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In Vitro Propagation of *Alternanthera sessilis L.* from Internode Explant

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Authors' contributions

This work was carried out in collaboration by both authors. Author DD designed and performed the study under the supervision of author PKB. Author DD worked out the statistical analysis, searched previous literature, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

An efficient protocol was developed for micropropagation of *Alternanthera sessilis* L. from internode explant. Callus was developed from the cut ends and surface of the internode explants when the Murashige and Skoog (MS) medium was supplemented with 0.5mg/L or 1.0mg/L 2,4-D. The greatest mean number of shoots (124) with the highest mean shoot length (9.3cm) was obtained when calli were cultured on the MS medium with 1.0mg/L each of 6-Benzylaminopurine and adenine sulfate. Early and maximum root induction was noted when shoots were cultured in half strength MS basal medium. *In vitro* regenerated plants were successfully hardened in a mixture of soil and cow dung in ratio of 3:1 and transferred to field. Cent percent plants survived in field condition, morphologically identical to parent plant and showed normal flowering.

Keywords: Alternanthera sessilis; medicinal plant; micropropagation; internode; callus; shoot bud induction.

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1. INTRODUCTION

Highly valuable medicinal plant *Alternanthera sessilis* L. belongs to the family-Amaranthaceae. The plant is considered as weed of rice in tropical regions and of other crops like sugarcane and bananas. Although it is a weed, it has medicinal properties. In Ayurvedic medicine *A. sessilis* is known as Matyakshika [1]. It is used as a remedy against intestinal disorders like Diarrhoea, dysentery and cramps. A decoction of the leaves is consumed to treat itchy and overheated skin [2]. In Ghana, a decoction with salt is taken to stop blood vomiting, a paste is used to draw out spines or any other object from the body and it is also used to cure hernia. In Senegal and India, the leafy twigs are grounded to a powder and applied on snakebites [3]. In Nepal, its root is used for the treatment of stomachache [4].The plant enhances the secretion of milk in new mothers and also used against intestinal inflammation, externally to treat wounds, hepatitis, tight chest, bronchitis, asthma, lung troubles, to stop bleeding and as a hair tonic [5]. Young shoots and leaves are eaten as vegetable in South-East Asia [6]. Leaf shows very high content of iron, vitamin A and dietary fiber. The plant contains protein and soups made with the leaves are given to anemic patients in rural areas. The plant is rich source of carotene, therefore it is used for curing night blindness. Leaf paste of *A. sessilis* inhibits the formation of the potent environmental carcinogen nitrosodiethanolamine from its precursors such as triethanolamine. The aqueous alcoholic extract of the entire plant exhibits hypothermic and histaminergic activities and relaxes smooth muscles. An ether extract of *A. sessilis* yields an active principle having anti-ulcerative property [7].

Earlier the plant has been found abundantly in different localities of Assam. But now-a-days due to various human activities the plant is disappearing from its habitats. Considering its depleting population from this region an attempt has been made for *in vitro* cultivation of this medicinal plant for large scale multiplication as well as conservation. The *in vitro* propagation is an alternative tool for large scale multiplication that increases the number of propagules for cultivation as well as aid the replacement of natural populations [8,9,10]. The present study is an attempt to develop a fast economically viable complete micropropagation protocol from internode explant of *A. sessilis*. However, few literatures are found available pertaining to the micropropagation of *A. sessilis* from shoot tip and nodal explants reported by Gnanaraj *et al*. [3], leaf explant reported by Singh *et al*. [11]. In the present study, using different growth regulators and their combinations shoot bud induction rate per explant has been tried to improve than the earlier works reported by different tissue culturists.

2. MATERIALS AND METHODS

2.1 Source of Explant

Plants of *A. sessilis* were collected from Khalihamari, Dibrugarh, identified and planted in Medicinal Plant Garden of Life Sciences Department, Dibrugarh University. Prior to surface sterilization shoots of *A. sessilis* were collected from the aforesaid garden of Life Sciences Department, Dibrugarh University.

2.2 Surface Sterilization of Explants

Internode portions (approx. 1cm) of collected shoots were isolated and washed thoroughly in running tap water for half an hour and then treated with 10% tween-20 for 5 min followed by seven times distilled water wash. Explants were surface sterilized in 0.1% $HgCl_2$ for 7 min in

front of laminar air flow and finally washed for five times (five minutes each) with sterile distilled water.

