- Short communication

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# Organogenesis of *Ammi majus* via Callus Culture from Leaf

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*Ammi majus* L. is an imperiled medicinal plant, which contains various medically important and uncommon ~~extraordinary~~ secondary metabolite compounds.. An efficient organogenesis protocol was established for *Ammi majus* through *in vitro* process of leaf callusing, shooting and rooting by using different combination of auxins and cytokinins. *In vitro* cultured juvenile leaf explants were used directly for callus formation on MS supplemented with different concentration of IAA, Kn and CH. Maximum percentage response for callus formation was 97.7 after 5 weeks of incubation on MS supplemented with 2 mg/l IAA, 2 mg/l Kn and 1000 mg/l CH. About 81% of calluses converted to shoot on callusing medium supplemented with 50 mg/l glutamine and 40 mg/l adenine, after 80 days of culture. Plantlets with shoot were transferred to half strength of MS with different concentration of IBA and glutamine. Maximum rooting of 75.96% was observed on half strength of MS supplemented with 0.2% of IBA and 100 mg/l glutamine. The present study gives reliable *in vitro* regeneration protocol for *Ammi majus* L. from leaf explants, which may be used for future commercial-scale propagation and genetic study.

*Ammi majus* L. also known as bishop's weed from Apiaceae family is one of the wild pharmacopoeial plant species. Native origin of *Ammi majus* is northern Africa, southern Europe and western Asia (Queensland). At least 16 psoralens have now been identified in *Ammi majus*. The seed contains furanocoumarins (Hamerski and Matern 1988), which stimulate pigment production in the skin that is exposed to bright sunlight (Bown 1995, Chevallier 1996). Thus, this specie is one of the richest known sources of linear furocoumarins. This furocoumarins when activated by sunlight acts as bactericidal, herbicidal, fungicidal, mollscicidal, larvicidal, nematicidal, insecticidal, ovicidal and viricidal. Therefore it is considered as a natural pesticide. An infusion is used to calm the digestive

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system, whilst it is also used in the treatment of asthma and angina (Chevallier 1996). Some isolated coumarin compounds showed anti-inflammatory and anti-viral activity (Abdelaal Selim and Hassan Ouf 2012). The yellowish brown powder of fruit is prepared for use in the treatment of leukoderma (vitiligo) (Ossenkoppele et al. 1991, Schoenberg and Sina 1947). Also in 1982, the FDA (USA) approved PUVA as a treatment of severe cutaneous psorasis. *Ammi majus* is often cultivated for its attractive flowering stems which are often sold in the markets (Bown 1995). In Europe the growth of *A. majus* is poor due to the cool climate (light frosts). Attempts to acclimatize *A. majus* in central european climatic conditions were not successful, the fruits failed to ripe in moderate climatic zones and plants were highly susceptible to infection (Purohit et al. 1995). Economic potential of the plant is and will continue to be high for the widespread use of its galenicals as well as its furanocumarins in the treatment of leucoderma. The plant is endangered because; the cultivation of the plant is not favored by the farmers. This is referred to two reasons: (i) the lack of knowledge for cultivation of this plant among the farmers and (ii) the improper harvest methods usually lead to the shedding of the fruits leading to their dispersal and infesting the field in the next season (Batanouny 1999).

A few scientists have reported *in vitro* tissue culture protocols with different combinations of plant growth hormones (Purohit et al. 1995, Ekiert and Gomółka 2000, Pande et al. 2000, Staniszewska et al. 2003) and reliable genomic DNA isolation protocol from different organs of the *A. majus* (Magdum 2013). The objective of the present study was to establish reliable regeneration protocol for *Ammi majus*, which can subsequently be used for easier cultivation, propagation and plant genetic study and engineering for their properties of secondary metabolic production and their enhancement.

The seeds of *Ammi majus* were procured from plants growing in Herbal Garden of Jamia Hamdard University, New Delhi, India. To obtain seedlings, seed were surface sterilized and inoculated on gelled agar MS media. The seedlings served as a source of explants. Culture media consisted of MS. Optimum level of auxin and cytokinin was standardized to obtain best regeneration. The media were adjusted to pH 5.7 prior to autoclaving for 15 min at 15 lb ps and 120°C. All cultures were maintained at 25°C under white fluorescent light (1500 Lux) with 10 hrs light and 14 hrs dark cycle. The seedlings served as a source of explants. Leaf explants of from young sterile *in vitro*-obtained seedlings of *Ammi majus* were excised and used for organogenesis experiments. Leaf segments cultured on MS media modified by the addition of 30 g/l sucrose, 7 g/l agar, and various combinations (Table 1) of IAA (0.5 - 3.0 mg/l), Kn (0.5 - 3.0 mg/l) and casein hydrolysate (0- 1000 mg/l). Induced calli

from leaf explants were subcultured in every 2 weeks interval. After 4 weeks, leaf explants

