IN-VITRO PROPAGATION OF CHAMOMILLA RECUTITA FROM CAPITULUM INFLORESCENCE: A MEDICINAL PLANT WITH MULTIPLE THERAPEUTIC APPLICATIONS

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ABSTRACT

A protocol has been developed for induction of somatic embryogenesis from whole inflorescence explants of *Chamomilla recutita* L. (chamomile). Chamomile is a well-known medicinal plant from the Asteraceae family often referred to as the "star among medicinal species." Nowadays, it is a highly favoured medicinal plant in folk and traditional medicine. Its multitherapeutic, cosmetic and nutritional values have been established through the years of traditional and scientific use and research. Chamomile has an established domestic (Indian) and international market, which is increasing day by day. Among the various major constituents, α-bisabolol and chamazulene have been reported to be more useful than others. Chamazulene occurs in the capitula of the flowers in minute quantities and has been demonstrated to exert anti-inflammatory activity *in-vivo*. Moreover, chamomile is a seasonal 4-5 months winter crop in India but is extensively required in various medicinal applications. Therefore, to increase the overall yield of this plant, its *in-vitro* propagation is needed. In the present study, somatic embryos were developed from capitulum explants after 2-4 weeks of culture on MS medium supplemented with 26.8 µM NAA and 11.5 µM Kin. The somatic embryos were further subcultured *in-vitro,* where new plantlets regenerated from embryos. It is concluded that *in-vitro* propagation is possible in case of chamomile and can be used to increase the overall yield of chamazulene present in the capitula of flowers as well as augment the overall yield of this important plant, which is conventionally propagated by seeds.

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Key words: Capitulum, *Chamomilla recutita*, Inflorescence, Plant regeneration , Somatic embryogenesis,
Abbreviations: NAA α-napthalene Acetic Acid, Kin Kinetin, MS medium Murashige and Skoog (1962) basal medium.

INTRODUCTION

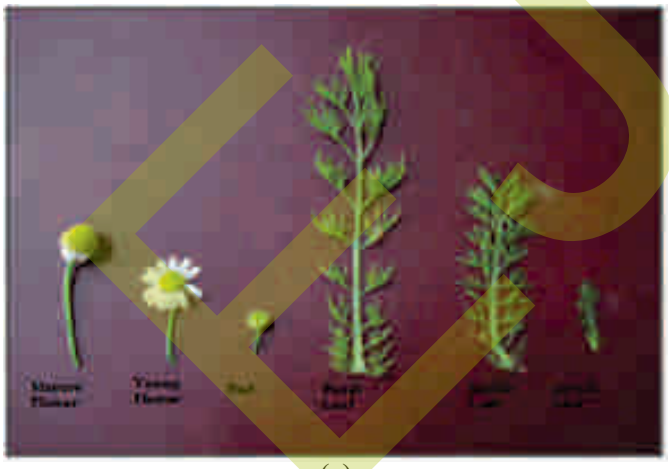
Chamomilla recutita (synonyms: *Matricaria recutita*, *Matricaria chamomilla*), commonly known as chamomile (also spelled camomile), German chamomile, Hungarian chamomile (kamilla), wild chamomile or scented mayweed, is an annual plant belonging to Asteraceae family. *Chamomilla recutita* is the most popular source of the herbal product chamomile, although other species are also used as chamomile. Chamomile is one of the important medicinal herbs native to southern and eastern Europe. Hungary is the main producer of the plant biomass. In Hungary, it also grows abundantly in poor soils and it is a source of income to poor inhabitants of these areas. Flowers are exported to Germany in bulk for distillation of oil (1).

It was introduced to India during the Mughal period, now it is grown in Punjab, Uttar Pradesh, Maharashtra and Jammu & Kashmir. It was introduced in Jammu in 1957 by Handa et al (2). The plant was first introduced in alkaline soils of Lucknow in 1964-65 by Chandra et al (3,4). There is a great demand for flowers of chamomile (Fig.1). Presently, two firms, namely, M/s Ranbaxy Labs Limited, New Delhi and M/s German Remedies are the main growers of chamomile for its flowers.

