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BRIEF COMMUNICATION

Direct organogenesis from mature leaf and petiole explants of *Eryngium foetidum* L.

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Abstract

Eryngium foetidum L. plants were regenerated from mature leaf and petiole explants through direct organogenesis without intervening callus phase. From leaf explants, adventitious multiple shoots raised on Murashige and Skoog (MS) medium supplemented with 4.43 μM benzylaminopurine (BAP) and 0.57 μM indole-3-acetic acid (IAA), whereas in petiole explants shoot regeneration occurred at 8.86 μM BAP and 0.57 μM IAA. 80 % of the leaf explants and 44 % of petiole explants produced shoots after four weeks of culture. The regenerated plants were rooted on MS medium supplemented with 2.46 μM indole-3-butyric acid and 2.88 μM gibberellic acid. The plants were successfully established in the soil and showed 70.9 % survival in the field.

Additional key words: growth regulators, *in vitro* cultivation, regeneration.

Plant regeneration via direct organogenesis is preferred over regeneration through somatic embryogenesis and callus culture. *Eryngium foetidum* is of special interest because it is an essential oil crop in India (Saikia and Shadeque 1996). Plant regeneration from callus cultures and through somatic embryogenesis of *Eryngium foetidum* has already been reported (Arockiasamy and Ignacimuthu 1998, Ignacimuthu *et al.* 1999). However, regeneration through direct organogenesis from mature leaves and petioles has not been reported so far. Hence the present study was initiated to obtain regeneration from petioles and leaves for genetic improvement of this species.

Mature plants of *Eryngium foetidum* were collected from Andaman Nicobar Islands and were grown in the garden of the Entomology Research Institute, Loyola College, Chennai, India. Mature leaf blades (7.0 × 2.0 cm) and petioles (0.5 -1 cm) were used as explants. They were washed in running tap water for 30 min, shaken with 0.1 % $HgCl_2$ plus a few drops of *Tween 20* for 5 min and then thoroughly washed with sterilised distilled water. The leaf blade was cut into four rectangular pieces (1.5 × 1.0 cm), which were placed on the medium with their abaxial surface in contact with the culture medium. The petiole explants were placed on the regeneration medium vertical as well as in horizontal position. The culture tubes (15 × 2.5 cm) containing 10 cm^3 of medium were closed with cotton plugs. In each experiment, twenty tubes each one containing one explant were used. The regeneration medium comprised Murashige and Skoog (1962, MS) salts and vitamins supplemented with different concentrations of N^6-benzylaminopurine (BAP; 0 - 13.31 μM) and BAP in combination with indole-3-acetic acid (IAA; 0.57 - 2.85 μM). The regenerated shoots were transferred to MS medium supplemented with different concentrations of indole-3-butric acid (IBA), IAA, and α-napthalene acetic acid (NAA), along with different concentrations of giberellic acid (GA_3) for root induction. The media were adjusted to pH 5.7 and solidified with 0.6 % agar (m/v) (*Hi-media*, Mumbai, India) and autoclaved at 121 °C for 15 min. All cultures were incubated under cool white-fluorescent light (16-h photoperiod with a quantum flux density of 30 μmol m^{-2} s^{-1}) and temperature of 27 ± 1 °C. The completely developed

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Abbreviations: BAP - N^6-benzylaminopurine; GA3 - gibberellic acid; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; NAA - α-naphthalene acetic acid; MS medium - medium after Murashige and Skoog (1962).
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plantlets were removed from the tubes, washed in running tap water and transferred to paper cups containing a mixture of sterilised garden soil and sand (1:1). They were maintained in a mist chamber under controlled temperature (27 ± 1 °C), 16-h photoperiod (irradiance of 30 μmol m^{-2} s^{-1}) and relative humidity 80 - 90 % for hardening. They were initially irrigated with half-strength MS basal medium followed by tap water after one week. Thereafter the acclimatised plants were shifted to a field and percentage of survival of the plantlets was recorded. The experiment was repeated at least three times with twenty replicates per treatment.

All explants gradually became dark brown after 5 d on the medium containing various growth regulators. Multiple shoot buds were initiated from the cut ends of the leaves after 15 d (Fig. 1*a*) and on the petiole explants after 25 d (Fig. 1*b*). Regeneration occurred in both the explants on medium containing different concentrations of BAP either alone (2.21 - 8.86 μM) or in combination with IAA (0.57 and 2.85 μM). However, the number of shoots varied with respect to type of explant and different concentrations of growth regulators (Table 1). The highest number of shoots (7.98 per explant) was produced from the leaf explants at 4.43 μM BAP and 0.57 μM IAA (Table 1). The regeneration of shoots occurred from leaves with about 80 % success and from petioles with only 44 % success. The highest number of shoots from

