

Technique for rapid micropropagation of *Alternanthera bettzichiana* (Regel) Nicols

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ABSTRACT

A simple and efficient micropropagation protocol was developed for *Alternanthera bettzichiana* using shoot tip as explants. The explants were culture in MS basal medium fortified with phytohormones and different concentration of cytokinins (BAP and KN). Maximum shoot proliferation from single explant was obtained on MS Medium incorporated with KN (5 mg/l) and MS medium with BAP Concentration (7 mg/l) showed highest and significant frequency of root proliferation. KN showed formation of small clusters only. Successful hardening-off is achieved using conditions of relatively low humidity. In Vitro regenerated plantlets were healthy and attained heights of 5-6 cm in 5 weeks which were transferred to field conditions after acclimatization with the success rate of 80 % without any seasonal constraints.

Key words: *Alternanthera bettzichiana*, Shoot tips, Shoot proliferation, Phytohormones.

INTRODUCTION

Tissue culture techniques being increasingly exploited for clonal multiplication and *in vitro* conservation of valuable indigenous germplasm threatened with extinction. Greater demand for these plants especially for the purpose of food and medicine is one of the causes of their rapid depletion from primary habitats. Plant cells and tissue cultures hold great promise for controlled production of a myriad of useful secondary metabolites¹. Micropropagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation². Many improved plant varieties has been regenerated using tissue and cell cultures techniques^{3,4}. The Amaranthaceae are mostly herbs but rarely also shrubs or small trees comprising 65 genera and 900 species further characterized

by the presence of betalains and p-plastids. The genus *Alternanthera*, a medicinally important member of family Amaranthaceae is widely used for the presence of volatile constituents, essential amino acids, flavone glycoside and steroids *Alternanthera bettzichiana*, a horticulture species of many forms, is often found as an escape, reported for its potential claims in certain viral diseases⁵ as an immunomodulator⁶, protective agent against cancer⁷, and in treatment of diarrhoea⁸. Although adventitious shoot regeneration is a basic prerequisite for the application of generic engineering to crop improvement, the production of plants from apical meristems and axillary buds has proven to be the most generally applicable and reliable method of *in vitro* micropropagation. This paper describes a rapid and efficient *in vitro* micropropagation system from shoot explants of *Alternanthera bettzichiana*.

EXPERIMENTAL

Materials and Method

Establishment of aseptic explant

Alternanthera bettzichiana growing wild in the field was collected and authenticated from Botanical Survey of India, Pune and planted at the herbal garden of our departmental laboratory. *Alternanthera bettzichiana*, much branched, diffuse herbs, stem ribbed, green; young branches white pubescent with spike like minute flower⁹. Apical meristem and shoot tips of the plant were used as explants and were first washed with detergent and rinsed under running tap water for 30 minutes. They were then dipped into 70% alcohol for one minute before surface sterilizing, using mercuric chloride (HgCl_2) 0.08% (w/v) for 5 minutes. After rinsing three times with sterile distilled water, the explants were again sterilized with Clorox 0.15% (w/v) for 15 minutes. They were then again rinsed three times with sterile distilled water. The explants were dried using sterile filter paper before inoculating into basic MS culture medium¹⁰ containing 3% (w/v) sucrose. The pH of the medium was adjusted to 5.7-5.8 followed by addition of 7.5 g l⁻¹ bacto agar before autoclaving at 121°C and 1.06 kg/cm² for 13 minutes using autoclave. All the cultures were incubated at 25 ± 2°C under 24-h photoperiod provided by white fluorescent light with a light intensity of 32.5 mE m⁻² s⁻¹. The aseptic shoots that were obtained after two weeks of cultures were used as the source of explants for the subsequent experiments.

