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REGENERATION OF PLANTLETS FROM LEAF DERIVED CALLUS OF *AMMI MAJUS*-A MEDICINAL PLANT

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ABSTRACT

An efficient protocol was developed for high frequency plant regeneration from leaf explants of *Ammi majus*. *Ammi majus* L. (Apiaceae) is a herb of pharmaceutical as well as ornamental interest and used in the treatment of leucoderma. Since in *Ammi majus* the seed set and germination is poor attempts were made to propagate the plants through tissue culture. The callus from cotyledonary leaves obtained on Murashige and Skoog's (MS) medium supplemented with α-indoleacetic acid (IAA) + Kinetin (Kn) + Casein hydrolysate (CH), differentiated shoots in 100% cultures when adenine and glutamine were added to the above medium. Plantlets resulted on MS medium with indolebutyric acid (IBA) and glutamine.

KEYWORDS:*Ammi majus*, **callus, regeneration, plantlet.**

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INTRODUCTION

Ammi majus L.(family Umbelliferae), commonly known as Bishop's weed, the honey plant, an important medicinal herb, constitutes the principal commercial source of xanthotoxin, a linear furanocoumarine with psoralen as the major compound used in the treatment of leucoderma. Psoralenes are useful in phyto-chemotherapy of numerous dermatological afflicitions, such as alopecia, areata, atopic eczema, lichenoides, mycosis fungoides, pityriasis etc. Fruits of this umbellifer are rich in coumarins and related compounds that have been used in the treatment of vitiligo and in formulations of sun-tan lotions. Besides photosensitizing and anti-proliferating properties, calcium antagonistic actions of these compounds, important in the field of therapeutics, have been reported. Leaves and flowers of *Ammi majus* exhibit marked potency in killing the snail vectors of schistomiasis. Flowers and umbel axes also contain high concentration of furanocoumarins.[1] The species is traditionally seed propagated that leads to genetic segregation and thus, does not ensure uniform yield of xanthotoxin. Attempts to acclimatize *A. majus* under various climatic conditions suffer a set back as the seed set, the only source of propagation, and germination is poor. Also the fruits fail to ripen in moderate climatic zones, and the plants become highly sensitive to infections. The chemical variability resulting from climatic and edaphic conditions affects the qualitative yield of the desired product. *I-vitro* propagation methods offer powerful tools for germplasm maintenance and multiplication. It has been shown that shoot organogenesis via callus culture can be used as an effective method for multiplication of medicinal plants.[2,3] The technique also facilitates production of uniform clones from highly heterozygous plants, and conservation of biodiversity of threatened species.[1,4,5]

MATERIALS AND METHODS

Mature seeds of *Ammi majus* were collected from herbal garden seed collection centre Hamdard University, New Delhi, surface sterilized with 1.0% Sodium hypochlorite and inoculated on MS medium. Hypocotyl, cotyledonary leaves and radical segments, from 3.5cm long seedlings were cultured on Murashige and Skoog's medium 1962[6] supplemented with IAA, Kn and CH. Green and compact callus produced by cotyledonary leaves was used for the morphogenetic studies. Murashige and Skoog's nutrient medium (MS) with 3% sucrose, gelled with 0.63% agar was used throughout. The pH of the medium was adjusted to 5.7 before autoclaving (121^0C for 15min). All the cultures were incubated at 25 ± 2^0C under white fluorescent cool light ($65\mu E/m^2/s$) with 14h light/10h dark cycle. Growth regulators in various combinations and concentrations were also added when required. For each set of experiment, percentage of cultures showing shoot regeneration and average number of shoots per culture obtained after another three weeks were analyzed. The shoots were rooted on MS medium supplemented with IBA and glutamine.

Transplantation

Hardening of the rooted shoots or plantlets was achieved by sequential transfer to half and one-fourth of MS medium and finally to medium lacking any organic constituents.

Table 1
Effect of growth reglulators on callus induction.

IAA (mg/L)	Kn (mg/L)	CH (mg/L)	Callus initiation %	Callus Morphology
1	0.5	500	49.20±0.70	Greenish Callus
0.5	1	500	55.80±4.21	Greenish Callus
1	1	500	58.37±3.55	Greenish Callus
2	1	1000	68.11±3.06	Greenish Callus
1	2	1000	82.03±2.65	Greenish Callus
2	2	1000	97.70±2.00	Greenish Callus
3	2	1000	79.44±2.42	Greenish Callus
2	3	1000	57.73±3.06	Greenish Callus
3	3	1000	39.21±5.61	Greenish Callus

Table 2
Effect of IAA/Kn on morphogenic potentiality of leaf segment of Ammi majus.