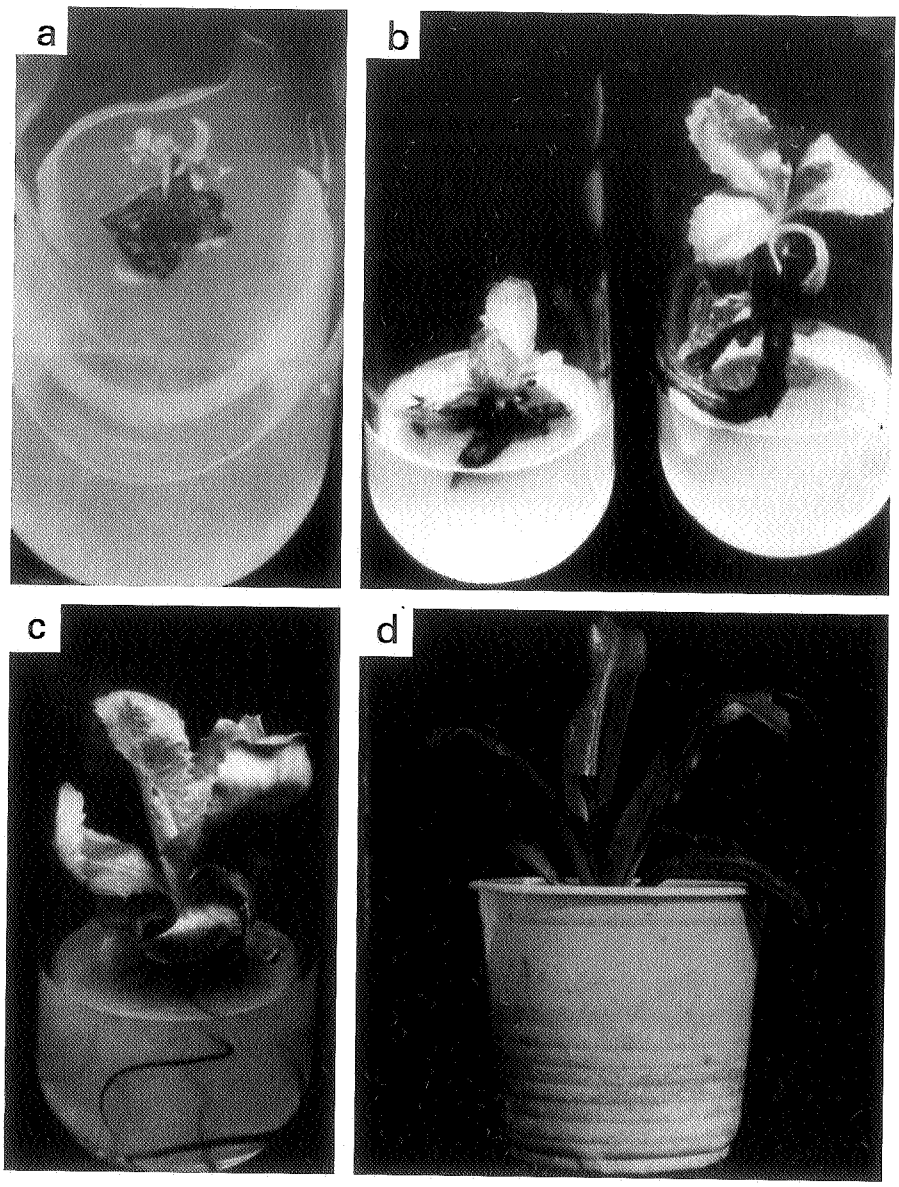

Fig. 1. Shoot regeneration from the cut end of the leaf explants of *Eryngium foetidum* after three weeks (*a*), petiole cultures showing regeneration at the cut ends after 25 d (*b*), complete plantlets showing root induction and elongation (*c*), and acclimatised plantlet *ex vitro* (*d*).

Table 1. Effect of BAP and IAA on plant regeneration from mature leaf and petiole explants in MS medium. Means ± SD, $n = 60$. Data were analysed by Duncan's multiple range test and means followed by identical letters were not statistically different within the column ($P < 0.05$). Percentage was calculated on the basis of total number of explants cultured and number of explants responded for shoot initiation. Data were scored after four weeks (++ - callus).