Chamomile has been used in herbal remedies for thousands of years and has been included in the pharmacopoeia of 26 countries (5).

(a)

(b)



(c)

Fig: 1 (a) Chamomile recutita Habit (b) Flowers (c) Flower and Leaf Morphology

It is an ingredient of several traditional, Unani and homeopathy medicinal preparations (6-9). As a drug, it finds use in flatulence, colic, hysteria and intermittent fever (10). The flowers of *M.* chamomilla contain the blue essential oil from 0.2 to 1.9% (11, 12) which finds a variety of uses. Chamomile is used mainly as an anti-inflammatory and antiseptic, also as an antispasmodic and mildly sudorific (13). The other pharmacological properties include carminative, healing, sedative and spasmolytic activity (14). M. chamomilla has been shown to exhibit both positive and negative bactericidal activity with *Mycobacterium tuberculosis*, *Salmonella typhimurium* and *Staphylococcus aureus.*

The international demand for chamomile oil has been steadily growing. As a result, the plant is widely cultivated in Europe and has been introduced in some Asian countries for the production of its essential oil. The oil is used as a mild sedative(15,16) and also for digestion(17-19) besides being antibacterial and fungicidal in action (20,21). In addition to pharmaceutical uses, the oil is extensively used in perfumery, cosmetics and aromatherapy,(22-24) and in food industry (25). Gowda et al (26)found that the essential oil present in the flower heads contains azulene and is used in perfumery, cosmetic creams, hair preparations, skin lotions, tooth pastes, and also in fine liquors (26). The dry flowers of chamomile are also in great demand for use in herbal tea, baby massage oil, for promoting the gastric flow of secretion and for the treatment of cough and cold (27).

With an ever-increasing global inclination towards herbal medicine, there is not only an obligatory demand for a huge raw material of medicinal plants, but also of the right stage when the active principles are available in optimum quantities at the requisite time for standardization of herbal preparations. Commensurate with this, the intervention of biotechnology or to be precise, plant tissue culture for accelerating clonal multiplication of desired clones and strains (high-yielding) of medicinal plants through micro propagation and their conservation through establishing Tissue Banks or Gene Banks are warranted in the right earnest. Ideally, the herbal plants should be grown under uniform environmental conditions and the planting material must have the same genetic make-up as of the selected high-yielding clones, which is possible when they are cloned through an in vitro strategy, that is, micro propagation, at least in cases where conventional vegetative propagation methods are insufficient or wanting to achieve the goal. In the

present study, a protocol has been developed for rapid in-vitro propagation of *Chamomilla recutita.*

MATERIALS AND METHODS

Plant material and disinfection

This research work was carried out in the School of Life Sciences, ITM University, Gwalior, MP, India. In this study, young inflorescences from *Chamomile recutita* were used as explants. All explants were washed under running tap water for 10 min then surface sterilized in a 70% ethanol for 60 seconds followed by sodium hypochlorite solution (NaCl with 1% active chlorine) for 10 min. This was followed by 2-3 rinses in distilled water.

Culture conditions

Immediately after surface sterilization, the explants were transferred to laminar airflow cabinet and further processes were carried out under aseptic conditions. The explants were carefully transferred to another Petridish containing sterilized blotting paper. All petals and sepals from the explants were excised with the help of sterilized blades and forceps and the explants were carefully inoculated on basal MS medium (Murashige & Skoog, 1962) supplemented with 26.8 µM NAA, 11.5 µM Kin, 3% (w/v) sucrose and solidified with 0.7% (w/v) agar. Sucrose was used as the carbon source and agar as solidifying agent. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 min and poured into 800x800mm glass jars (50 ml medium per jar and four explants per jar). Inoculated jars were sealed with Parafilm™and incubated at 25°C on tissue culture racks under controlled light regime (16:8 h light/dark photoperiod) supplied by cool-white fluorescent lamps and the changes were observed.

Somatic embryo development

After 2-4 weeks in culture all callus tissues (with or without somatic embryos at globular stage) were transferred to solid media having the same composition as the induction medium from which they were removed. Culture in jars was maintained for 4-8 weeks at 25°C under controlled light regime (16:8 h light/dark photoperiod) supplied by cool-white fluorescent lamps.