**Table 1. Effect of different concentration for IAA, Kn, CH, adenine and glutamin on shooting response and effect of different concentration of IBA and glutamine on rooting response of *Ammi majus*.**

| IAA (mg/l) | Kn (mg/l) | CH (mg/l) | IBA (mg/l) | Glutamine (mg/l) | Adenine (mg/l) | Shoot Initiation (%) | Root Initiation (%) |
|---|---|---|---|---|---|---|---|
| Shoot induction | | | | | | | |
| 0.5 | 1 | 500 | - | Nil | Nil | 20.52 ± 6.14 | - |
| 0.5 | 2 | 1000 | - | 20 | 20 | 47.65 ± 5.79 | - |
| 1 | 1 | 500 | - | Nil | Nil | 30.87 ± 6.75 | - |
| 1 | 2 | 1000 | - | 50 | 40 | 72.43 ± 4.50 | - |
| 2 | 2 | 1000 | - | 50 | 40 | 81.32 ± 3.54 | - |
| 1 | 2 | 1000 | - | 75 | 60 | 69.95 ± 3.90 | - |
| 2 | 2 | 1000 | - | 75 | 60 | 70.65 ± 4.07 | - |
| 1 | 2 | 1000 | - | 100 | 80 | 54.55 ± 5.02 | - |
| 2 | 2 | 1000 | - | 100 | 80 | 56.23 ± 3.23 | - |
| Root induction | | | | | | | |
| - | - | - | 0.0 | 100 | - | - | 16.87 ± 5.80 |
| - | - | - | 0.2 | 100 | - | - | 75.96 ± 3.11 |
| - | - | - | 0.4 | 100 | - | - | 54.92 ± 4.54 |
| - | - | - | 0.6 | 100 | - | - | 38.90 ± 4.37 |

The results are the mean SE of 5 replicates, SE : Standard error.

were converted in to green calli. These calli were cut to 300 mg pieces and transferred to shoot induction media. MS supplemented with similar quantity of IAA, Kn and CH in addition of amino acids, filter-sterilized Adenine and glutamine were also added to some media after autoclaving. There were 5 replicates of each treatment. Adventitious shoot cultures were subcultured in every 2 weeks interval. Observed shoots were subcultured onto stock culture media for root formation. Induced shoots of about 4 cm in length were transferred to root induction media containing half strength of MS in addition 10 g/l sucrose and 7 g/l agar with various concentrations of IBA (0.1 - 0.4 mg/l ) and glutamine. Selected rooted plantlets were acclimatized in sterile soil containing small pot covered with plastic to maintain humidity placed in the culture room for 3 weeks and then placed in sunlight. The regeneration experiment was carried out with three sets of replicates from independently obtained calli.