BAP [μM]	IAA [μM]	Explants forming shoots [%] petiole	leaf	Number of shoots [explant^{-1}] petiole	leaf	Shoot length [cm] petiole	leaf
0	0	0	0	0	0	0	0
2.21	0	12	25	0.80 ± 0.21ad	0.70 ± 0.14a	0.51 ± 0.02a	0.97 ± 0.21a
4.43	0	28	30	1.72 ± 0.50ac	4.31 ± 0.72b	0.79 ± 1.13ab	2.10 ± 0.07b
6.64	0	30	28	1.05 ± 0.32ad	3.92 ± 0.23'b	1.05 ± 0.21be	2.01 ± 0.15b
8.86	0	38	0	4.32 ± 0.97b	++	1.90 ± 0.05c	0
13.31	0	0	0	++	++	0	0
2.21	0.57	30	20	1.21 ± 0.31ac	0.75 ± 0.03a	0.91 ± 0.14b	0.78 ± 0.12a
4.43	0:57	34	80	3.01 ± 0.23b	7.98 ± 0.38c	1.21 ± 0.02d	2.51 ± 0.14c
6.64	0.57	40	65	3.21 ± 0.156b	4.49 ± 0.27b	1.32 ± 0.54dc	2.02 ± 0.13b
8.86	0.57	44	0	6.37 ± 0.48c	++	1.97 ± 0.14c	0
2.21	2.85	0	0	++	++	0	0
4.43	2.85	10	0	0.50 ± 0.01d	++	0.70 ± 0.27a	0
6.64	2.85	14	40	1.21 ± 0.71ade	1.73 ± 0.25d	1.02 ± 0.13e	1.02 ± 0.21a
8.86	2.85	22	-	2.05 ± 0.36e	++	1.50 ± 0.34cde	0

Table 2. Effect of different auxins on root induction from regenerated shoots in MS medium. Means ± SD, $n = 60$. Data were analysed by Duncan's multiple range test and means followed by identical letters were not statistically different within the column ($P < 0.05$). Percentage was calculated on the basis of total number of explants cultured and number of explants responded for root induction. Data were scored after four weeks.

Auxin	[μM]	Regenerants from leaves rooted cultures [%]	root number [culture^{-1}]	Regenerants from petioles rooted cultures [%]	root number [culture^{-1}]
Control	0	12	3.21 ± 0.15a	5	2.34 ± 0.64ai
IBA	0.04	35	8.24 ± 2.05b	18	4.78 ± 1.76jf
	0.49	55	34.27 ± 3.47c	39	9.14 ± 1.34k
	2.46	100	64.45 ± 3.98d	85	26.34 ± 2.38e
	4.92	70	22.14 ± 2.97e	58	14.54 ± 1.56m
IAA	0.05	25	5.12 ± 0.97g	7	1.31 ± 0.31n
	0.57	15	3.41 ± 0.72fa	25	4.41 ± 0.72f
	2.85	24	7.21 ± 0.43bi	16	3.14 ± 0.38f
	5.70	0	0	10	2.01 ±0.10qa
NAA	0.05	22	6.14 ± 2.14gi	10	4.31 ± 2.14fi
	0.53	34	11.21 ± 2.31h	35	6.78 ± 1.44k
	2.86	28	6.13 ± 2.03i	40	11.32 ± 1.24r
	5.37	0	0	12	4.32 ± 0.94f

petioles (6.3 per explant) was produced at 8.86 μM BAP and 0.57 μM IAA. The orientation of the explants in the medium might affect the morphology of the plant. But in this case, when the petiole explants were placed on the medium vertically as well as horizontally (Fig. 1*b*), the regenerated plants showed normal morphology in contrast to our earlier findings, wherein the root explants placed in a horizontal position showed aberrant morphology of leaves (Arockiasamy and Ignacimuthu 1998). The repeated subcultures of three or four clumps of shoots in the same medium promoted further proliferation of shoots. There are many reports similar to this study showing adventitious organogenesis from leaf of *Arachis hypogea* (Eapen and George 1994), *Mentha piperita* (Caissard *et al.* 1996), *Pelargonium domesticum* (Boase *et al.* 1996), and in petiole explants of *Carica papaya* (Jeyasankar and Yadava 1996) and *Petasites hybridus* (Wildi *et al.* 1998).

Shoots regenerated from both the explants, which attained the length of 2 - 3 cm were excised and cultured

on MS medium with or without growth regulators. In order to induce roots and elongation of shoots simultaneously they were cultured on MS medium supplemented with different concentrations of IAA, IBA and NAA plus 2.88 μM GA_3 (Table 2). Addition of 2.46 μM IBA and 2.88 μM GA_3 to the medium was the most effective (Fig. 1*c*). Similar results were found in *Anacardium occidentale* (Boggetti *et al.* 2001). A total of 585 plantlets were transferred to soil and 415 plants were successfully hardened (Fig. 1*d*) The fully developed plantlets when transferred to *ex vitro* conditions exhibited 70.9 % survival in the paper cups (Fig. 1*d*). The regenerated plants were fertile and normal.

In conclusion we observed that the leaf explants produce more shoots than the petiole explants. The growth regulators namely BAP and IAA were found to be effective in promoting shoots.

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