In vitro cultivation of medicinal plant *Anethum graveolens* L.

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Abstract:

Micropropagation is one of the reliable methods of *in vitro* studies by which large number of pathogen free plants can be produced. Micropropagation is the true-to-type propagation of a selected genotype using *in vitro* culture technique. The survival, multiplication and field establishment of cultures depend upon a variety of factors such as origin of cultures, physiological stages of explant, endogenous hormone level and culture environment like nutrient medias, photoperiod, CO_2 concentration, temperature, etc. Each species is unique in these requirements. Medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. For present study an annual aromatic herb Dill (*Anethum graveolens* L.) was selected which belong to Apiaceae family. For micropropagation MS media was supplemented with 2,4-dichlorophenoxy acetic acid (2, 4-D). 2, 4-D was used in different concentrations. On increasing concentration of 2-4 -D a gradual decrease in the percentage of explants forming callus was noted.

Keywords: *Anethum graveolens* L., micropropagation, 2,4- dichlorophenoxy acetic acid.

Introduction:

Natural products had been indispensably used by many cultures and traditions in folklore medicines for thousands of years. The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. For present study an annual aromatic herb Dill (*Anethum graveolens* L.) was selected which belong to Apiaceae family. *Anethum graveolens* L. is an annual aromatic branched herb known for culinary use, since ancient times. Dill is native of South-east Europe and is cultivated commercially in most parts of Europe. The species of family Apiaceae are well known source of many important herbal products (Ekiert, 2000). Its foliage, fruits and their volatile oil are extensively used for culinary and medicinal purposes. This multipurpose herb has history of associated for the making of perfumery, insecticides, and traditional Iranian medicine.

Micropropagation is one of the easiest and fastest methods of producing numerous plantlets in limited period (Bhojwani and Razdan, 1996). *In vitro* procedures have been adopted for large scale propagation of medicinal

plants (Borthakur *et al.*, 2000; Liu *et al*, 2003; Aricat *et al.*, 2004). Tissue culture techniques have the potential to provide secure conservation method. The term plant tissue culture broadly refers to the *in vitro* multiplication of plants through plant parts (tissue, organs, embryo, single cells, protoplasts etc) on nutrient medium under aseptic conditions.

Callus is an unorganized mass of cells and formed by the vigorous division of plant cells. Callus cultures are a potential source of pharmaceutical important plant metabolites. Biosynthesis of secondary metabolites can improve in callus through feeding culture medium by certain nutrients, precursors or elicitors as their building blocks or certain intermediary compounds of their biosynthesis cycle (Namdeo, 2007). Callus culture should produce an embryo and subsequently plantlets. Establishment of callus culture has considerable potential as an alternative to traditional agriculture for the production of plant secondary metabolites and obtaining useful drugs. The advantages of callus culture is provide continuous and constant year that supply of natural plant products, easy method for isolation of medicinally important active compound in pure form; remove all limitations of plant field cultivation (Berlin, 1988; Mulder-Krieger *et al,* 1988; Nin *et al.*, 1996).

Material and Method:

Dill (*Anethum graveolens* L.) plants were grown and maintained in the Department of Botany, Gujarat University, Ahmedabad. Dill plants were grown (5.6 pH) in sandy loam soil with 42% to 45% humidity. Temperature was maintained at 30° C to 35° C and plants were irrigated at every 3 days intervals.

In the present study, MS (Murashige and Skoog's, 1962) medium were used for best response in the explants on optimized PGRs concentration. In the present study, 2,4- dichlorophenoxy acetic acid (2, 4-D) were used in different concentrations i.e. 2, 4,6,8,10 mg/ml.