2.3 Callus Culture and Shoot Bud Induction

Murashige and Skoog (MS) medium [12] was used for *in vitro* culture of *A. sessilis*. Medium pH was maintained at 5.7 and sucrose and agar were used at concentrations 30 gm/L and 8 gm/L respectively. Culture medium was sterilized in autoclave at 121ºC and 15 psi pressure for 15 min. For callus induction MS medium was supplemented with 2,4-D at concentrations 0.5 mg/L and 1.0 mg/L. After callus induction, calli were taken out aseptically, cut into small sections (approx. 1cm in diameter) and inoculated onto MS medium supplemented with BAP (0.5mg/L, 1.0mg/L and 1.5mg/L), Adenine sulfate (Ads) (0.5mg/L, 1.0mg/L and 1.5mg/L), TDZ (Sigma-Aldrich) (0.5mg/L, 1.0mg/L and 1.5mg/L), BAP + Ads (0.5mg/L+0.5mg/L, 1.0mg/L+1.0mg/L and 1.5mg/L+1.5mg/L) and BAP + KIN (0.5mg/L+0.5mg/L, 1.0mg/L+1.0mg/L and 1.5mg/L+1.5mg/L).

2.4 Rooting of Shoots

For root induction shoots (9-10cm) were isolated from callus and cultured in half strength MS basal medium, MS medium supplemented with 0.5 mg/L NAA and MS medium supplemented with 0.2 gm/L Activated Charcoal (AC).

2.5 Hardening and *in Vivo* Transfer of *in Vitro* Grown Plants

Sufficiently rooted plantlets were removed from medium and root portions were carefully washed with distilled water to remove the media adhering to roots. For hardening *in vitro* regenerated plantlets were first cultured in distilled water for one week in incubation room without plugging the cultured test tube. After one week plants were transplanted in earthen pots containing soil and cow dung in ratio 3:1 and maintained in agro net house for one week and finally transferred to field condition. *In vitro* raised plants were irrigated regularly with 10ml of tap water.

2.6 Culture Condition

All the cultures were incubated at 25ºC temperature, 65-70% humidity and under white light at intensity of 2000 lux provided from white fluorescent lamps with 16 hrs. photoperiodic duration.

In the present study ten replicas were prepared for each treatment and each treatment was repeated for thrice. Only Hi media grade chemicals were used in the study and to make the culture process economically viable 3% commercial sugar was tested as substitute for 3% sucrose (Hi media grade).

3. RESULTS AND DISCUSSION

Internode explant was found suitable for large scale propagation of *A. sessilis*. MS medium supplemented with 0.5 mg/L and 1.0 mg/L 2,4-D could be used for callus induction. In presence of 2,4-D in culture medium all the treated explants showed callogenesis within one week. However, earlier it has been reported that MS medium supplemented with 1 mg/L BAP and 1mg/L 2,4-D is the most suitable medium formulation for callus induction from leaf

