callus induction study with different concentrations and combinations of auxins 2,4-D and naphthalene acetic acid (NAA). Among the different treatments, leaf explant showed optimum callusing in cultures supplemented with 3 mgL^{-1} of 2,4-D alone. Treatments with NAA alone showed very poor callusing response. When combinations of 2,4-D (3 to 5 mgL^{-1}) and NAA (3 to 5 mgL^{-1}) were used, root formation was induced along with callusing. The fresh calli produced in all the treatments were white and hard which turned brown after two weeks.

Key words: *Saraca indica*, callus induction, 2,4-D, NAA, leaf explants

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INTRODUCTION

Saraca indica auct, non Linn., commonly called as Ashoka (Hindi), Asogam (Tamil), Kankeli (Sanskrit) is one of the most legendary and sacred trees of India. It is a medium sized evergreen tree belonging to leguminosae family. Its bark has high commercial value and is a major ingredient of the well-known Ayurvedic preparations "Ashokarishta" and "Ashokaghrita". The drug extracted from the bark is useful in treating menorrhagia, leucorrhoea, internal bleeding, haemorrhoids and haemorrhagic dysentery. The bark contains tannins (6%), catechol, haematoxylin, a ketosterol, a crystalline glycosidal constituent, a saponin ($C_{10}H_{21}O_{14}$), leucoanthocyanidins, an organic calcium compound ($C_6H_{10}O_5Ca$) and an organic iron compound [1, 2, 3]. The presence of a powerful oxytocic principle in the bark has also been reported [4]. Flowers possess antibacterial and anticancer activities [5, 6]. Continuous exploitation of the bark has threatened this species in its natural habitat. Hence application of biotechnological methods will be an alternative method for propagation of *Saraca indica*. In this study we report an efficient method for callus induction in *S. indica*.

EXPERIMENTAL SECTION

Explant preparation

Tender leaf bits, stem bits and flower buds were used as explants. The explants were washed with the liquid detergent Tween 20 (few drops per 100ml tap water) for 3 min and then rinsed in running tap water for 10 min. The cleaned explants were surface sterilized with aqueous 0.1% mercuric chloride solution for 4 min followed by 4 rinses in sterile distilled water. After trimming the cut ends, surface sterilized explants were inoculated on the culture medium.

Media and callus induction

Murashige and Skoog (1962) basal medium [7]containing 3% sucrose and 0.8% agar supplemented with different concentrations (1-5 mgL^{-1}) and combinations (1:1 and 2:1) of 2,4-Dichlorophenoxy acetic acid (2,4-D) and Naphthalene acetic acid (NAA) were prepared for callus induction. The pH of medium was adjusted to 5.8 before autoclaving at 120^{o}C for 20 min. The explants were inoculated in the medium and incubated in the dark at 25 ± 2^{o}C. Cultures were observed daily for finding out the number of days taken for callus initiation. The number of explants producing callus was recorded 45 days after inoculation. Fresh weights of callus were taken 60 days after inoculation. The calli were washed free of media and weighed after removing the excess moisture on the surface using a blotting paper. The relative growth rate of callus was calculated using the formula: (Final weight-Initial weight)/Number of days. Callus index was calculated by taking the product of relative growth rate and callusing percentage.

RESULTS AND DISCUSSION

Explant standardization

Callus induction was first studied using leaf, stem and flower bud explants cultured in MS basal medium supplemented with different concentrations (2-5 mgL^{-1}) of 2,4-D. Tender leaf explant (Figure 1) and stem explants showed 100% callusing in all treatments, whereas callusing was not effective with flower buds (Table 1). Leaf explants (Figure 2) and stem explants showed profuse callusing in MS medium supplemented with 3 and 4 mgL^{-1} 2,4-D (Table 1).

Table 1: Effect of 2,4-D on callus induction in leaf, stem and flower bud explants of *Saraca indica*

Explant	Concentration of 2,4-D (mgL^{-1})	Callusing %	Size of callus
Leaf	2	100	++
	3	100	+++
	4	100	+++
	5	100	++
Stem	2	100	++
	3	100	+++
	4	100	+++
	5	100	++
Flower bud	2	33.3	+
	3	33.3	+++
	4	16.7	+
	5	-	-

- No callus formation, + small size, ++ medium size, +++ profuse callusing

Figure 1: Tender leaf explant

Figure 2: Callus from leaf explant

Callus induction in leaf explants

Leaf explant was used for further studies of callus induction in cultures supplemented with different concentrations and combinations of 2,4-D and NAA. MS basal medium without any growth regulators was taken as control. This treatment did not induce any callus formation. Callus was successfully induced using 2,4-D (2 to 5 mg L^{-1}) alone whereas only a few explants produced callus when NAA (1 to 5 mg L^{-1}) alone was used. The fact that callus was induced by 2,4-D but not by NAA suggests that leaf explants of *Saraca indica* are auxin specific. This fact was previously observed in the callus induction from cotyledon, hypocotyl and root explants of *Medicago littoralis* [8] and also in the callus culture of cotyledon explants of *Parkia biglobosa* (Jacq.) Benth [9].

