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In vitro propagation of guayule (*Parthenium argentatum*) – a rubber yielding shrub

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ABSTRACT

Nodal explants (0.5 to 0.8 cm long) isolated from 2-year old shrubs of guayule, Parthenium argentatum Gray, when cultured on MS medium supplemented with different concentrations of KN, BAP, 2,4-D, 2,4-D + BAP, NAA and NAA + BAP produced callus tissues and shoots simultaneously with varying frequencies. Shoots were regenerated with a high frequency (80-88%) from callus on MS medium containing NAA + BAP with or without glutamine. Addition of glutamine to these media improved considerably the number of shoots formed from a known amount of callus. Shoots could be regenerated from 200 day old callus cultures with a very high frequency but the organogenetic capacity declined thereafter. Increase in the concentration of sucrose (upto 4%) significantly enhanced the shoot forming ability of callus, but higher concentrations (6%) suppressed it. Rooting was induced only in dark when IAA, IBA and NAA were used, but 2,4-D could induce them both in light and dark. The system is suitable for the mass propagation of this important rubber yielding plant.

ABBREVIATIONS

MS, Murashige and Skoog (1962); IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; NAA, α-Naphthaleneacetic acid; 2,4-D, 2,4-Dichlorophenoxy-acetic acid; KN, Kinetin; BAP, 6-Benzylaminopurine.

INTRODUCTION

Parthenium argentatum Gray (Asteraceae), commonly known as guayule, is an important rubber producing shrub. The plant is generally grown under semi-arid or arid regions for the production of natural rubber. Plants in the field may be established from nursery grown seedlings and cuttings (Nishimura et al. 1944). One major limitation for the commercial production of guayule rubber is its low rubber yields and inconsistent field establishment by direct seeding. This is because of susceptibility of seedlings to water and salinity stresses (Tipton, 1988). Therefore, there is a need to generate variation in guayule which can resist water and salt stresses. Efficient methods of plant regeneration from tissue cultures are necessary for the selection and/or the creation of useful variants. Subramanian and Subba Rao (1980) and Zavala et al. (1980) reported Parthenium callus cultures that develop roots and shoots. The present paper describes a method for the mass propagation of guayule by in vitro organ and callus cultures.

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MATERIALS AND METHODS

Nodal explants (from 3rd to 12th node below the shoot apex) measuring 0.5 to 0.8 cm were collected from 2-year old shrubs of guayule (Parthenium argentatum Gray), washed under running tap water and then surface sterilized with 0.1% mercuric chloride for 6-8 minutes. The explants were washed with sterile distilled water thoroughly and inoculated (one into each test tube containing 15 ml of agar medium) onto MS medium containing 2% sucrose and various concentrations and combinations of hormones (Table 1). The pH of the medium was adjusted to 5.8 before solidifying with 0.8% agar. Callus tissues initiated from the nodal explants on MS media containing 0.5 mg/l KN or 0.5 mg/l BAP or 0.5 mg/l 2,4-D or 1 mg/l 2,4-D + 2 mg/l BAP were subsequently transferred and maintained on 0.2 mg/l NAA + 0.2 mg/l IAA + 1 mg/l BAP. Callus was subcultured every 25-30 days. The cultures were maintained at 25 ± 2°C with a photoperiod of 16 hours light (2000 lux, fluorescent light). For generation of shoots, approximately 250 ± 20 mg of callus was used. Explants, callus and regenerated shoots for rooting were scored at the end of the fourth week. For rooting of the in vitro derived shoots, half the MS salt concentration but with full strength of iron (Fe EDTA) was used with varying concentrations of auxins either in light (2000 lux) or in dark. All experiments were repeated at least once.

RESULTS AND DISCUSSION

The influence of different hormones on the morphogenetic response of nodal explants of guayule is given in Table 1. Increasing concentrations of KN and BAP (0.5 to 2 mg/l) decreased the frequency of callus initiation but increased the formation of multiple shoots directly from the nodal explants. Still higher concentrations (3 and 5 mg/l) suppressed callus formation. BAP was found to be better compared to KN for the production of multiple shoots. 2,4-D or NAA alone, or 2,4-D and BAP together produced only callus but no organogenesis. NAA (0.5 mg/l) and BAP (3 mg/l) combination however, showed the highest (72%) frequency of response (Table 1) from nodal explants.

