

A protocol for *in vitro* regeneration of *Eryngium foetidum* L.

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Eryngium foetidum L. is an important plant cultivated as leafy vegetable and for its essential oil, which is of high economical value in international market. Multiple shoots (17.8) were obtained through direct regeneration from leaf excised from field grown plants of *E. foetidum* and cultured on Linsmaier and Skoog's Basal Medium (LSBM) fortified with BAP (1.5 mg l^{-1}) and PVP (250 mg l^{-1}). After subculture on the same medium, the multiple shoots formed roots, thereby eliminating an additional step of *in vitro* rooting. The well-developed plants were hardened and successfully transferred to field with 88% survival frequency.

Keywords: *Eryngium foetidum*, leaf, direct regeneration, multiple shoots, survival frequency

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Eryngium foetidum L. an aromatic plant, grown as a leafy vegetable, commonly known as Spiny coriander and Culantro; belongs to the family Umbelliferae¹. It is one of the rare, biennial, indigenous species distributed in continental Tropical America and the West Indies². Its essential oil, consisting of about 40 compounds, has high economic value in international and domestic markets³⁻⁵. Besides its use as a condiment for culinary purposes, medicinal properties have also been attributed to it⁶. Moreover, it is extensively used in perfumery and cosmetic industry⁷. At present, a report is available on regeneration of plants through leaf callus culture and direct organogenesis from root explant of *E. foetidum*⁸. However, regeneration through direct organogenesis from leaf explant has not been reported so far. The present study is an attempt to develop a repeatable protocol on plant regeneration through direct organogenesis of leaf explant.

Eryngium foetidum L. was collected from Dandeli and maintained in the greenhouse at Department of Botany, Bangalore University, Bangalore. The mature leaves measuring $7.0 \times 2.0 \text{ cm}$ were removed from the branches of potted plants, washed thoroughly in tap water, surface sterilized using liquid detergent Labolene-5% (v/v), given a rinse in 70% ethanol for 30 sec, disinfected in 0.1% mercuric chloride (HgCl_2) for 3 min and rinsed several times in sterile water to remove the traces of HgCl_2 . The leaf was then cut into segments measuring $1.5 \times 1.0 \text{ cm}$ and inoculated on Linsmaier and Skoog's Basal Medium (LSBM) fortified with Poly Vinyl Pyrrolidone (PVP) 250 mg l^{-1} and different concentrations of growth regulators BAP and Kinetin (Kn) ranging from 0.5 - 2.5 mg l^{-1} separately to study their effect on multiplication potential.

The culture bottles were closed with plastic caps and sealed with klin wrap and maintained in the culture room at a temperature $25 \pm 2^\circ\text{C}$ under built-in white fluorescent light at a photon flux density of 30 - $50 \mu\text{Em}^{-2}\text{s}^{-1}$ under a photoperiodic regime of 16 h light and 8 h dark cycles. The well developed complete plantlets were transferred to pots containing potting mixture of peat:sand:perlite (2:1:1 v/v) for hardening. The hardened plants were transferred to field for acclimatization and their survival frequency was recorded.

For histological studies, the leaf explants were fixed in FAA (Formalin-Acetic Acid-Alcohol-5:5:90 v/v) for 24 h and dehydrated through ethanol-butanol series and embedded in paraffin. Small blocks containing sample were prepared and sections were taken at 10 - 12μ and stained in haematoxylin and made permanent with DPX. Detailed observations were made under the microscope.

The experimental data were analysed statistically by one-way analysis of variance (ANOVA) to determine the variation in the number of multiple shoots and roots between and within the treatments (Table 1). For each treatment 10 replicates were used and each experiment was repeated thrice.