IAA/KN	%Regeneration	Av. No. of shoots/cultur after 6 weeks	Av. Ht.(cm.) of regenerated shoots after 6 weeks
0.0/0.0	0.0±0.0	0.0±0.0	0.0±0.0
0.0/0.5	3.0±0.1	1.2±0.3	0.2±0.2
0.0/1.0	5.0±0.3	2.0±0.7	0.7±0.1
0.0/2.0	18.0±0.2	3.5±0.2	0.7±0.1
0.0/5.0	20.0±0.3	4.5±0.2	1.0±0.2
0.2/1.0	56.0±0.2	7.9±1.1	2.0±0.1
0.2/2.0	75.0±0.4	9.2±0.3	2.5±0.1
0.2/5.0	77.0±1.0	6.5±0.3	2.8±0.2
0.5/0.5	81.0±0.3	8.5±0.2	3.3±0.3
0.5/1.0	88.0±0.2	8.5±1.2	5.8±0.2
0.5/2.0	93.3±0.2	15.3±1.2	8.5±0.3
0.5/5.0	77.0±0.5	7.4±1.1	7.0±0.1
1.0/0.5	35.0±0.5	4.5±0.3	4.2±0.2
1.0/1.0	23.0±0.4	3.9±0.4	2.1±0.2
1.0/2.0	0.0±0.0	Callus	Callus
1.0/5.0	0.0±0.0	Callus	Callus

