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RAPID MULTIPLICATION OF MATURE FLOWERING PLANT OF *Eryngium foetidum* L. BY *IN VITRO* TECHNIQUE

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Abstract- An *in vitro* protocol has been developed for rapid multiplication of mature flowering plant of *E. foetidum*. The nodal and scape explants derived from 10-12 day old inflorescence explant were cultured on Murashige and Skoog's (MS) medium supplementing with different concentrations and combinations of BAP and Kinetin. Both the explant types produced direct adventitious shoots varying in their number. Each adventitious shoot possessed a rosette of leaves with apical or axillary inflorescence or both inflorescence with well-developed rooting system. The entire process of adventitious shoot formation and mature plantlet development occurred within 3-4 weeks of culture. The well-organized shoots were individually excised and potted in the soil. Up to 95% of the plants survived under field conditions.
Keywords- *Eryngium foetidum* L., Adventitious shoots, *in vitro* flowering, rapid multiplication

Introduction*Eryngium foetidum* L**.** (Apiaceae) is a biennial, rare, endemic plant having aromatic properties. It is used widely in ethnic medicine and extensively used as a culinary herb. Though the herb is indigenous to the Caribbean, Latin America and far East, its pungent and unique aroma which is similar to the common coriander makes it an indispensable condiment in the cuisine of Thailand, Malaysia, Singapore, Latin America and US. The plant is a source for three kinds of essential oil from different parts viz. leaf, root and seed. These essential oils are of high value in the International trade for their application in perfumery and pharmaceutical industries [1]. The plant finds extensive usage in ethnomedicinal practices for treating various ailments like cold, cough, fever, ear pain, respiratory disorders, diabetes, lowering of cholesterol, diarrhoea and convulsions[2-6]. The pharmaceutical properties of this plant are highly valued in Indo-China, Mexico, Nicaragua, Guiana, and Brazil. The leaves possess anti-inflammatory and analgesic properties [7]. The roots of the plant is highly valued for its officinal uses as a nervine tonic, aphrodisiac, expectorant, diuretic, diaphoretic and stomachic[8].
E. foetidum is rare in India and localized to small pockets of Tamil Nadu, Kerala, Karnataka, Assam, Andaman and Nicobar Islands. The availability of the plant is restricted to certain regions because of low seed viability and long growth phase. Hence its utility for commercial applications is been greatly hindered. In order to achieve bulk propagation and rapid multiplication of *E. foetidum* plants, an *in vitro* approach has been established for obtaining mature flowering plants having high vigor in a short duration of time.

Materials and methods

Source of explant

E. foetidum plants were collected from Kundapur, Karnataka and maintained in the garden, Department of Microbiology and Biotechnology, Bangalore University, Bangalore for experimental purpose. A voucher specimen was deposited at the National Ayurveda Dietetics Research Institute, Bangalore for identification and authentication of the plant. (No - RRCBI-Mus/09).

Explant preparation

Scape (peduncle) explants (approx. 10-12 mm in length) without any apparent buds and nodal explants (approx. 6-8 mm in length) were used for culture from 10-12 days old inflorescence. The explants were washed in running tap water for 15 mins followed by soaking in Tween -20 detergent for 5 mins, surface sterilized in 70% alcohol for 2-3 mins and rinsed twice with sterile distilled water. They were further surface sterilized with 0.1% mercuric chloride solution for 2-3 mins and rinsed with sterile distilled water for three times and were cultured on the media.

Media and culture conditions

The Murashige and Skoog media [9] containing the MS salts and other organic addenda supplemented with 6-benzyl amino purine (BAP) alone (1mg l^{-1} and 1.5 mg l^{-1})

and in combination with kinetin (Kn) viz. 2 mg l^{-1} BAP and 1 mg l^{-1} of kinetin were used .The adjuvants like coconut milk (10-15%) and Polyvinyl pyrrolidone (PVP) were used. The P^H of the media was adjusted to 5.8 and gelled by adding 8 g l^{-1} of agar. The media was sterilized by autoclaving at a pressure of 1.06 kg cm^{-2} for 20 min.

The culture tubes (25 mm x 200 mm) and culture bottles (53 mm x125 mm; 2 mm thick) were used as culture vessels. Culture tubes were closed with polypropylene autoclavable caps while polypropylene autoclavable lids were used for culture bottles. An external wrapping with a cling film was done to prevent contamination. The cultures were incubated at 23±2^{0}C with 16/8 h photoperiod under white fluorescent tubes at a photosynthetic photon flux of 25 µmol $m^{-2}s^{-1}$.

Field establishment

The mature plants bearing inflorescence with well developed roots were transferred to small pots containing sand and soil (1:1) for field establishment.

Experimental design

The experiments were conducted using a completely randomized design. Each treatment was done using 20 explants and repeated at least twice. The data was evaluated by analysis of variance.