Somatic embryo maturation and germination

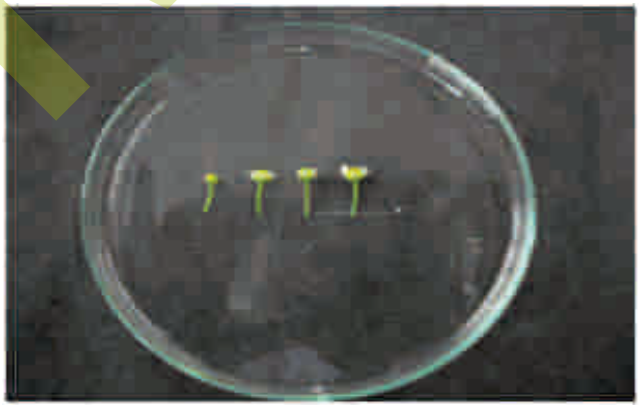
Cotyledonary stage embryos were dissected from callus tissues and transferred onto solid induction medium where they were maintained for 4-5 weeks at 25°C under controlled light regime (16:8 h light/dark photoperiod) supplied by cool-white fluorescent lamps.

Sub-Culturing of Roots, Shoots and Embryos

The jars containing developing embryos were opened under aseptic conditions and the embryos taken out very carefully using sterilized forceps. Roots and shoots were separated very carefully from the embryos. All three were sub-cultured in separate jars containing MS media.

RESULTS

After 7-10 days of culturing, some of the cultured explants (Fig. 2a) showed adaptation to the environment while others showed necrosis (browning of explants) (Fig. 2b). In 15-18 days, early stages of callus formation were observed (Fig. 2c, d). Complete callus formation occurred within 4 weeks of culture initiation (Figs.2e,f). The combination of an auxin with a cytokinin was more favorable for callus induction than addition of only one plant growth regulator. Somatic embryogenesis in callus tissue was initiated within 30 days of explant culture (Fig. 2g). Embryoids neither formed nor developed on media not containing growth regulators or supplemented only with auxin or cytokinin. The plumules and radicles were excised from the embryos and sub-cultured separately (Fig. 2h). Leaves could be seen arising from the plumule, roots developing from the radicle. whereas callus forming on embryos within 2 weeks of subculturing (Fig. 2i).



(a)

(b)

(c)

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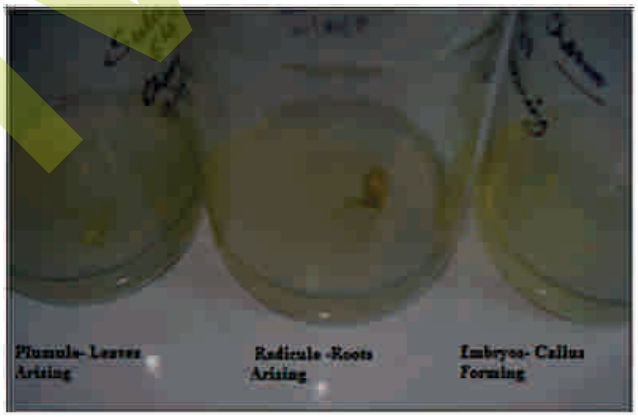
(g)

(h)



(i)

Fig.2 (a) Sterilized capitula from Chamomile as explants. (b) Inoculation of explants on MS medium. (c,d) early. and (e,f) late stages of callus formation from cultured explants. (g) somatic embryogenesis from transferred callus tissue. (h,i) Sub-culturing of excised radicle, plumule and embryos