explants of *A. sessilis* [11]. Internode derived callus of *A. sessilis* was found colourless compact and non-embryogenic in nature (Fig. 1b). *De novo* shoot bud regeneration was observed in all the combinations of plant growth regulators evaluated, but there was variation in the number of induction of shoot buds from the callus. Callus showed green segment development from 6 days to 18 days when transferred to shooting medium. Green pigment production in non-embryogenic callus is required for the growth and formation of shoots and roots in Oat [13]. Plant regeneration is characterized by the development of green sectors distributed discretely throughout the callus. This leads to shoot differentiation independent of root development. In the present study it was noted that the most suitable combination of growth regulator for d*e novo* shoot bud differentiation was BAP (1.0mg/L) in combination with Ads (1.0mg/L). An average of 124.47 shoot bud differentiation was recorded from the treatment of 1mg/L BAP in combination with 1mg/L Ads (Fig.1e). However, MS medium enriched with 1mg/L each of IAA and BAP can trigger differentiation of 10 shoots per stem explant of *A. sessilis* [11] and MS medium enriched with 2mg/L BAP and 1.5mg/L BAP can trigger differentiation of 23.4 shoots per shoot tip and 15.2 shoots per nodal explant of *A. sessilis* respectively [3]. Thus, the rate of maximum shoot differentiation (124.47) recorded in the present study is significantly higher than the aforesaid earlier works. It was noted that increased concentrations of BAP and Ads (1.5 mg/L+ 1.5mg/L) reduced the number of shoot bud induction and shoot length in *A. sessilis* as shown in Table 1. Combination of BAP and KIN was also found to induce significant number of shoot buds from internode derived calli as compared to rest of the medium formulations tested in the study. An average of 50.43 and 52.83 shoot buds differentiation was recorded at concentrations BAP 0.5mg/L + KIN 0.5mg/L and BAP 1.0mg/L + KIN 1.0mg/L respectively. BAP at concentration 2.0mg/L and 1.5mg/L could trigger highest frequency of shoot proliferation (94.3 ± 0.43) and (90.4 ± 0.82) from shoot tip and nodal explant of *A. sessilis* [3] respectively while maximum number of shoot bud have developed from leaf derived callus of *A. sessilis* cultured on half strength MS medium supplemented with 1 mg/L IAA and 1 mg/L BAP [11]. Similar observation has been made in case of *A. versicolor* [14]. In *A. versicolor* highest proliferation of shoot bud from leaf and internode explant has been obtained when cultured on MS medium supplemented with 1mg/L IAA and 1mg/L BAP. High cytokinin:auxin ratio favours shoot regeneration in Amaranthus [15]. It was found that combination of BAP and Ads also positively influenced shoot length (Table 1). Calli cultured onto MS medium supplemented with BAP and Ads also showed early response for shoot bud differentiation as compared to other medium formulations evaluated in the present study.

In the present study three experiments were set for root induction, of which shoots cultured onto half strength MS basal medium showed early and maximum root induction (Table 2). Thus, the present study for the first time has established that *in vitro* root induction from shoot of *A. sessilis* can be achieved in hormone free half strength MS medium. Root induction was recorded on second day of incubation of shoots on half strength MS basal medium. Root length of shoots cultured onto half strength MS basal medium was found maximum (7.49cm) as compared to other treatments. Less number of root induction was noted on MS medium supplemented with NAA. While in case of *Amaranthus paniculatus* hypocotyl segments formed roots on B5 medium supplemented with NAA [16]. Shoots of *A. sessilis* cultured on half strength MS medium supplemented with 3 mg/L IBA have been found to induce highest number of roots [3]. While optimal rooting of shoots on half strength MS medium supplemented with 1mg/L IBA has been reported in *A. sessilis* [11].