Results and Discussion

Adventitious shoots from inflorescence:-

a) Scape explant:

Adventitious shoots were initiated from the cut ends of scape explant within 4-6 days of culturing on MS medium with BAP and Kinetin at different concentrations and combinations (Table1).The adventitious shoots produced were 3-4 in number per explants (Fig. A). The adventitious shoots bolted out elongated stem like structure/s (Fig. B) in 8-10 days of culturing. These structures subsequently produced adventitious buds of 3-4 in number at nodal regions within 10-12 days of culturing. Each adventitious bud possessed a rosette of 6-8 leaves, an apical inflorescence having numerous and distinct white florets within 10-12 days of culturing (Fig. D). The MS medium supplemented with BAP (1 mg l^{-1}) and Kinetin (1 mg l^{-1}) was found to be the best for production of maximum number of adventitious shoots per explant (3.3±0.95) (Table1).

b) Nodal explant:

Adventitious shoots were directly produced from the proximal ends of nodal explants after 4-6 days of culturing. 2-4 adventitious shoots per explant were produced (Fig. F). Each adventitious shoot possessed a rosette of 5-6 leaves, 1-2 inflorescence (apical or axillary) after 8-12 days in culture (Fig. G and H respectively). The medium suited to elicit highest number of shoots (3.7±1.5) was with BAP (3 mg l^{-1}) and Kinetin (1 mg l^{-1}) (Table 1).

The adventitious shoots and adventitious buds produced from scape and nodal explants developed roots on the same medium. In both the explants roots were initiated in 6-10 days of culturing. Initially the roots were white in color and turned brown within 14-15 days of culturing (Fig .C and I). In the present study the addition of PVP as an adjuvant enhanced the response for adventitious shoot formation as also observed by earlier workers [10]. In this study PVP could also reduce the leaching of phenolics and essential oil to the medium as already reported [1].

Earlier report for multiple shoot formation and *in vitro* flowering in *E. foetidum* had advocated the use of cytokinin (TDZ) along with an auxin [11] whereas, in the present finding the vital role of cytokinin/s has been highlighted for obtaining the flowering response in the adventitious shoots. A much cost effective and time saving step in the present study is the absence of rooting step and rooting medium, thereby reducing the time taken for the whole plant formation.

Field establishment

The adventitious shoots were transferred to sand and soil (1:1) mixture and maintained for a week before transplanting them to field conditions (Fig. E and J). In the present study the acclimatization process has been greatly reduced and the rate of survival of plants was above 90% from both explants. Earlier work on somatic embryogenesis showed a survival of 80-90% [12-14].

Off late, *E. foetidum* is becoming a plant of rare occurrence in Indian subcontinent and is very sparsely distributed only at specific regions. Therefore, there is a need to take up a firm footing on the conservation and sustainable development of this plant. The present communication presents a reproducible and effective method for obtaining mature flowering plants of *E. foetidum*. Rapid regeneration via adventitious shoot formation, which avoids callus phase is preferred as it minimizes the risk of somaclonal variation [15]. In conclusion, a simple but efficient protocol by direct plant regeneration is been developed.