DISCUSSION

Tissue culture is the culture and maintenance *in vitro* of plant cells or organs in sterile, nutritionally and environmentally supportive conditions. It has applications in research and commerce. In commercial settings, tissue culture is often referred to as micro propagation, which is really only one form of a set of techniques. Micro propagation refers to the production of whole plants from cell cultures derived from explants, the initial piece of tissue put into culture of meristem cells. In literature, there are reports on tissue culture and micro propagation of chamomile using various plant parts as explant sources. Stem and leaf explants have been used for the induction of callus cultures; this approach has been taken by Reichling and Baker (28) for the establishment of callus cultures from two chamomile varieties (E40 and BK2) on a modified MS medium supplemented with 2.4 µM NAA (28). Reichling et al. (29), isolated a callus culture from surface-sterilized shoot segments of the chamomile variety BK2 on a modified MS medium supplemented with NAA (27.7 µM) and Kin (11.9 µM) (29). Somatic embryogenesis has also been induced from disc florets of capitulum inflorescence of chamomile (30). Szoke et al(31, 32) obtained callus tissues from root, stem and flower clusters of wild chamomile. They studied the dynamics of growth of callus tissues on the basic growth medium containing 2,4-Dichlorophenoxyacetic acid (2, 4-D) and Kin in light and dark. It was observed that the growth of inflorescence callus, either cultured in light or dark, was sensitive to added growth regulators. It grew better with kinetin + 2, 4-D. Cellarova et al (33) have dealt with the possibility of morphogenesis induction in callus tissue cultures of some representatives of *M. chamomilla*. Shoots in calli have been induced by 0.1 mg/L Kin or by combination of 0.5 mg/L Kin and 0.5 mg/L NAA added to Murashige and Skoog medium. Rhizogenesis took place without any other addition of auxin. So far, there has been no report of chamomile micro propagation from the whole capitulum (flower) head and this study entails an efficient method for *in-vitro* propagation of chamomile from the whole capitulum inflorescence.

The multiple uses of medicinal plants in traditional systems of medicine such as Ayurveda, Unani, and Siddha have increased their commercial demand resulting in their over-exploitation. Because of destructive harvesting, the natural populations of a number of medicinal plants are rapidly disappearing and they are recognized as 'vulnerable'. The development of efficient micro propagation protocols for such species would play a significant role in meeting the requirements for commercial cultivation, thereby conserving the species in their natural habitat.

CONCLUSION

In the present study, an efficient protocol was developed for rapid micro propagation of chamomile, a winter crop, from whole capitulum inflorescence. It can be concluded from the study that micro propagation can be used in chamomile as an alternative to conventional propagation by seeds to increase the yield not only of chamazulene but the entire herb for various medicinal uses.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Svab J. New aspects of cultivating chamomile. Herba Polonica. 1979:25:35-39.
2. Handa KL, Chopra IC, Abrol BK Introduction of some of the important exotic aromatic plants in Jammu and Kashmir. Indian Perfumer. 1957:1:42-49.
3. Chandra V.Cultivation of plants for perfumery industry at Lucknow. Indian Perfumer.1973: 16:40-44
4. Chandra V, Singh A, Kapoor LD.Experimental cultivation of some essential oil bearing plants in saline soils, Matricaria chamomilla L. Perfum Essent Oil Rec. 1968:59:871
5. Pamukov D, Achtardziev CH Natural pharmacy (in Slova). 1st ed. Priroda, Bratislava 1986.
6. Das M, Mallavarapu GR, Kumar S. Chamomile, (Chamomilla recutita): Economic botany, biology, chemistry, domestication and cultivation. J Med Aromat Plant Sci. 1998: 20:1074-1079.
7. Kumar S, Das M, Singh A, Ram G, Mallavarapu GR, Ramesh SJ. Composition of the essential oils of the flowers shoots and roots of two cultivars of Chamomilla recutita. J Med Aromat Plant Sci. 2001:23: 617-623.
8. Lawrence BM. Progress in Essential Oils. Perfume Flavorist. 1987:12:35-52.
9. Mann C, Staba EJ The chemistry, pharmacology and commercial formulations of chamomile. In: Craker LE, Simon JE, eds. Herbs, Spices and Medicinal Plants- Recent Advances in Botany, Horticulture and Pharmacology. Oryx Press: Phoenix; 1986.

10. Tyihak E, Sarkany-Kiss J, Verzar-Petri G. Phytochemical investigation of apigenin glycosides of Matricaria chamomilla. Pharmazie.1962:17:301-304.

11. Bradley P The British herbal compendium. In: Bradley P, eds. 1 ed., British Herbal Medicine Association: London;1992.