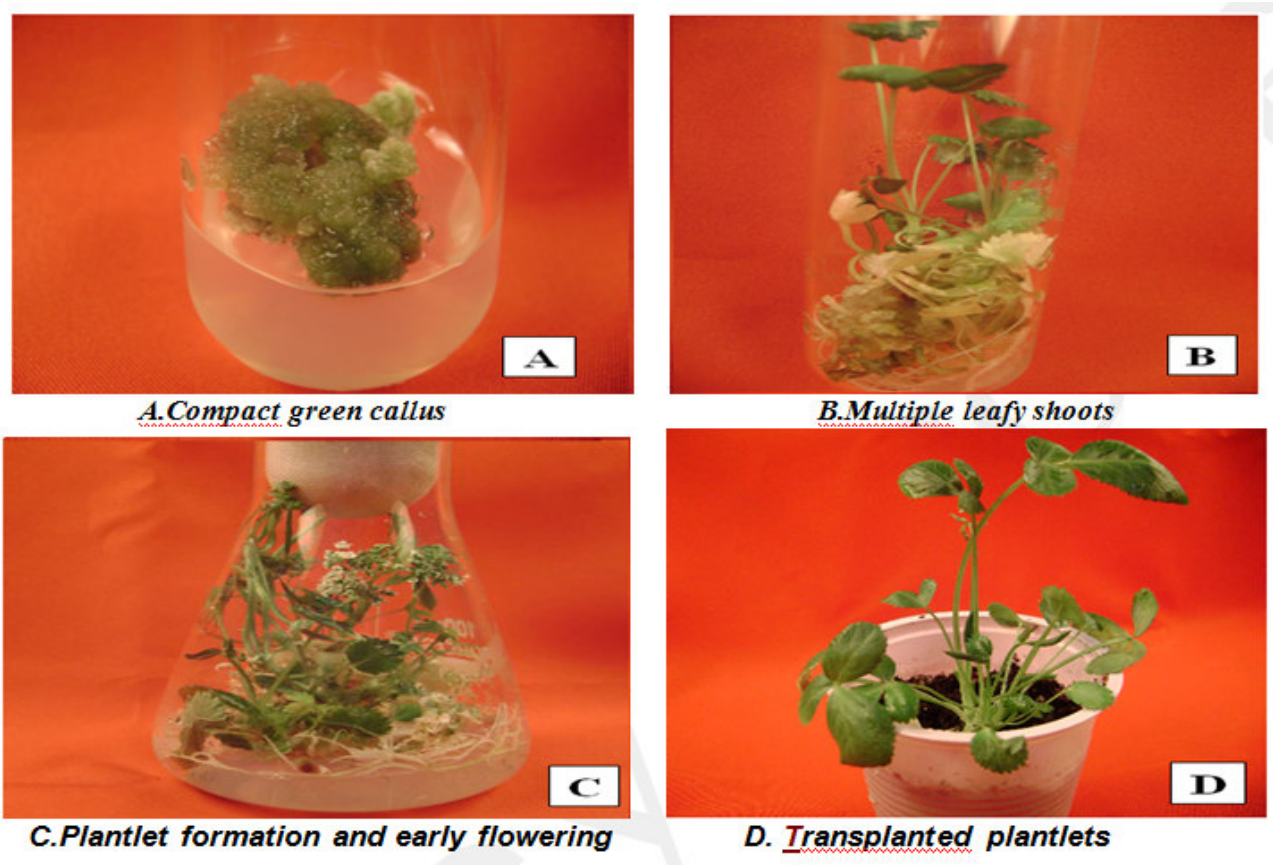

Figure 1
Morphogenic response of leaf explants of Ammi majus cultured on:MS (Sucrose 3%) + IAA (2.0 mg/l) + Kn (2.0 mg/l) + Adenine (40.0 mg/l) + CH (1000 mg/l) + Glutamine (50 mg/l)

RESULTS AND DISCUSSION

On MS basal medium, the seeds germinated after two weeks of planting. Callusing initiated at the cut ends of hypocotyls segments, as well as from the cotyledonary leaves after 10 days on MS medium supplemented with IAA (2.0), Kn (2.0) and CH (1000). The callus from the hypocotyls segments turned brown and succumbed within 15 days, whereas, the cotyledonary leaves produced a green and compact callus that could be further multiplied. The cotyledonary leaves callus transferred to MS medium containing IAA (2.0), Kn (2.0), Ad (40) and CH (1000), differentiated multiple shoots in 50% cultures. Adventitious shoot formation from leaf explant was also reported in *Withania somnifera*.[7,] Lowering of IAA concentration to 0.5 and adding glutamin (50) raised the regeneration frequency to 100% with 10 shoots per culture. The shoots regenerated were rooted on MS basal with IBA (0.1) and glutamine (100). After three weeks, these plantlets flowered *invitro*. Experimental observations support that MS medium containing low concentration of auxin along with cytokinine increases the rate of shoot multiplication.[8,9,10] Nodal segments cultured on MS medium + CH and supplemented with Kinetin (Kn) resulted in the growth of axillary buds but the percent regeneration, number of shoots per culture and shoot length was very low. However, presence of IAA along with Kn in the MS + CH significantly improved the percent regeneration and number of shoots per culture. The leaf segments cultured on MS medium supplemented with IAA (0.5 mg/l) + Kn (2.0 mg/l) + CH (1000 mg/l) proved best and exhibited 93% regeneration with 16.2 shoots per culture after 6 weeks. Kn at higher concentrations resulted in considerable reduction in shoot length. Addition of IAA exceeding 0.5 not only adversely affected differentiation but also gave abnormalities in shoots. Rooting was obtained in 93% cultures on MS + IBA (0.2) + Glu (100). IBA was most effective in inducing rooting of several other plants.[11,12,13,14,15] The efficiency of other cytokinins was compared with Kn. The explant grown on BA (0.5, 1.0, 2.0 and 5.0) with IAA (0.5) produced fascinated shoots with callus and proved inferior to Kn; this is in contrast to earlier report where superiority of BA over Kn and Zeatin is reported.[7] The shoots obtained on MS + IAA (0.5) + Kn (2.0) + CH (1000) when transferred after 5 weeks to MS + IAA (0.5) + Kn (2.0) + Ad (40) + CH (500) + Glu (50), produced flower buds in umbels in 60% cultures after 10 weeks. Reduction in IAA from 0.5 to 0.25 enhanced the flowering percentage to 92 and reduced the time required to induce flowers to 6 weeks. After another week the bisexual flowers opened in 5-6 umbels per culture. With the onset of flowering, yellowing or shedding of leaves also commenced. Green oblong fruits were observed on the same medium after 4-5 weeks. Simultaneously, the shoots elongated further but started turning yellow. Fully mature and viable fruits were obtained in 40% cultures. The overall success of, the tissue culture raised plants, depends upon successful transplantation to the field. The heterotopy mode of nutrition and poorly developed mechanism to control water loss renders micropropagated plants vulnerable to transplantation shock that results in low survival in the field. Therefore,

hardening of plantlets was done by gradually reducing the nutrient levels in the medium. The regenerated plantlets with well developed shoots and roots were transferred to pots containing sterile vermiculate for hardening at diffuse light conditions. The potted plantlets were covered with polythene membrane to ensure high humidity and watered every three days with half strength MS-salt solution free of sucrose. The acclimatized plants of *A. majus* were transferred to field where 75% of the plants survived. The protocol described is an efficient and could be used as a means of propagation and multiplication of *Ammi majus*- a potential medicinal plant for commercial exploitation.

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CONFLICT OF INTEREST

Conflict of interest declared none

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