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CLONAL PROPAGATION OF *ALTERNANTHERA SESSILIS* -A BIOPHARMA CEUTICALLY POTENT HERBAL MEDICINAL PLANT

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The nodal explants of *Alternanthera sessilis* were grown *in vitro* in Murashige and Skoog medium supplemented with BAP (0.5-2.0 mg/l), 2, 4-D (0.5-5 mg/l), Kn (0.25-3 mg/l), NAA (0.25-1.5 mg/l) and IAA (0.25-3 mg/l). The combined effect of Kn (0.8 mg/l) and IAA (0.4 mg/l) induced higher frequency of shoot formation from each explant within 8 weeks. Repeated subculturing of the plantlets at 3 weeks of intervals for over a year enabled mass multiplication of shoots without any evidence of decline. The supplementation of 2, 4-D (4 mg/l) alone showed the highest rate of callus induction. Optimum rooting was recorded when the excised shoots were transferred to the medium supplemented with IAA (0.25 mg/l) and NAA (0.25 mg/ l). IAA (3mg/l) alone also induced rooting from shoots and callus. The rooted plantlets were subsequentely transferred to soil mixture for hardening.

Keywords : *Alternanthera sessilis* ; Micropropagation.

Introduction

Tissue culture techniques are being increasingly exploited for clonal multiplication and *in vitro* conservation of valuable indigeneous germplasm threatened with extinction[1]. Micropropagation offers a great potential for large scale multiplication of medicinals and other economic crops. *Alternanthera sessilis* belongs to the family Amaranthaceae occuring in temperate, tropical and sub-tropical regions in both hemispheres. *Alternanthera sessilis* are good suppliers of iron, proteins, vitamin and minerals[2]. These plants are also recommended by physicians in constipations, piles, colies, diabetes, tuberculosis, anaemia, blurred vision, in aedema adeuretic in heart and kidney ailments along with vitamine-c. The seeds are very good emitic and are also given in hydrophobia, used antidote in snake bite and scorpian sting[3]. The present work has been designed to study the clonal multiplication of *A. Sessilis* from nodal explants along with the callus induction for biochemical studies.

Material and Methods

The plants are found in wild state growing here and there in Assam throughout the year. The freshly collected explants were washed with 1% (v/v) tween-20 for 1 minutes, surface sterilised with 0.1% $HgCl_2$(w/v) for 5 minutes. The disinfactant was removed by several successive washes with sterile distilled water. About 0.5-0.8 cm nodal explants were inoculated on Murashige and Skoog[4] basal medium containing 3% sucrose(w/v), 0.8% agar and various concentration of BAP (0.5-2.5 mg/l), 2, 4-D(0.5-5mg/l), Kn(0.25-5mg/ l), NAA (0.25-1.5 mg/l) and IAA (0.25-3mg/ l) as plant growth regulators.

The pH of the medium was adjusted to 5.8 with IN sodium hydroxide or 1N hydrochloric acid before the addition of agar powder, autoclaved at 151b pressure for 15 minutes. Cultures were incubated under 16/8 hour light and darkness with temperature 25± 2°C.

Results and Discussion

After 4 weeks of culture the branched axillary shoots proliferated upon the nodal explants were separated for further multiplication (Fig.1). These explants were cultured in Murashige and Skoog medium supplementing various concentration of BAP (0.5-2.0 mg/1), 2,4-D(0.5-5 mg/1), Kn (0.25-3 mg/1), NAA (0.25-1.5 mg/1) and IAA (0.25-3 mg/1).

Table 1 : Combined response of different concentration of NAA, IAA and BAP with Kn in shoot multiplication of *Alternanthera sessilis* (mean value of three replication).

MS basal supplemented with Growth regulators. (mg/l)				Shoot regeneration (%)	Shooting/explant	Root induction (%)
Kn	BAP	IAA	NAA			
0.25	0.5	-	-	35.0	2	0
0.5	1.0	-	-	56.0	3	0
1.0	1.5	-	-	28.0	6	0
1.5	2.0	-	-	20.0	4	0
0.8	-	0.4	-	68.8	7	30
0.45	-	0.5	-	20.0	2	40
0.9	-	0.45	-	40.0	4	30
2.0	-	1.0	-	51.0	3	10
3.0	-	2.0	-	35.0	3	18
0.5	-	0.5	-	10.0	3	20
0.5	-	1.0	-	22.0	4	20
0.5	-	1.5	-	10.0	1	12
0.5	-	2.0	-	10.0	1	12
-	-	2.0	-	10.0	1	20
-	-	3.0	-	20.0	2	50
-	-	0.25	0.25	10.0	2	60
0.5	-	-	0.5	28.4	3	10
0.5	-	-	1.0	32.0	3	12
0.5	-	-	1.5	14.9	4	20
0.5	-	-	2.0	14.2	3	40