The callusing percentage was higher (100%) when 2,4- D (2 to 5 mgL^{-1}) alone was used compared to other treatments as shown in Table 2. The number of days taken for callus initiation was lower, ie.31.5, 32.7 and 31.8 days

in treatments supplemented with 2, 3 and 4 mgL^{-1} of 2,4-D respectively. Treatments containing 2,4-D and NAA in the ratio 2:1 showed the slowest response to callus initiation (33.8 to 39.3 days) compared to other treatments (Table 2). Combinations of 2,4-D and NAA (3,4 and 5 mgL^{-1}) in the ratio 1:1 produced callusing along with some amount of root formation.

The fresh calli produced in all the treatments were white and hard which turned brown after two weeks. The highest fresh weight of callus, relative growth rate and callus index was observed in cultures containing 3 mgL^{-1} of 2,4-D. Statistical analysis (ANOVA) of callus index shows that all the treatments shown in Table 2 are significantly different with respect to control at $P < 0.05$. Though the callus index of treatments containing 3, 4 mgL^{-1} of 2,4-D alone and those containing 3 and 4 mgL^{-1} of 2,4-D and NAA (1:1) did not vary significantly among themselves, the highest value is observed in treatment with 3 mgL^{-1} of 2,4-D (Table 2). The relative growth rate was also highest (13.14) in this treatment. Hence, the treatment containing 3mgL^{-1} 2,4-D may be considered as the best one for callus.

Table 2: Effect of different concentrations and combinations of 2,4-D and NAA on callus induction in leaf explant of *Saraca indica*

Conc. of Growth regulators		Callusing % **	Days for callus initiation *	Callus fresh weight mg *	Relative growth rate mg/day *	Callus index ***
2,4-D (mgL^{-1})	NAA (mgL^{-1})					
2,4-D alone						
1	-	66.7	36.0 ± 3.6	177 ± 26	7.38±0.46	492^{cd}
2	-	100.0	31.5 ± 0.5	302 ± 35	10.59±1.24	1059^{b}
3	-	100.0	32.7± 1.0	359 ± 50	13.14±1.72	1314^{a}
4	-	100.0	31.8 ± 0.4	339±43	12.03±1.42	1203^{ab}
5	-	100.0	33.7 ± 2.0	264±29	10.05±1.05	1005^{b}
2,4-D and NAA in the ratio 1:1						
2	2	83.3	33.2 ± 1.5	135±36	4.96±1.40	413^{d}
3	3	100.0	33.3 ± 2.1	336±76	12.47±2.01	1247^{ab}
4	4	85.7	34.0 ± 1.8	334±28	12.82±0.48	1099^{ab}
5	5	100.0	34.3 ± 1.9	161±23	6.48±1.02	648^{c}
2,4-D and NAA in the ratio 2:1						
1	0.5	50.0	37.0 ± 3.6	127±39	5.4±0.86	270^{e}
2	1	100.0	39.3 ± 5.1	104±54	4.92±1.69	492^{cd}
3	1.5	83.3	33.8 ± 1.9	305±48	11.64±1.41	970^{b}
4	2	83.3	34.7 ± 1.9	261±51	10.24±1.50	853^{bc}
5	2.5	75.0	36.2 ± 0.8	147±47	6.15±1.86	461^{cd}

** Mean ±Standard deviation for n = 6; ** Mean values for n = 6 to 20; ****
Means within column followed by the same letter do not differ significantly at P < 0.05

CONCLUSION

In conclusion, the present study offers a viable protocol for callus induction of *Saraca indica* using leaf explant. This could be exploited further for regeneration of plantlets for micropropagation studies as well as for secondary metabolite production. The callus produced will be a potential source of phenolic compounds, which is the major active principle in this medicinal tree. Also suspension culture of the cells will act as a biochemical factory for the production of the active principles.

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