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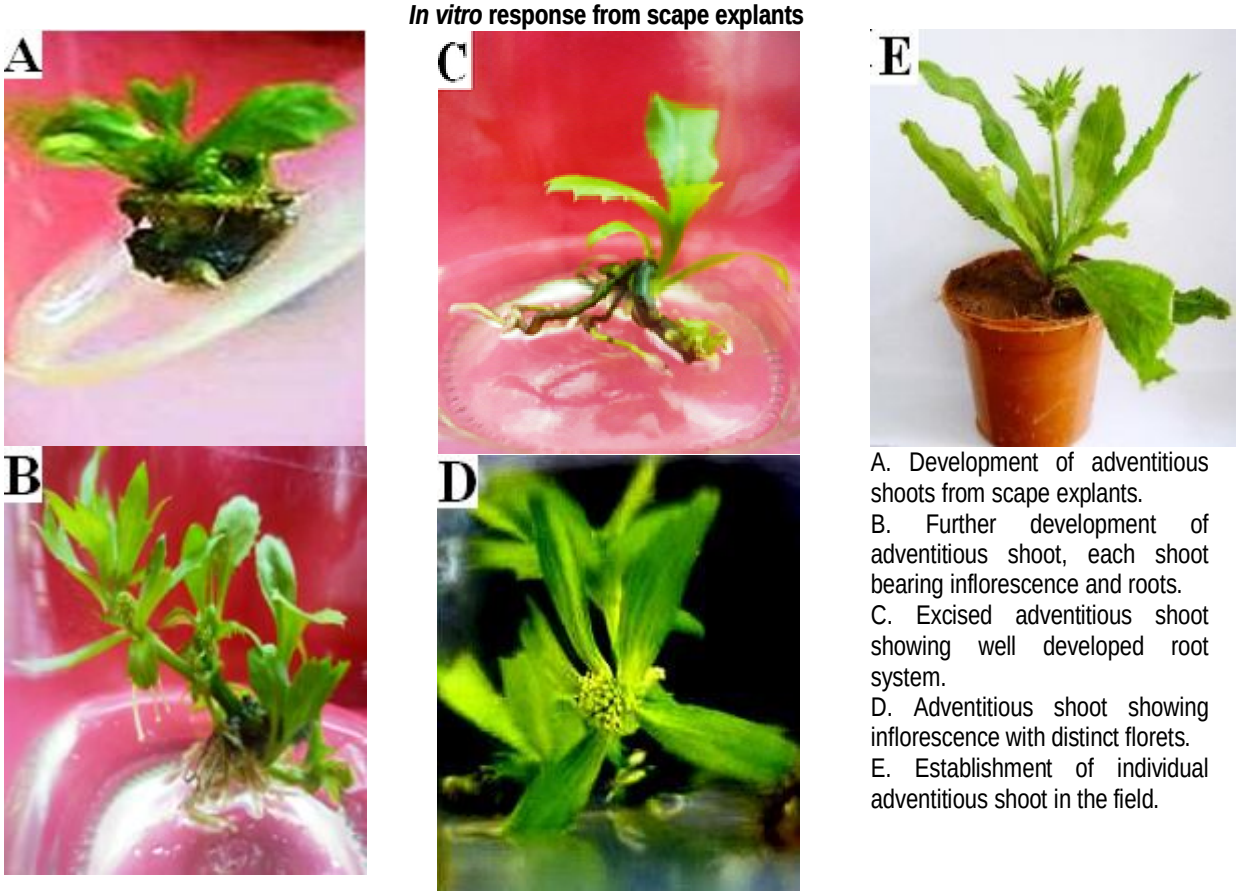
Table 1- Effect of BAP and Kinetin on shoot length, shoot number, root formation and inflorescence formation on adventitious shoots produced from nodal and scape explants of E. foetidum (after 4 weeks)

Source of explants	Sl. No.	Supplements	No. of shoots/explant	Shoot length (in cm)	No. of inflorescence	No. of roots/explant
Nodal explants	1.	BAP(2mgl^{-1})	3.1±1.10	3.65±0.67	2.2±1.14	7±1.05
	2.	BAP(1mgl^{-1})+ Kn(1mgl^{-1})	3 ±1.05	4.15±0.75	2.6±0.84	7.1±1.10
	3.	BAP(2mgl^{-1})+ Kn(1mgl^{-1})	3.3 ±1.16	3.5±1.08	2±0.67	7±0.94
	4.	BAP(3mgl^{-1})+ Kn(1mgl^{-1})	3.7 ±1.16	3.8±0.92	2.4±1.07	7.3±1.16
Scape explants	1.	BAP(2mgl^{-1})	3.1±1.10	3.9±0.78	2.2±0.92	7.5±1.27
	2.	BAP(1mgl^{-1})+ Kn(1mgl^{-1})	3.3±0.95	3.8±0.92	2.1±0.74	6.9±1.52
	3.	BAP(2mgl^{-1})+ Kn(1mgl^{-1})	2.9±0.88	4±0.67	2.5±0.85	7.1±0.99
	4.	BAP(3mgl^{-1})+ Kn(1mgl^{-1})	2.6±0.67	3.8±0.79	2.2±1.14	7.5±1.27

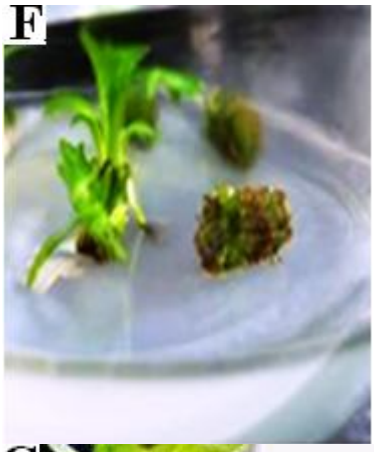
Data are presented as mean ± SD based on 20 explants.

***In vitro* response from scape explants**



A. Development of adventitious shoots from scape explants.
B. Further development of adventitious shoot, each shoot bearing inflorescence and roots.
C. Excised adventitious shoot showing well developed root system.
D. Adventitious shoot showing inflorescence with distinct florets.
E. Establishment of individual adventitious shoot in the field.

***In vitro* response from nodal explants**



A. Initiation of adventitious shoots from nodal explant.
G. Adventitious shoot bearing apical inflorescence with distinct florets.
H. Adventitious shoot bearing axillary inflorescence.
I. Excised adventitious shoot with profuse rooting.
J. Mature adventitious shoot established in the field.