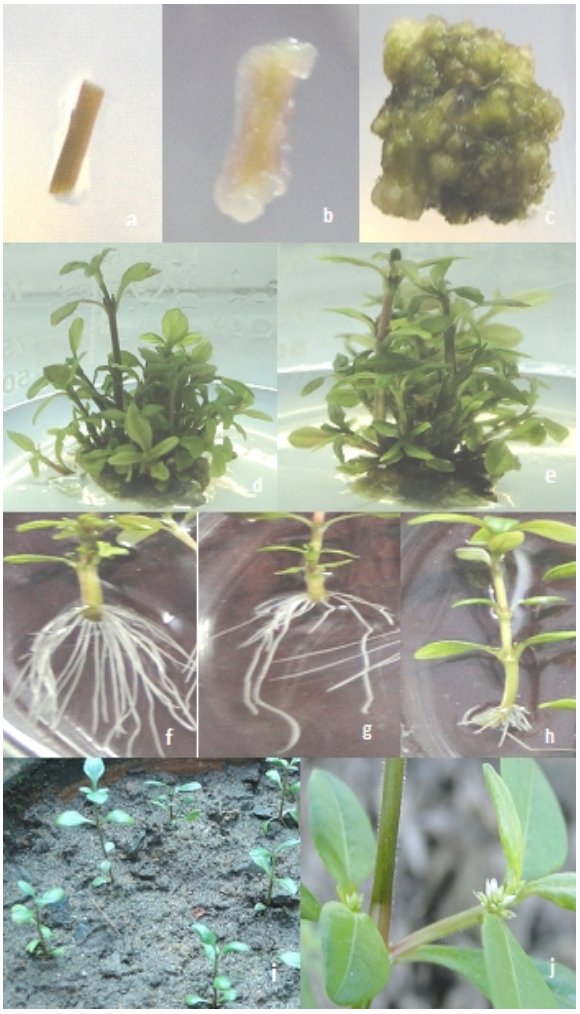

Fig. 1. *In vitro* propagation of *A. sessilis*.
1a. internode on culture medium, 1b. non-embryogenic callus, 1c. green sector development on callus,
1d. and 1e. de novo organogenesis in presence of BAP+Ads,
1f. rooting on ½ strength MS basal medium, 1 g. rooting in AC enriched medium, 1h. rooting in NAA enriched medium,
1i. hardening of plantlets, 1j. flowering of field transferred in vitro raised plant.

Effect of 3% sucrose (Hi media grade) and 3% commercial sugar on various morphogenic differentiation was tested in the present study and in both the treatments similar results for callus induction, shoot bud and root differentiation were recorded. This first report on use of 3% commercial sugar instead of using Hi-media grade 3% sucrose for culture of *A. sessilis* will make *in vitro* culture economically more viable. During hardening 100% *in vitro* regenerated plants survived. 100% survival rate was recorded in field condition. *In vitro* regenerated *A. sessilis* plants were morphologically identical to parent plant and showed normal flowering in natural condition (Fig. 1j).

Table 1. Effect of different PGRs on shoot bud differentiation from internode derived callus of *A. sessilis*

Sl. No.	Control	PGRs (mg/L)				Time taken for shoot differentiation (days)	Av. No. of shoot/callus ± s.e	Av. length of shoot (c.m.) ± s.e
		BAP	KIN	Ads	TDZ			
1	Control	-	-	-	-	-	-	-
2	-	0.5	-	-	-	18-22	24.67 ± 0.40	2.14 ± 0.45
3	-	1.0	-	-	-	15-19	28.03 ± 0.71	4.38 ± 0.05
4	-	1.5	-	-	-	22-28	23.33 ± 0.25	4.24 ± 0.01
5	-	0.5	0.5	-	-	15-19	50.43 ± 0.74	4.56 ± 0.03
6	-	1.0	1.0	-	-	12-16	52.83 ± 0.59	5.41 ± 0.03
7	-	1.5	1.5	-	-	20-26	51.30 ± 0.25	5.56 ± 0.03
8	-	0.5	-	0.5	-	10-18	113.13 ± 0.75	7.33 ± 0.04
9	-	1.0	-	1.0	-	10-15	124.47 ± 0.59	9.30 ± 0.05
10	-	1.5	-	1.5	-	15-18	117.87 ± 0.58	7.61 ± 0.03
11	-	-	-	0.5	-	17-23	26.03 ± 0.35	4.31 ± 0.03
12	-	-	-	1.0	-	18-25	31.60 ± 0.66	5.38 ± 0.03
13	-	-	-	1.5	-	20-26	25.63 ± 0.29	5.23 ± 0.01
14	-	-	-	-	0.5	15-23	5.40 ± 0.23	3.37 ± 0.03
15	-	-	-	-	1.0	16-21	6.03 ± 0.29	3.61 ± 0.03
16	-	-	-	-	1.5	23-28	5.13 ± 0.18	2.92 ± 0.03

Table 2. Effect of medium composition on root induction of shoots of *A. sessilis*

Sl. No	Treatment	Time taken for root induction (days)	Av. No. of root/ shoot ± s.e.	Av. Length of root (cm)± s.e.
1	½ MS basal	2-3	28.10 ± 0.35	7.49 ± 0.05
2	MS + AC (0.2gm/L)	3-5	19.10 ± 0.36	4.57 ± 0.04
3	MS + NAA (0.5 mg/L)	3-5	9.13 ± 0.33	3.77 ± 0.04

4. CONCLUSION

The findings of the present study would certainly provide an economically viable alternative method of propagation of this elite medicinal plant in order to improve its multiplication rate as well as for its conservation. This protocol may find application in re-culture of cryopreserved *A. sessilis* explant for plant conversion.

COMETING INTERESTS

Authors have declared that no competing interests exist.

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