Table 1. Morphogenetic response of nodal explants of guayule to different hormonal treatments

Hormonal conc. (mg/l)	No. of explants inoculated	Percentage of explants giving		No. of shoots formed per explant
		Callus	Shoots	
No hormone	20	-	-	-
0.5 KN	15	75	-	-
1.0 KN	18	35	10 (elongated)*	1-2
2.0 KN	15	5	25 (elongatged)	1-2
3.0 KN	20	-	30 (dwarf)**	2-3
5.0 KN	22	-	41 (dwarf)	2-4
0.5 BAP	24	90	-	-
1.0 BAP	25	30	20 (elongated)	1-2
2.0 BAP	30	5	48 (elongated)	2-4
3.0 BAP	26	-	49 (dwarf)	5-8
5.0 BAP	29	-	59 (dwarf)	8-12
0.5 2,4-D	18	60	-	-
1.0 2,4-D	20	70	-	-
1.0 2,4-D + 0.5 BAP	22	32	-	-
1.0 2,4-D + 1 BAP	28	44	-	-
1.0 2,4-D + 2 BAP	31	50	-	-
2.0 2,4-D + 3 BAP	32	56	-	-
0.5 NAA	20	92	-	-
1.0 NAA	26	90	-	-
0.5 NAA + 0.5 BAP	20	50	-	-
0.5 NAA + 1 BAP	18	46	50 (elongated)	3-6
0.5 NAA + 2 BAP	30	46	56 (elongated)	8-12
0.5 NAA + 3 BAP	35	65	72 (dwarf)	14-18

* 2 to 4 cm in length; ** 0.5 to 1 cm in length

Table 2. Influence of glutamine and sucrose on organogenetic response of node derived callus of guayule

MS Medium with hormones (mg/l)	Sucrose conc. (g/l)	Age of callus (days)	No. of calli cultured	% Frequency of calli showing shoot regeneration	No. of shoots formed per callus mass (250 ± 20 mg)
0.1 BAP	20	50	18	49	3-4
1.0 BAP	20	50	20	58	6-8
0.1 NAA + 2.5 BAP	20	50	18	67	5-8
0.5 NAA + 3 BAP	20	50	18	70	8-11
0.1 NAA + 2.5 BAP + 200 glutamine	20	50	20	88	13-15
0.1 NAA + 2.5 BAP + 200 glutamine	20	100	25	84	22-25
0.1 NAA + 2.5 BAP + 200 glutamine	20	200	23	85	22-25
0.1 NAA + 2.5 BAP + 200 glutamine	20	250	20	30	10-15
0.02 NAA + 2.5 BAP	20	50	18	75	9-12
0.02 NAA + 2.5 BAP + 200 glutamine	20	50	18	75	18-20
0.1 NAA + 2.5 BAP	5	60	16	-	-
0.1 NAA + 2.5 BAP	10	60	20	32	3-4
0.1 NAA + 2.5 BAP	20	60	20	65	5-8
0.1 NAA + 2.5 BAP	30	60	18	80	12-15
0.1 NAA + 2.5 BAP	40	60	19	85	12-14
0.1 NAA + 2.5 BAP	60	60	25	24	3-4

The effect of sucrose, BAP, NAA + BAP, NAA + BAP + glutamine on organogenetic response of node derived callus of guayule is shown in Table 2.

BAP at the concentrations used (0.1 and 1 mg/ml) exhibited 49-58% frequency of shoot regeneration. The frequency of response increased considerably (70%) when a combination of NAA (0.5 mg/l) and BAP (3 mg/l) was used. Addition of 200 mg/l glutamine greatly improved the number of shoots formed per callus piece. The callus has retained the capacity to differentiate shoots continuously for over 200 days through 8 passages. Each callus mass (250 ± 20 mg) produced 13-15 shoots with a mean height of 0.5 cm in four weeks. The number of regenerants almost doubled (22-25) during the 2nd and subsequent passages on the same medium. However, the regenerating ability of the callus declined considerably by 250 days (10 passages). The callus grown on MS medium containing 2 mg/l 2,4-D + 0.4 mg/l KN was friable, watery, brownish and found to be recalcitrant. On the other hand, callus grown on 0.2 mg/l IAA + 0.2 mg/l NAA + 1 mg/l BAP was creamy white, compact and nodular with green patches. This callus showed a very strong morphogenetic ability for over 200 days in culture. Replacement of NAA with IAA did not suppress the frequency

of response, but incorporation of glutamine doubled the number of shoots formed per callus mass. Sucrose at 0.5% level failed to evoke any organogenetic response, but higher concentrations (upto 4% level) increased the frequency of response. However, 6% sucrose drastically reduced the shoot forming potentiality of these cultures (Table 2).

Shoots obtained via direct regeneration from nodal axillary buds or adventitous buds formed on callus were transferred to MS agar media (half strength except Fe EDTA) fortified with different concentrations of auxins (Table 3). IAA, IBA and NAA when added did not induce roots in light. Increasing concentrations of IAA enhanced the frequency of root formation in shoots when incubated in dark. IBA produced roots only at 0.06 mg/l level but not at other concentrations. While lower concentrations of NAA (0.02 to 0.06 mg/l) failed to initiate roots, higher concentrations were very effective. On the other hand, 2,4-D produced roots both in light as well as in dark, the frequency response being higher in light. Lower concentrations of 2,4-D (0.04 to 0.06 mg/l) seemed to be better compared to higher concentrations for rooting of shoots. Few cultures also showed callusing at the base of the shoots (Table 3).