Organogenesis / Induction of multiple shoots formation

The apical meristem and shoot tips were cultured on MS medium supplemented with various concentrations of BAP and KN using a factorial experimental design. The pH of the culture media was adjusted to 5.7-5.8 with 0.1M NaOH or 0.1 M HCl prior to autoclaving at 121°C and 1.06 Kg/Cm² for 13 minutes using autoclave. Ten explants were used for each medium treatment and the experiment was repeated three times. The cultures were incubated at 25 ± 2°C under continuous illumination with cool white fluorescent tubes at an intensity of 32.5mE M⁻² s⁻¹. Responses of the culture were observed and recorded over a period of five weeks. The shoot length and number of shoots

formed were recorded. Explant that produced more than two shoots was considered as multiple shoots. The *in vitro* regenerated plantlets were transferred to root trainer cups containing a mixture of sterile soil and sand (3:1) for hardening. They were irrigated every 24 hrs. with a solution containing MS salts at half strength. High humidity levels were maintained by covering the pots with polyethylene bags. Progressive puncturing of polyethylene bags decreased the humidity levels and simultaneously they were exposed to sunlight. After four weeks the hardened plants were transferred to nursery.

RESULTS AND DISCUSSION

Within 5 weeks, an average of 3 to 5 shoots were formed from each apical meristem of *Alternanthera bettzichiana* when they were cultured on MS supplemented with 1.0 to 10 mg/l of BAP and with KN respectively. The addition of KN, as low as 1 mg/l, into MS medium containing BAP, however, did not show significant influence on multiple shoots formation from the apical meristem explants (Table 1). This observation suggested that the induction of multiple shoots formation of *Alternanthera bettzichiana* depended only on the presence of BAP in the culture medium. The apical meristem and shoot tips culture on basic MS medium with BAP produced significant shoots with complete root system (Fig. 1). All the multiple shoots

Table 1: Number of shoots formed *Alternanthera bettzichiana* when cultured in MS medium supplemented with only BAP (1.0-10.00 mg/L) within five weeks

S No.	MS + BAP (mg/l)	Number of shoots Mean ± SEM
1.	00	1.0 ± 0.13
2.	01	2.0 ± 0.21
3.	02	3.0 ± 0.14
4.	03	5.0 ± 0.21
5.	04	5.0 ± 0.21
6.	05	8.0 ± 0.29
7.	06	9.0 ± 0.21
8.	07	16.0 ± 0.74
9.	08	11.0 ± 0.29
10.	09	12.0 ± 0.39
11.	10	11.0 ± 0.25

formed in MS media supplemented with only BAP of 7.0 and 9.0 mg/l showed formation root system (fig. 1), while with KN showed formation of small clusters, and accompanied with swelling and callus growth without root system. It is observed that high concentration of cytokinin generally inhibited root formation of plants ¹¹. More multiple shoots formed when the explants were cultured in MS Medium supplemented with only BAP at an average concentration of 7.0 and 9.0 mg/l. However the number of shoots formed over and above this range of BAP concentration was significantly different. Small shorter shoots were formed as the concentration of with KN present in the culture

medium. Result indicated that culture medium MS with 7 mg/l of BAP was sufficient to produce multiple shoots culture of *Alternanthera bettzhiana*. Results are very indicative for the varying proportion of growth multiplication in average concentration of only BAP and KN as shown in table 1 and 2. Su *et al* ¹² reported that MS medium added with low concentrations of BAP produced normal shoots and roots for *Typhonium flagelliforme*. The Same phenomena was observed for *Alternanthera bettzhiana* and all its *in vitro* multiple shoots were normal with complete root system when average concentration of BAP was added into the culture medium.

