SHORT COMMUNICATIONS

In vitro Conservation of Exotic Mint Germplasm

Neelam Sharma, S Vimala Devi and Richa Satsangi
Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012

Key Words: Mint, Volatile Oil, *In vitro* Conservation

Mentha L. (Lamiaceae), commonly known as mint, is a taxonomically complicated genus that includes around 25 species of aromatic perennial and annual herbs apart from hybrids such as spearmint (*M. spicata*), peppermint (*M. pipperita*) and threatened species of global conservation interest (*M. gattefossei and M. requienii*). The plant is a native of temperate and subtemperate regions of the world.

All *Mentha* species contain varying amount of volatile oils including menthol, and other isolates like menthone, piperitone, iso-menthone, methyl acetate, carvone, linalyl acetate and linalol. The mint oil has a fine odour and also it is an excellent carminative, antiseptic preservative and gastrostimulant. Hence, it has a diverse utilization commercially in herbal products as well as in flovouring of foods, beverages, tobacco, mouthwashes, dental creams and medicinal products. This wider range of utilization has led to extensive cultivation of *Mentha* in many parts of world. It is now widely cultivated in USA, Europe, South and South-east Asia and Latin American countries. The whole plant including the freshly sprouted flowering buds have been used for commercial extraction of volatile oils.

The USDA-ARS National Clonal Germplasm Repository, Corvallis maintains world's largest germplasm repository on *Mentha*. It consists of about 580 mentha accessions representing 40 taxa and 130 interspecific hybrids. The germplasm is being maintained as clonal-field grown, clonal-*in vitro* maintained or potted plants (Chambers and Hummer, 1992).

Mint germplasm including many cultivars, even the two commercial types-peppermints and spearmints are sterile. These are conventionally propagated vegetatively by running stolons and offshoots. Plant tissue culture is a unique technique for multiplication of true to type plants and maintains the integrity of the genotype. Mints have been amenable to *in vitro* technique, making *in vitro* propagation and conservation of germplasm, a viable alternative to clonal field maintenance (Reed 1999, Tariqual Islam *et al.*, 2003). Tissue culture provides an easy and inexpensive way for international exchange of disease-free material.

One such international germplasm exchange, *Mentha* germplasm form USDA, Corvallis were received through germplasm exchange division, National Bureau of Plant Genetic Resources (NBPGR) in the form of *in vitro* collections and as live materials. From the live material, single nodal segments were surface sterilized (0.1% mercuric chloride for 7-10 minutes) and used for *in vitro* culture establishment. The material received as *in vitro* material was subcultured on Murashige and Skoog's 1962 (MS) medium supplemented with 0.5 mg/ l of 6-benzyladenine (BA) and 0.1 mg/ l of indole-3-butyric (IBA) for contamination free establishment. This was further subcultured using single nodes for initiation and maintained for 3 wks for establishment and to detect and eliminate contamination, if any. The cultures were maintained at 25 ±2°C under 16 h photoperiod (30 mmol/ m^2 / s^1) provided by cool white fluorescent lamps (Philips, Mumbai). As a part of long-term study, the healthy proliferating cultures after 3 wks were transferred to low temperature modules (4°C) for conservation. Periodic monitoring for survival of cultures was carried out.

A total of 22 accessions comprising EC 388948, EC 388950, EC 388951, EC 388953, EC 388955 to EC 388961, EC 388963, EC 388964, EC 388966 to EC 388969, EC 388971, EC 388972, EC 388974, EC 388978 and EC 388980 were established *in vitro*.

Twenty-two accessions of above mentioned exotic germplasm material were maintained in culture room by periodic subculture at 4-16 months interval. In the present study, low temperature incubation at 4°C without light has been found to be beneficial for mint conservation by prolonging subculture interval, as new sprouts were observed after 8-12 months. This study using slow growth technique by low temperature incubation has helped in conserving

twenty-two accessions of mint germplasm for eight years so far. In an earlier study, the beneficial effect of low temperature and diffused light on conservation of four mint species was reported (Reed 1999). The reduction of light intensity or total darkness in conjunction with low incubation temperature has been reported to effectively slow down the growth rate and enhance conservation period in many medicinal species (Chandel *et al.,* 1996, Sharma 2001). However, cold tolerant level may vary from species to species and accession to accession.

Plant tissue culture technique, besides its advantage in propagation and conservation, also has potential, if needed, to introduce genetic variability in mentha genotypes through somaclonal variations, wide hybridization (embryo rescue), somatic hybrids or transgenic plants. It is very important to conserve exotic germplasm with or without currently identified economically important traits. In India exotic mint germplasm has been utilized for crop improvement programme (Gupta, 1995). In this context, the conserved exotic germplasm of *Mentha* may serve as a nuclear material for utilization for genetic manipulation and crop improvement programme.

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Evaluating Germplasm Introductions of Underutilized Plants

RS Rathi, HL Raiger, BS Phogat and RP Dua
National Bureau of Plant Genetic Resources, New Delhi–110 012

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Germplasm introduction is the key to diversifying the crop composition and broadening the genetic base of existing crops in any area. Germplasm introduction and evaluation has led to introduction of several new crops and improvement of the existing crops in India. 3361 accessions of different underutilized plants have been introduced during the last fifty years (RC Agrawal, Personal Communication). Maximum introductions were made in faba bean (1016), followed by those in amaranth (645), su-babool (488), chenopods (216) and jojoba, in the descending order. Most of the accessions came from USA (1205), Syria (360), Australia (252), UK (180), Bulgaria (188) and Germany (154).

Evaluation of the introductions of underutilized plants under the aegis of All India Coordinated Research Project on Underutilized Crops led to identification of 395 promising lines (Anon. 2004), mainly in adzuki bean (92), winged bean (68), amaranth (72) and rice bean (53). Four of the promising lines were released for cultivation. Two new plants, namely, jojoba and paradise tree were adopted for cultivation. Project on cultivation and oil extraction has been taken up by Government of Rajasthan while paradise tree has been adopted by NOVOD Board and the Departments of Forest of Tamil Nadu, Karnataka, Orissa and Maharashtra for large scale cultivation. Seeds of jojoba contain 45 per cent oil suitable