12. Mann C, Staba EJ The chemistry, pharmacology and commercial formulations of chamomile. In: Craker LE, Simon JE, eds. Herbs, spices and medicinal plants- recent advances in botany, horticulture and pharmacology. Haworth Press Inc: USA; 2002.

13. Mericli AH. The lipophilic compounds of a Turkish Matricaria chamomilla variety with no chamazuline in the volatile oil. Int J Crude Drug Res. 1990:28:145-147.

14. Salamon I. Chamomile a medicinal plant. J Herbs Spices Med Plants. 1992:10:1-4.

15. Gould L, Reddy CV, Compreht FF. Cardiac effect of chamomile tea. J Clin Pharmacol. 1973:13:475-479.

16. Das M, Mallavarapu GR, Kumar S. Isolation of a genotype bearing fascinated capitula in chamomile (Chamomilla recutita) J Med Aromat Plant Sci. 1999:21:17-22.

17. Gasic O, Lukic V, Adamovic R, Durkovic R. Variability of content and composition of essential oil in various chamomile cultivators (Matricaria chamomillaL.) Herba Hungarica. 1989:28:21-28.

18. Lal RK, Sharma JR, Misra HO. Vallary: An improved variety of German chamomile. Pafai J.1996:18: 17-20.

19. Lal RK, Sharma JR, Misra HO, Singh SP. Induced floral mutants and their productivity in German chamomile (Matricaria recutita) Indian J Agric Sci. 1993:63: 27-33.

20. Shahi NC. Traditional cultivation of Babunah Chamomilla recutita (L.) Rauschert syn. Matricaria chamomilla L. Lucknow. Bull Medico-Ethnobotanical Res. 1980:1:471477.

21. Sharma A, Kumar A, Virmani OP. Cultivations of German chamomile- a review.Curr Res Med Aromat Plants.1983:5: 269-278.

22. Anonymous:A superior variety of German chamomile identified. CIMAP Newsletter.1993:20: 13.

23. Earle L Vital oils. 1st ed., Ebury Press, London.1991.

24. Masada Y Analysis of essential oils by gas chromatography and mass spectrometry. 1st ed., John Wiley and Sons Inc., New York.1976.

25. Misra N, Luthra R, Singh KL, Kumar S, Kiran L. Recent advances in biosynthesis of alkaloids . (In: Nanishi K, O-Methcohn, eds. Comprehensive) natural product (CONAP), Elsevier Publisher, Oxford. pp. 2569.1999.

26. Gowda TNV, Farooqi AA, Subbaiah T, Raju B. Influence of Plant density, Nitrogen and Phosphorus on growth, yield and essential oil content of chamomile (Matricaria chamomilla Linn.) Indian Perfumers.1991:35: 168-72.

27. Anonymous. Azulene in pharmacy and cosmetics. Dragoco Rep. 1969:16: 23-5.

28. Reichling J, Becker H. Callus culturen von Matricaria chamomilla. J. Planta Med. 1976:30: 258-268.

29. Reichling J, Bisson W, Becker H. Vergleichende Untersuchungen zur Bildung und Akkumulation von etherischem Öl in der intakten Pflanze und in der Calluskultur von Matricaria chamomilla. Planta Med. 1984:56: 334-337.

30. Kintzois S, Michaelakis A Induction of somatic embryogenesis and in vitro flowering from inflorescences of Chamomile (Chamomilla recutita L.). Plant Cell Reports. 1999:18:684-690.

31. Szoke E, Verzar-Petri G, Shavarda AL, Kuzovkina IN, Smirnov AM. Differences in the essential oil composition in isolated roots, root callus tissues and cell suspensions of Matricaria chamomilla. Izvestiia Akadmii Nauk SSSR-Seriia Biolgicheskaia. 1980:6:943-949.

32. Szoke E, Kzovkina IN, Verzar-Petri G, Smirnov AM. Tissue culture of the wild chamomile. Fiziologiya Rastenii (Mosc) 1977:24: 83240.

33. Cellarova E, Grelakova K, Repcak M, Honcariv R. Morphogenesis in callus tissue cultures of some Matricaria and Achillea species. Biol Plant. 1982:24:430-436.