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Short Communication

Effect of adjuvants and nitrogen sources on *in vitro* shoot regeneration and clonal propagation of medicinally important plant *Eryngium foetidum* L.

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***In vitro* regeneration of medicinally important plant *Eryngium foetidum* L. was established by developing a protocol for successful faster clonal propogation. Young leaves of the plant were taken and cultured on 1x MS medium supplemented with different concentrations and combinations of 6- Benzyl amino purine (BAP), 1-Napthylacetic acid (NAA) and Kinetin (Kin). Leaves cultured on 2mg/L BAP and 1mg/L NAA showed better response in short duration of time when compared to other concentrations and combinations. Induced shoots obtained from standardized media were used as explant and cultured on 1x MS medium containing 2mg/L BAP and 1mg/L NAA fortified with 10% of different nitrogen sources like tryptone, peptone, beef extract, yeast extract and adjuvants like coconut milk, tomato juice and banana pulp to observe the growth response. It was observed that media containing 10% coconut milk and 10% peptone showed better result when compared to other adjuvants and nitrogen sources. The well developed plantlets were hardened and successfully transferred to the field with 85% survival rate.**

Key words: *Eryngium foetidum,* nitrogen source, adjuvants, clonal propagation.

The genus *Eryngium* L. belonging to the sub family Saniculoideae of Apiaceae, is represented by 317 taxa wide spread throughout central Asia, America central and south east Europe (Worz 2004). It is been used in traditional medicines worldwide (Köpeli 2006). *E. foetidum* L (culantro) known as spiny coriander biennial, endemic plant having aromatic properties, it is widely used in ethnic medicines and extensively used as a culinary herb (Ignacimuthu *et al* 1999, Chandrika *et al* 2011). It has valuable essential oils used in pharmaceutical perfumery and flavor industries (Wong *et al*, 1994, Pino *et al* 1997, Mohammed 1992, Barbara *et al* 2011). The plant finds extensive usage in ethno medicine practices for treating various ailments like cold, cough, respiratory disorders and also used as antidote for snake bites (Saenz *et al* 1997). *E. foetidum* is rare in India and localized in few parts of south India, Western Ghats, Andaman & Nicobar, & Assam. The availability of plant is restricted to certain regions because of low

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seed viability and long growth phase. Clonal multiplication from the young leaves derived from field grown plant to ensure genetic stability and to show that these plants can be made available to pharmaceutical, perfumery, flavor and chemical industries for commercial exploitation. The present study was started with the aim to check the effect of nitrogen source and adjuvants on *in vitro* shoot regeneration of medicinally important plant *Eryngium foetidum* L.

Materials and Methods

Eryngium foetidum L. a medicinally important plant, was collected from its habitat from Western Ghats, Sakleshpur, Hassan district and maintained in green house in Genohelix Biolabs, Bangalore. The young leaves were harvested from plant maintained at green house, washed thoroughly under running tap water, surface sterilized using liquid detergent 2% (v/v) Savlon, 6-8 drops of Tween-20 for 15 mins, rinsed with 70% ethanol for 30 secs, disinfected with 0.05% (w/v) $HgCl_2$ for 6 mins and rinsed in sterile water several times to remove the traces of $HgCl_2$. The sterilized leaves were excised on a sterile petriplate into small pieces and were inoculated on MS basal medium (Murashige and Skoog, 1962) with 3% sucrose fortified with different concentrations and combination of BAP, NAA and Kinetin. The cultures were maintained in the culture room at a temperature of 25±2°C, light intensity of 1000 LUX, relative humidity between 50 - 60%, under photo-periodic regime for 16 hours light and 8 hours dark cycle. After 16 days of incubation shoot induction were observed on 1X MS basal medium with 3% sucrose fortified with 2mg/l BAP and 1mg/l NAA, which were used as a source material for the further experiment.

MS basal medium with 3% sucrose, 2mg/l BAP and 1mg/L NAA fortified with different adjutants like (10% coconut milk, 10% tomato juice, 10% raw banana pulp) and nitrogen sources like (10% peptone, 10% beef extract, 10% tryptone, 10% yeast extract) were prepared separately and autoclaved at 121° C for 15 mins at 15 pounds after adjusting the pH to 5.8.The induced shoot from the leaf explants were used as a source material and were excised and inoculated on the prepared medium containing different adjuvants and nitrogen sources. The cultures were maintained under standard controlled culture conditions and data were collected at regular intervals.