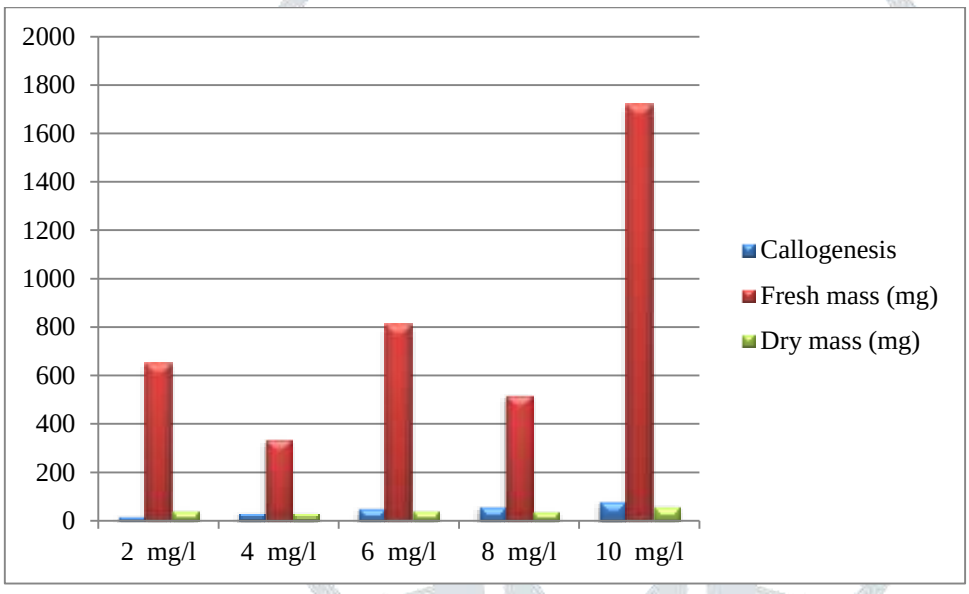
Result and discussion:

Natural products like plant extract, pure compounds and standardized extracts, provided unlimited opportunities for new drug discoveries because of the unmatched chemical diversity they can provide (Cos *et al.*, 2006). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The dill essential oil has hypolipidemic activity, could be used as a cardio-protective agent in decreasing cholesterol (Hajhashemi and Abbasi, 2008). Fruits of this plant are a rich source of different groups of metabolites, e.g. volatile oil, coumarins, flavonoids, phenolic acids, fatty oil and minerals (Heywood, 1971).

During present investigation it was observed that direct organogenesis thorough nodal explants were grown on different phytohormone concentrations were expanded in size within 15 days of inoculation. Callus was initiated on medium containing 2, 4-D alone and with NAA. On increasing concentration of 2-4 -D a gradual decrease in the percentage of explants forming callus was noted. Higher concentrations of 2-4 D were induced

callus formation. The colour of callus was light green yellowish. Within 3 weeks of cultured period, 89% callogenic response was achieved when the medium was fortified with 2, 4-D at different concentrations for nodal explants (Fig-1). 2, 4-D is a synthetic auxins; its role in callus induction was particularly observed in experiments. Efficacy of exogenous 2, 4-D has also been reported with other medicinal plants. Results described by Mungole *et al*., 2009 and Hassan *et al*., 2009 were also resulted in using this synthetic plant growth regulator in the culture medium for *Ipomea obscura*, *Withania somnifera*, *Cardiospermum halicacabum Linn.* and *Abrus precatorius* respectively.

Fig-1: Effect of different concentrations of 2, 4-D on callogenesis and biomass of *Anethum graveolens* L.



Dill has reported as anticancer (Zheng *et al.*, 1992), anti-diabetic (Panda, 2008), antioxidant (Al-Ismail and Aburjai, 2004; Styanarayana *et al.*, 2004; Bahramikia and Yazdanparast, 2009), antisecretory (Hosseinzadeh *et al.*, 2002), antosplasmodic (Naseri and Heidari, 2007); cytotoxic to human lymphocytes (Lazutka *et al.*, 2001), insecticidal (Mazyad *et al.*, 1999; Khalaf , 2004; Chauby, 2008; Seo *et al.*, 2009) and diuretic (Mahran *et al*., 1992). Monsefi *et al.* (2006) reported female rates to access the effects of *Anethum graveolens* L. On female reproductive system, it has been found that dill can be used as a regulatory agent of the menstrual cycle.

Due to all these advantages, various methods of clonal propagation of medicinal plants have been reported. It can be achieved through rapid proliferation of shoot tips and axillary buds in culture (Tejavathi *et al*. 2001; Salvi *et al*. 2002). Recently , *in vitro* propagation procedures have been adopted for large scale propagation of several medicinal plant(Borthakur *et al*, 2000; Reddy *et al*, 2001; Liu *et al*, 2003; Aricat *et al*, 2004; Jabeen

et al,2007; Keng *et al*,2009). Several reports are available on the regeneration of varoius medicinal plants through callus culture (Saxena *et al*. 1997; Patra *et al*. 1998) Shekhawat *et al*. (2002) developed a protocol for *Azadirachta indica.* Sachdev *et al*. (2002) studied that embryogenesis and plantlet formation in callus culture of *Gloriosa superba.*

Conclusion: *Anethum graveolens* L. (dill) has been used in ayurvedic medicines since ancient times and it is a popular herb widely used as a spice and also yields essential oil. Micropropagation is one of the easiest and fastest methods of producing numerous plantlets in limited period. It was observed that direct organogenesis thorough nodal explants were grown on different phytohormone concentrations were expanded in size within 15 days of inoculation. Callus was initiated on medium containing 2, 4-D alone and with NAA. On increasing concentration of 2-4 -D a gradual decrease in the percentage of explants forming callus was noted.