Table 2 : Response of 2,4-D, IAA and Kn on callus induction and its growth :

MS basal + Growth regulators (mg/l)	Callus induction%	Fresh Weight (gm)	Dry weight (gm)
2,4 - D (0.5)	40	0.980	0.590
2,4 - D (1.0)	20	1.260	0.890
2,4 - D (1.5)	30	1.000	0.686
2,4 - D (2.0)	30	1.360	0.990
2,4 - D (2.5)	25	0.900	0.580
2,4 - D (3.0)	40	1.400	1.050
2,4 - D (3.5)	65	1.200	0.890
2,4 - D (4.0)	80	4.500	1.660
2,4 - D (4.5)	50	3.700	1.900
2,4 - D (5.0)	20	0.975	0.620
Kn (0.5) + IAA (0.25)	30	0.780	0.490
Kn (1.0) + IAA (0.5)	25	0.700	0.425
Kn (1.5) + IAA (1.0)	20	0.869	0.602
BAP (1.0) + IAA (0.5)	40	3.360	2.10
BAP (1.5) + IAA (1.0)	45	1.270	0.850
BAP (2.0) + IAA (1.5)	25	0.472	0.156

Fig. 1. Regeneration of shoot after 4 weeks of culture.

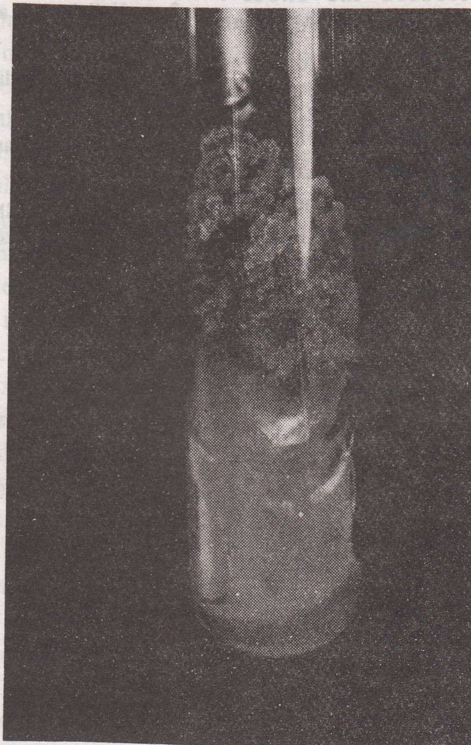

Fig. 2. Callus induced in MS basal supplemented with 2,4-D (4 mg/l).

Subsequently, mass propagation of shoot was achieved by repeated subculture in the media supplemented with Kn (0.8 mg/l) and IAA (0.4 mg/l) exhibiting mean 7 shoot per explant within 8 weeks of culture (Table 1). In the combined effect of BAP (1.5 mg/l) and Kn (1.0 mg/l), the shoot number increased to near per explant 6 (Table 1) while BAP along with IAA shows callusing (Table 2). BAP (1.5 mg/l) in presence of IAA (1.0 mg/l) was found to be the best combination for callus induction (45%) (Table 2). Increase in the concentration of 2, 4-D (0.5-5 mg/l) alone enhanced the growth of callus. 2,4-D (4 mg/l) was found to be the optimum concentration for highest percent (80%) induction (Table 2, Fig. 2). The growth of the callus in term of weight was recorded to be the highest (4.50gm) at 2, 4-D (4 mg/l, Table 2). Increase in the concentration of BAP (0.5-2.0 mg/l) in the medium decreased the shoot multiplication, while it enhanced the callus induction (Table 1).

Rooting was obtained in MS basal containing 100 mg/l meso-inositol, 3% (w/v) sucrose and varied concentration of IAA and NAA. Addition of IAA or IBA was generally proved beneficial for rooting[5]. Optimum rooting was recorded when the excised shoots were transferred to the medium supplemented with IAA (0.25 mg/l) along with NAA (0.25 mg/l) (Table 1). IAA (3 mg/l) alone was also found to be able to induce rooting (50%) (Table 1). After 8 weeks, the rooted plantlets were removed from the culture tubes, repeatedly washed the roots with distilled water and subsequently transferred to the potting mixture for hardening.

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