Results and discussion

Shoot induction were observed from the leaf explant on 1X MS basal medium with 3% sucrose fortified with 2mg/l BAP and 1mg/l NAA after 16 days of culture (Fig. 1). It was observed that media containing 10% coconut milk and media containing 10% peptone (Fig. 2-3) showed better result when compared to other adjuvants and nitrogen sources (Table 1). The well developed plantlets were hardened and successfully transferred to the field with 85% survival rate. (Fig 4)

E. foetidum is a medicinal plant having great importance in various fields in flavor industries, perfumanry industries and pharmaceutical industries due to over exploitation there is a great reduction of the plants in the nature. So the present objective was taken to multiply the plants under *in vitro* conditions and to see the effects of different nitrogen source and adjuvants on the growth and development of the plants. The shoot induction from inoculated leaves was well achieved on MSBM containing 2mg/L BAP and 1 mg/L NAA compared to other concentrations of growth regulators. This does not coincide with the earlier finding in petioles and nodal explants raised on MSBM supplemented with NAA and

TDZ. (Yasseen Mohamed-Yasseen 2002). According to the findings of Gayatri *et al* (2006) direct regeneration from leaves was able to regenerate on LSBM with 1.5mg/l BAP and 250mg/l PVP which do not coincide with present findings .In present study different nitrogen sources and adjuvant were used out of which 10% Peptone and 10% coconut milk containing medium showed best result with more multiple shoots and with roots. In the present study blackening of the medium was not observed as it was reported earlier (Yasssem Mohamed-Yassem 2002, Daniel *et al*, 1997, Gaytri *et al*, 2006). The addition of the coconut milk in medium has helped in the increased proliferation of the cells which agrees with the findings of Pollard *et al* (1961) which reduces the secretion of the metabolites into the medium. Use of Peptone has also shown the similar result seen in coconut milk containing medium. In the present findings the proliferation of shoot and root was best observed in peptone containing medium than coconut milk containing medium. The use of adjuvants and nitrogen source in media helps in faster propagation of the plantlets and it can fulfill the needs of pharmaceutical, perfumery and flavor industries.

Table 1 : Effect of different adjuvants and nitrogen sources used along with standardized media on shoot and root formation on *Eryngium foetidum* L.

	Culture duration (days)	6-Benzyl amino purine (µM)	Napthalene acetic acid (µM)	No. of shoots per explant $X^* \pm SE$	No. of roots per explants $X^* \pm SE$
1x MS media with adjuvants					
10% Coconut milk	23	2	1	3.04 ±0.68	4.46 ±0.83
10% Tomato juice	26	2	1	2.65 ±0.34	3.32±0.82
10% Banana	32	2	1	1.14 ±0.89	2.64 ±0.53
1x MS media with nitrogen source					
10% Peptone	20	2	1	3.46 ±0.45	4.82 ±0.64
10% Beef extract	23	2	1	2.29 ±0.71	3.5 ±0.69
10% Yeast extract	39	2	1	1.3 ±0.81	1.1 ±0.43
10% tryptone	48	2	1	1.1 ±0.91	1.7 ±0.41
Control	30	2	1	1.5±0.21	1.8±0.56

*Mean of 10 replications; SE, Standard error.

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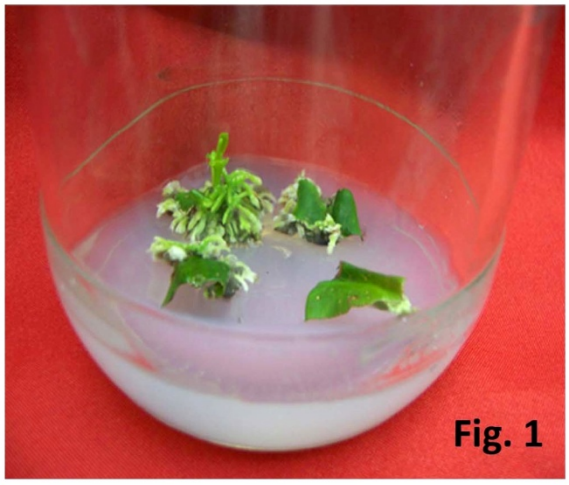

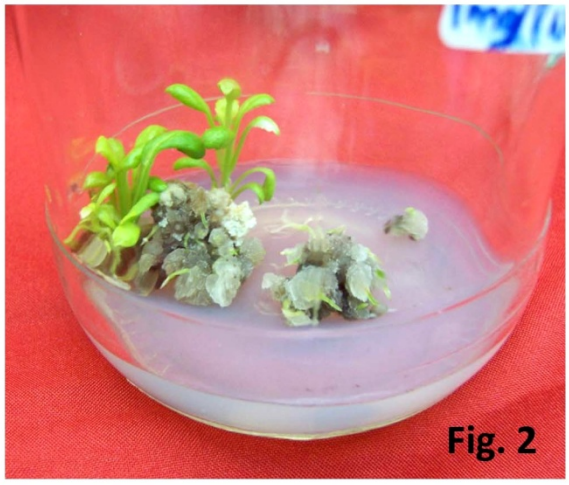

Figures - 1: Shoot induction from leaf inoculated on 1x MS media with 3% sucrose and 1% agar fortified with 2mg/L BAP and 1mg/L NAA. 2-3: Development of shoot on 10% peptone containing media. 4: Hardened plantlet.

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