In the present investigation, mature leaf (Fig. 1) excised from the *in vivo* plants of *Eryngium foetidum* L. were cultured on LSBM fortified with PVP (250 mg l^{-1}) and different concentrations of BAP and Kn

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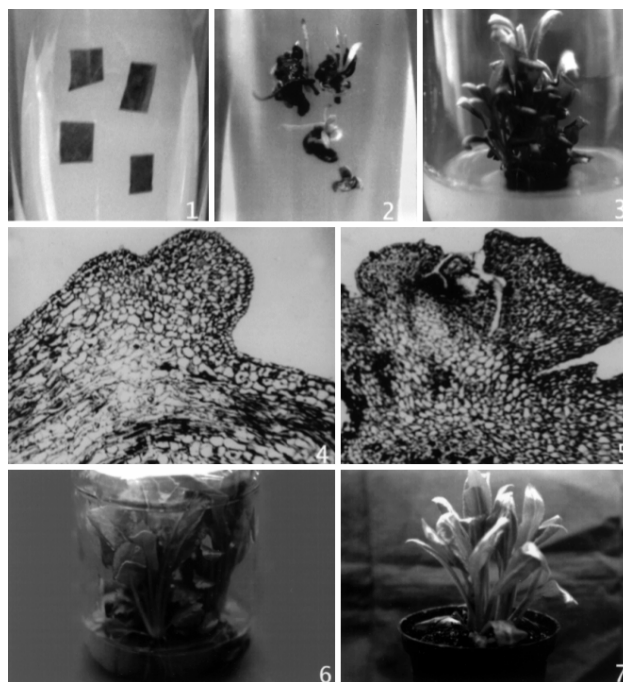
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ranging from 0.5-2.5 mg L⁻¹ separately to study their *in vitro* micropropagation potential.

After 15 days of culture, shoot initiation (Fig. 2) was observed without callus phase with varying percentages of initiation response. However, plant regeneration from callus, and stem and leaf explants has been earlier reported^{7,8}. In the present study, highest percentage of shoot initiation response (88%) was noticed on LSBM fortified with BAP (1.5 mg L⁻¹) and PVP (250 mg L⁻¹) and the lowest percentage of initiation response (38%) was recorded on LSBM fortified with BAP (2.5 mg L⁻¹) and PVP (250 mg L⁻¹) when compared to other concentrations of BAP and Kn tried (Table 1). This does not coincide with the earlier findings in which petiole and nodal explants raised on MSBM supplemented with NAA and TDZ were used for direct regeneration⁹. Further, it was observed in the present study that the initiation and multiplication of shoots were found to be best when PVP (250 mg L⁻¹) was incorporated into the medium. This coincides with the earlier findings that the incorporation of PVP into the medium as additive reduced tissue blackening and favoured healthy shoot proliferation from the explants of *Culantro*^{9,10}. After 45 d of culture, maximum number of multiple shoots (17.8) was observed on LSBM fortified with BAP (1.5 mg L⁻¹) and PVP (250 mg L⁻¹) (Fig. 3).

In the present study, histological studies revealed that the origin of shoot buds is from the proliferated mesophyll cells of the leaf explant (Figs 4 & 5). This is in contrast with the earlier report that the origin of embryoids was from parenchymatous cells on the surface of the callus derived from mature leaf explant

of *E. foetidum*.⁷ Further, direct shoot formation on explant tissue is commenced by cell division and formation of meristematic primordia comprising numerous cells. Similar results have been reported by Broertjes and Keen¹¹ to suggest that directly regenerated shoot buds may always be formed from one or few cells that originate from a single cell.



Figs. 1-7—*In vitro* regeneration of *E. foetidum*: 1, Leaf explant at culture; 2, Shoot initiation after 15 d of culture; 3, Multiple shoot formation after 45 d of culture; 4, Proliferated mesophyll cells of leaf explant; 5, Shoot bud initiation from proliferating mesophyll cells; 6, Root formation after 60 d of culture; & 7, Hardened plantlets ready for transfer to field.