Table 3. Response of excised shoots to rooting treatments on agar medium*

Treatment (mg/l)	% of cultures rooting at cut end		No. of roots per culture	No. of cultures callusing at shoot base
	Light	Dark		
0.02 IAA	-	-	-	-
0.04 IAA	-	50	1.0	-
0.06 IAA	-	15	1.0	-
0.08 IAA	-	65	2.5	-
0.1 IAA	-	70	2.5	-
0.5 IAA	-	75	3.0	10
0.02 IBA	-	-	-	-
0.04 IBA	-	-	-	-
0.06 IBA	-	41	2.5	-
0.08 IBA	-	-	-	-
0.1 IBA	-	-	-	-
0.5 IBA	-	-	-	-
0.02 NAA	-	-	-	-
0.04 NAA	-	-	-	-
0.06 NAA	-	-	-	-
0.08 NAA	-	70	3.5	-
0.1 NAA	-	72	5.5	-
0.5 NAA	-	74	6.5	15
0.02 2,4-D	35	38	3.5	-
0.04 2,4-D	70	45	4.5	-
0.06 2,4-D	72	50	8.5	-
0.08 2,4-D	60	50	9.0	10
0.1 2,4-D	55	42	8.0	20
0.5 2,4-D	48	30	4.0	35

* Data collected from 10 cultures per treatment

Arreguin and Bonner (1950) established Parthenium cultures from stems. Zavala et al. (1980) obtained axenic cultures from seedling explants or its cotyledons or leaf blades, petioles and roots (Wickham et al. 1980). Wickham et al. (1980) reported maximum callus formation and growth on MS medium supplemented with 0.5 mg/l 2,4-D and 1 mg/l BAP for Parthenium hysterophorus. On the other hand, Subramanian and Subba Rao (1980) noticed good growth of the callus on MS medium containing 0.18 mg/l IAA and 0.02 mg/l KN for the same species. Parthenium argentatum callus cultures exhibited superior growth with a combination of kinetin (1 mg/l), NAA (0.2 mg/l) and inositol (2 mg/l) (Zavala et al. 1980). But, enhanced callus growth has been achieved in the present study on MS medium containing 0.2 mg/l IAA, 0.2 mg/l NAA and 1 mg/l BAP (data not shown).

While lower concentrations of KN, BAP (0.5 mg/l), 2,4-D (1 mg/l) and NAA (0.5 mg/l) seemed to be good for callus initiation, higher concentrations (except 2,4-D or 2,4-D + BAP) favoured root formation from nodal explants (Table 1).

Subramanian and Subba Rao (1980) reported shoot buds on stem callus tissues of P. hysterophorus on a medium incorporated with IAA (1.75 mg/l) plus either KN (1.01 mg/l) or BAP (1.13 mg/l). Shoot formation was achieved from root or hypocotyl derived callus tissues of P. argentatum on Mahlberg's medium fortified with KN (0.1 mg/l), 2,4-D (0.2 mg/l), NAA (0.2 mg/l) and 2 mg/l inositol (Zavala et al. 1980). Dastoor et al. (1981) obtained shoots from callus cultures grown on MS medium containing 0.1 mg/l KN and 10 mg/l 2(3,4-dichlorophenoxy) triethylamine derivative. Staba and Nygaard (1983) reported shoot differentiation on MS medium supplemented with 0.1 mg/l BAP. Increasing concentrations of BAP enhanced the percent frequency of shoot regeneration in the present study. Addition of glutamine (200 mg/l) not only resulted in the highest frequency (84-88%) of shoot formation but also the number of shoots formed per callus mass greatly improved. Carbohydrate concentration in the medium also influenced the differentiation of shoots and 4% sucrose was found to be optimum (85% frequency).

Zavala et al. (1980) reported the formation of roots from callus cultures on a solid medium containing inositol (1-2 mg/l) or inositol and casein hydrolysate (1 mg/l), and different concentrations of 2,4-D, KN or NAA. Differentiation of roots has been shown also from callus tissues grown on MS medium containing 0.1 mg/l each of 2,4-D, BAP and kinetin or 2,4-D and 6-dimethylaminopurine (Wickham et al. 1980). Staba and Nygaard (1983) reported roots from shoots grown in liquid RT medium with 0.05 mg/l BAP. Contrary to the above reports, present studies showed that MS static medium containing 0.5 mg/l IAA or NAA was the best for root initiation in shoots grown in dark.

About 100 regenerated plants were transferred to pots with 60-70% success rate. Morphological variations have been observed among the regenerants (data not shown). This report, thus demonstrates successful regeneration of whole plants with high frequency and their subsequent transfer to soil.

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