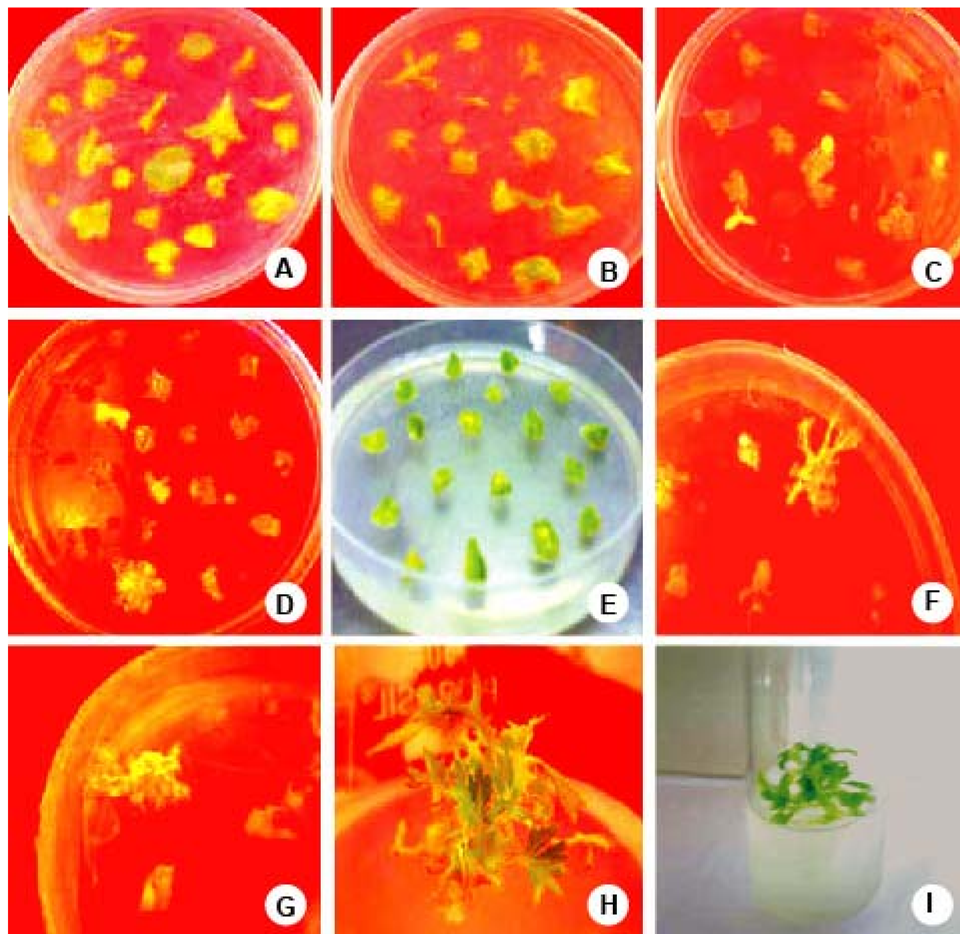


Fig. 1. Juvenile leaves of *Ammi majus* were cultured on MS supplemented with different combination of IAA, Kn and casein hydrolysate (CH) for callus induction (A, B). 97.7% green calli appeared after 2 weeks (C, D). After same size cutting of calli, they were cultured for shoot induction on different combination of hormones (E). Initiation of multiple shoots from green callus (F, G). Regeneration of multiple shoots from *Ammi majus* (H). Regenerated shoots were cultured in the rooting medium (I).

After sterilization within a week seed germination started and after 2 weeks developed juvenile plantlets were obtained. Observed seed germination rate was 59%, with no contamination, obtained successfully. Then, juvenile leaves were cultured on different combination of IAA, Kn and CH (Fig. 1A, B). Green callus from leaf explants were observed on the cut surfaces within 2 weeks. After 4 weeks, leaf explants converted into green calli and best calli formation obtained with 97.7% on MS containing 2 mg/l IAA and 2 mg/l Kn with 1000 mg/l CH (Fig. 1C). Five weeks were required for sustained growth before transferring to

shooting media (Fig. 1D). IAA concentration decreased and increased by 1 mg/l in callus induction media affects on percentage callus induction with 82.03 and 79.44, respectively. Different combination of IAA, Kn, CH, adenine and glutamine were used and the highest regeneration 81.32% (Table 1) were observed using MS supplemented with 2 mg/l IAA, 2 mg/l Kn, 1000 mg/l CH, 50 mg/l glutamine and 40 mg/l adenine (Fig. 1F, G). Sustained shooting also needs 5 weeks of incubation (Fig. 1H). The effect of high amino acid concentration resulted in reducing percentage of shooting. Three combinations of IBA (0.1 - 0.4 mg/l) and glutamine were used for rooting experiments (Fig. 1I). Best rooting was observed with 75.96% induction using half strength of MS, 10 g/l sucrose and 7 g/l agar supplemented with 0.2 mg/l IBA and 100 mg/l glutamine (Table 1). Rooted plants were acclimatized on sterile soil watered every day in culture room. After 3 weeks plants were transferred to the field with 60% survival rate.

In the present study, we standardized media composition for organogenesis of *Ammi majus* from leaf. Knowing medicinal importance of *Ammi majus*, hairy root culture option is also emerging to get rapid and high yield (Królicka et al. 2001). Purohit et al. (1995) reported a high frequency of regeneration of callus from cotyledonary leaves of *Ammi majus* on MS supplemented with IAA + Kn + casein hydrolysate (CH), differentiated shoots in 100% cultures when adenine was added to the above medium. The regeneration protocol of *A. majus* can be exploited to multiply elite genotypes and also for developing *in vitro* strategies for the conservation of this useful medicinal plant. This study has also opened new frontiers for genetic manipulation of *Ammi majus* for disease, pest resistance or enhancing secondary metabolites, using a rapid regeneration protocol.

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