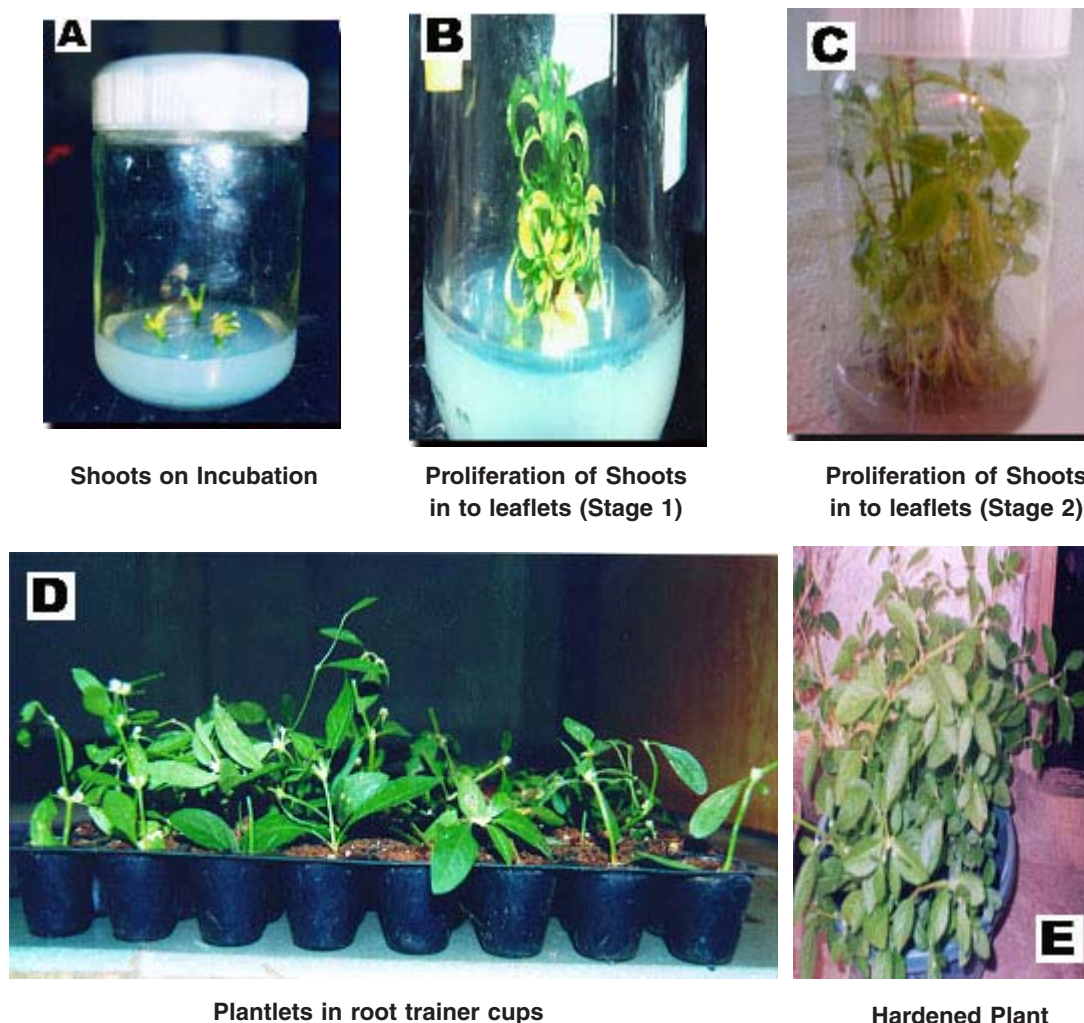


Fig. 1: Stages of in vitro propagation of *A. bettzhiana*

Table 2: Number of shoots formed *Alternanthera bettichiana* when cultured in MS medium supplemented with only KN (1.0-10.00 mg/L) within five weeks

S No.	MS + KN (mg/l)	Number of Shoots Mean \pm SEM
1.	00	1.0 \pm .013
2.	01	3.0 \pm 0.14
3.	02	4.0 \pm 0.25
4.	03	6.0 \pm 0.47
5.	04	9.0 \pm 0.39
6.	05	12.0 \pm 0.51
7.	06	11.0 \pm 0.39
8.	07	9.0 \pm 0.33
9.	08	7.0 \pm 0.29
10.	09	5.0 \pm 0.39
11.	10	4.0 \pm 0.25

An *in vitro* propagation of *Alternanthera bettichiana* could be achieved by culturing the aseptic apical meristem and shoot tips explants on MS medium supplemented with 7.0mg/l of BAP.

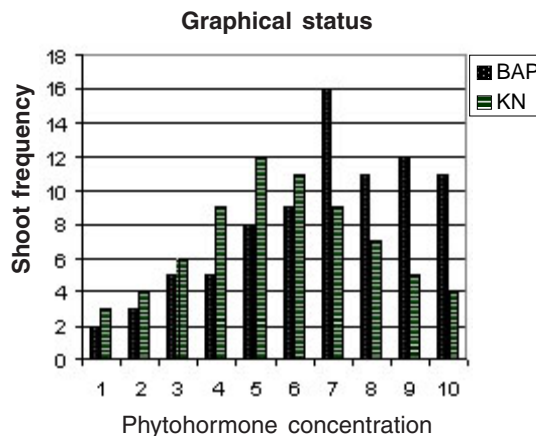


Fig. 2: Shoot tips propagation

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