Table 1—Effect of different concentrations of growth regulators on initiation and multiplication of shoots from leaf explants of *E. foetidum*

Basal media	BAP (mg L ⁻¹)	Kn (mg L ⁻¹)	PVP (mg L ⁻¹)	Initiation Response (%)	No. of shoots/culture X±SD	No. of roots/culture X±SD
LS	0.5	0	250	56	4.8± 0.63	3.3± 0.57
LS	1.0	0	250	68	10.6±1.43	6.7± 0.89
LS	1.5	0	250	80	17.8±1.96	11.1±1.23
LS	2.0	0	250	71	11.3± 0.88	6.7±0.71
LS	2.5	0	250	62	7.7±0.73	4.5±0.49
LS	0	0.5	250	35	4.1±0.75	1.9±0.52
LS	0	1.0	250	44	3.8 ± 0.69	3.6 ± 0.57
LS	0	1.5	250	58	6.7 ± 0.93	4.3 ± 0.79
LS	0	2.0	250	69	9.4 ± 0.98	5.2 ± 0.64
LS	0	2.5	250	61	5.1 ± 0.65	3.1 ± 0.32
Note:	X :	Mean of 10 replications				
	SD :	Standard Deviation				

After 60 d of culture, the multiple shoots formed roots (11.1) on the same medium (Fig. 6), thus eliminating a step of *in vitro* rooting thereby reducing the cost of micropropagation. This does not coincide with the findings of Arockiasamy and Ignacimuthu⁸, who have used separate rooting medium for the initiation of roots in *E. foetidum*. Further, the statistical analysis of the data revealed that LSBM fortified with BAP (1.5 mg L⁻¹) and PVP (250 mg L⁻¹) is the best medium for maximum number of multiple shoot (17.8) and root (11.1) formation when compared to other concentrations of growth regulators (Table 1). The well-developed plantlets were hardened (Fig. 7) and successfully transferred to the field with 88% survival rate.

The paper describes a repeatable protocol for rapid clonal multiplication from the mature leaf derived from field grown plant to ensure genetic stability. These plants can be made available to the pharmaceutical and chemical industries for commercial exploitation.

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References

- 1 Saikia A & Shadeque A, Propagation methods of some under-exploited leaf vegetables of northeast India, *J Hill Res*, 9 (1996) 59-61.
- 2 Adams C D, *Flowering plants of Jamaica* (University of West Indies Press, Mona, Jamaica WI) 1971.
- 3 Wong K C, Feng M C, Sam T W & Tan G L, Composition of the leaf oil of *Eryngium foetidum* L. from Cuba, *J Essent Oil Res*, 6 (1994) 369-374.
- 4 Pino J A, Rosado A & Fuentes, Composition of the leaf and root oils of *Eryngium foetidum* L., *J Essent Oil Res*, 9 (1997) 467-468.
- 5 Mohammed M, Post-harvest constraints and solutions involved in export marketing of selected speciality and exotic vegetables from the West Indies, *Acta Horticulturae*, 318 (1992) 355-362.
- 6 Saenz M T, Fernandez M A & Garcia M D, Anti inflammatory and analgesic properties from leaves of *Eryngium foetidum* L. Apiaceae, *Phytotherapy Res*, 11 (1997) 380-383.
- 7 Ignacimuthu S, Arockiasamy S, Antonysamy M & Ravichanran P, Plant regeneration through somatic embryogenesis from mature leaf explants of *Eryngium foetidum*, a condiment, *Plant Cell Tissue Organ Cult*, 56 (1999) 131-137.
- 8 Arockiasamy S & Ignacimuthu S, Plant regeneration from mature leaves and roots of *Eryngium foetidum* L. a food flavouring agent, *Curr Sci*, 75 (1998) 664-666.
- 9 Yasseen Mohamed-Yasseen, *In vitro* regeneration, flower and plant formation from petiolar and nodal explants of Culantro (*Eryngium foetidum* L.), *Society in vitro Biol* (2002) 423-426.
- 10 Daniel B, Simpson B, Lawrence B, Nair G M & Edison S, Rapid *in vitro* multiplication of *Eryngium foetidum* L., an aromatic spice, through shoot multiplication and organogenesis, *Proc Natl Sem Biotechnol Spices Arom Plants*, Calicut, India (1997) 51-55.
- 11 Broertjes C & Keen A Adventitious buds: Do they develop from one cell? *Euphytica*, 29 (1980) 73-87.