References:

1. Al-Ismail, K. Aburjai. T. 2004. Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds, Journal of Science of Food and Agriculture, 84:173-178.
2. Aricat, N. Jawad, F. Karam, N. Shibli. R. 2004. Micropropagation and accumulation of essential oils in wild sage (*Salvia Fruticosa* Mill), Journal of Scientia Horticulturae**,** 100: 193-202.
3. Bahramikia, S. Yazdanparast. R. 2009. Efficacy of different fractions of *Anethum graveolens* leaves on serum lipoproteins and serum and liver oxidative status in experimentally induced hypercholestero-laemic rat models, American Journal of Chinese Medicine, 37: 685-699.
4. Berlin, J. 1988. Formation of secondary metabolites in cultured plant cells and its impact onPharmacy, In: Biotechnology in Agriculture and forestry 4-Medicinal and Aromatic Plants I (Bajaj TPS Ed) Springer-Verlag, Berlin, 37-59.
5. Borthakur, M. Dutta, K. Nath, S. and Singh. R. 2000. Micropropagation of *Eclipta alba* and *Eupatorium adenophorum* using a single step nodal cutting technique. Journal of Plant Cell Tissue and Organ Culture**,** 62: 239-242.
6. Bhojwani, S. Razdan. M. 1996. Journal of Plant Tissue Culture: Theory and Practice, Elsevier Publications, Amsterdam, the Netherlands.
7. Ekiert, H. Gomołka. E. 2000. Coumarin compounds in *Ammi majus* L. callus cultures. Journal of Pharmazie, 55: 684–687.
8. Chaubey, M. 2008. Fumigant toxicity of essential oils from some common spices against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Journal of Oleo Science, 57: 171-179.
9. Cos, P. Vlietinck, A. Berghe, D. Maes. L. 2006. Anti-infective potential of natural products: How to develop a stronger *in vitro* proof-of-concept, Journal of Ethanopharmacology, 106: 290-302.
10. Hassan, M. Azam, F. Chowdhury, M. Rahmatullah. M. 2009. Callus induction of *Abrus precatorius*: screening of phytohormones, American- Eurasian Journal of Sustainable Agriculture, 3(3): 512-518.
11. Hajhashemi, V. Abbasi, N. 2008. Hypolipidemic activity of *Anethum graveolens* in rats, Phytotherapy Research Journal, 22(3): 372-375.
12. Heywood, V. 1971. The Biology and Chemistry of the Umbelliferae, Academic Press INC, New York.
13. Hosseinzadeh, H. Karimi, G. Ameri. M. 2002. Effects of *Anethum graveolens* L. seed extracts on experimental gastric irritation models in mice, Journal of BMC Pharmacology and toxicology, 2: 21.
14. Jabeen, N. Shawl, A. Dar. G. 2007. Micropropagation of *Inula racemosa* Hook, A valuable medicinal plant, International Journal of Botany**,** 3: 296-301.
15. Khafagi, I. Dewedar, A. Amein. M. 2003. Oppertunities of finding novel anti-infective agents from plant cell cultures, Journal of Current Medicinal Chemistry Anti-Infective Agent, 2: 191-211.
16. Keng, C. Yee, L. Pin. P. 2009. Micropropagation of *Gynura procumbens* (Lour.) Merr. -an important medicinal plant, Journal of Medicinal Plants Research, 3(3): 105-111.

17. Lazutka, J. Mierauskiene, J. Slapsyte, G. Dedonyte. V. 2001. Genotoxicity of dill (*Anethum graveolens* L.), peppermint (*Mentha piperita* L.) and pine (*Pinus sylvestris* L.) essential oils in human lymphocytes and *Drosophila melanogaster*, Journal of Food and Chemical Toxicology, 39: 485-492.
18. Liu, C. Murch, S. Demerdash, M. and Saxena. P. 2003. Regeneration of the Egyptian medicinal plant *Arstemisia judaica* L., Journal of Plant Cell Reports**,** 21: 525-530.
19. Mahran, G. Kadry, H. Isaac, Z. Thabet, C. Al-Azizi M. El-Olemy. M. 1992. Investigation of diureic drug plats, Phytochemical investigation and pharmacological evaluation of *Anethum graveolens* L., *Apium graveolens* L., *Daucus carota* L. and *Eruca sativa* Mill, Journal of Phytotherapy Research, 5: 169-172.
20. Mazyad, S. El-Serougi, A. Morsy. T. 1999. The efficacy of the volatile oils of three plants for controlling *Lucilia sericata*, Journal of Egyptian Society of Parasitology, 29: 91-100.
21. Monsefi, M. Ghasemi, M. Bahaoddini. A. 2006. The effects of *Anethum graveolens* L. on female reproductive system. Phytotherapy Research, 20(10):865–868, doi: 10.1002/ptr.1959.
22. Mulder-Krieger, T. Verpoorte, R. Barhein Svedsen, A. Scheffer. J. 1988. Production of essential oils and flavours in plant cell and Tissue cultures, A review, Plant cell Tissue and Organ Culture, 13: 85-154.
23. Mungole, A. Awati, R. Dey, S. Chaturvedi, A. Zanwar. P. 2009. *In vitro* callus induction and shoot regeneration in *Ipomoea obscura (L.)*, potent Indian medicinal plant, Indian Journal of Science and Technology, 2(8): 24-26.
24. Murashige, T. Skoog. F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Journal of Physiologia Plantarum, 15:473-497.
25. Namdeo, A. 2007. Plant Cell Elicitation for Production of Secondary Metabolites: A Review, Pharmacognosy Reviews, 1: 69-79.
26. Naseri, M. Heidari. A. 2007. Antispasmodic effect of *Anethum graveolens* fruit extract on rat ileum, International Journal of Pharmacology, 3: 260-264.
27. Nin, S. Morosil, E. Schiff, S. Bennici. A. 1996. Callus culture of *Artemisia absinthium* L: initiation, growth optimization and organogenesis, Journal of Plant Cell Tissue and Organ Culture, 45: 67-72.
28. Patra, A. Rai, B. Rout, G. Das. P. 1998. Successful plant regeneration from callus cultures of *Centella asiatica* (Linn.) Urban, Journal of Plant Growth Regulation, 24: 13–16.
29. Panda, S. 2008. The effect of *Anethum graveolens* L. (dill) on corticosteroid induced diabetes mellitus: involvement of thyroid hormones, Journal of Phytotherapy Research, 22(12):1695–1697.
30. Reddy, S. Rodrigues, R. Rajashekharan. R. 2001. Shoot organogenesis and mass propagation of *Coleus forskohlii* from leaf derived callus, Journal of Plant Cell Tissue and Organ Culture, 66: 183-188.
31. Salvi, N. George, L. Eapen. S. 2002. Micropropagation and field evaluation of miccropropagated plants of turmeric, Journal of Plant Cell Tissue Organ Culture, 68: 143–151.
32. Sachdev, K. Prakash, H. Prasad T. Sathyanarayana. B. 2002. Somatic embryogenesis and plantlet regeneration from node and leaf callus cultures of *Gloriosa superba*, Journal of Plant Biology, 29 (2): 179-190.
33. Saxena, C. Palai, S. Samantaray, S. Rout, G. Das. P. 1997. Plant regeneration from callus cultores of *Psoralea corylifolia* Linn. Journal of plant growth regulation, 22: 13–17.
34. Satyanarayana, S. Sushruta, K. Sarma, G. Srinivas, N. Subba. R. 2004. Antioxidant activity of the aqueous extracts of spicy food additives--evaluation and comparison with ascorbic acid in *in vitro* systems, Journal of Herbal Pharmacotherapy, 4: 1-10.
35. Shekhawat, G. Batra, A. Mathur. S. 2002. A reliable *in vitro* protocol for rapid mass propagation of *Azadirachta indica* Juss, Journal of Plant Biology, 29: 109-112.
36. Seo, S. Kim, J. Lee, S. Shin, C. Shin, S. Park I. 2009. Fumigant antitermitic activity of plant essential oils and components from Ajowan (*Trachyspermum ammi*), Allspice (*Pimenta dioica*), Caraway (*Carum carvi*), Dill (*Anethum graveolens*), Geranium (*Pelargonium graveolens*), and Litsea (*Litsea cubeba*) oils against Japanese termite (*Reticulitermes speratus* Kolbe), Journal of Agriculture and Food Chemistry, 57: 6596-6602.
37. Tejavathi, D. Sowmya, R, Shailaja. K. 2001. Micropropagation of *Bacopa monnieri* using shoot tip and nodal explant, Journal of Tropical Medicinal Plants, 2 (1): 39-45.

38. Zheng, G. Kenney, P. Lam. L. 1992. Anethofuran, carvone and limonene: Potential cancer chemoprotective agents from dill weed oil and caraway oil, Planta Medica, 58(4):338-341.