# Mint (*Mentha* spp) germplasm conservation in Brazil

**Silva, D. B.; Vieira, R. F.; Alves, R. B. N.; Mendes, R. A.; Cardoso, L. D.; Queiroz, L.; Santos, I. R. I.**
*Embrapa Recursos Genéticos e Biotecnologia, C.P. 02372, Brasília, D.F., 70770-900, rfvieira@cenargen.embrapa.br*

**ABSTRACT**: **Mint (*Mentha spp*) germplasm conservation in Brazil .** The main goal of this project was to establish a mint germplasm collection and to evaluate it in different Brazilian environmental conditions. In 2002 and 2003, mint germplasm accessions were introduced from Purdue University, USA, Campinas Agronomic Institute (IAC), São Paulo, SP, CPQBA of Campinas University, Campinas, SP, Ceará Federal University (UFC), Fortaleza, CE, Brasília University (UnB), Brasília, DF, Paraná Agronomic Institute (IAPAR), Londrina, PR, West Paraná State University (Unioeste), Cascavel, PR to Embrapa Genetic Resources and Biotechnology. All accessions were obtained and propagated by cuttings, except accessions from Kew Garden (CM60 and 61), and Feltrini (CM58), which were propagated by seeds. The total germplasm collection included 14 species and 67 accessions, which are maintained at field and greenhouse conditions. Nineteen accessions have not been identified yet. Twenty-seven accessions from USA have been maintained at *in vitro* conditions. The following species were included in this collection: *Mentha aquatica* L. (5); *Mentha arvensis* L. (6); *Mentha campestris* Schur. (1); *Mentha canadensis* L.(2); *Mentha* cf. ×*gracilis* Sole (1); *Mentha* cf. *spicata* L. (6); *Mentha longifolia* L. (2); *Mentha* ×*piperita* L. (12); *Mentha* ×*piperita* subsp. *citrata* Ehrh. (2); *Mentha* ×*villosa* Hudson (4); *Mentha rotundifolia* (L.) Hudson. (2); *Mentha* sp. (19); *Mentha suaveolens* Ehrh. (3); *Mentha suaveolens* Ehrh. × *M. aquatica* L. (1); and *Mentha sylvestris* L. (1).

**Key words:** conservation, genetic resources, germplasm, *Mentha*.

## INTRODUCTION

The genus *Mentha*, a member of the family Lamiaceae and the tribe Mentheae, is divided into five sections and consists of approximately 25 species (Harley and Brighton, 1977). Most *Mentha* species are characterized by a great morphological variation which is reflected on the high number of different taxonomic rank names attributed to mint plants during the past 200 years. Furthermore, the hybridization, that occurs frequently when the species of section *Mentha* are in contact, contributes to the complex variation patterns characterizing most wild populations. Apart from their high morphological variability, most mint species are characterized by a great chemical diversity with respect to their essential oil constituents. The great differences in the essential oil composition found in members of the genus are reflected in the number of commercial essential oils obtained from them (carvone, dihydrocarvone, menthone, menthol, pulegone, linalol and linalyl acetate) (Kokkini, 1992). From these, menthol is the main commercial product. It is produced in specialized glands present in the leaves and flowers of the plant and has an array of applications in the food, cosmetic and pharmaceutical industries.

In Brazil, mint species were first cultivated in small-scale plantations by the Japanese immigrants that brought it to the interior of the state of São Paulo in the beginning of the 20$^{th}$ century (Maia, 1998). Brazil was once an important world producer and exporter of mint essential oils. At the time of the second world war the country was producing over 3000 tons of menthol per year, most of which was bought by the United States of America (Clark, 1998). After 1974 there was a pronounced drop in the production of raw mint oil in the country, caused by the reduction in the plantation areas and by the low technological level of the Brazilian growers (Czepak, 1998).

Also, the production of synthetic menthol contributed to the decrease in the market value of this compound and discouraged mint cultivation in the country. However, synthetic menthol is contaminated with toxic molecules during its production process, making its use inadequate in numerous food and pharmaceutical products (Maia, 1998). Therefore, despite the predominance of synthetic menthol in the world market, the production of natural menthol continues to have a series of advantages over the synthetic product, due to its purity and superior quality. Currently, Brazil imports almost all the menthol that is consumed here and mint cultivation is restricted to small plantations and backyards, where the majority of the genotype has been conserved, in most cases, inadequately. Considering the tremendous demand for natural and

Recebido para publicação em agosto/2004
Aceito para publicação em julho/2006

organic products, the high level of technological knowledge existing today on the production and processing of mint, Brazil could recover its position as a big world mint producer in a short period of time. *Mentha* must be considered an important species for the conservation of genetic resources of medicinal and aromatic plants. The collection and introduction of new genetic materials of *Mentha* is fundamental to obtaining genetic pool adequate for further research in the area of plant breeding for the selection of genotypes with superior quality. Several Brazilian institutions have maintained under precarious conditions, small mint collections, with minimal or no potential value.

The objective of this work was to gather at Embrapa Genetic Resources and Biotechnology the germplasm of mint cultivated throughout the country, to form a germplasm collection in the field and *in vitro.*

## MATERIAL AND METHOD

### Plant material

In 2002, twenty-seven mint accessions were introduced from Purdue University, USA. In 2003, forty-five mint accessions were collected in Brazil at Campinas Agronomic Institute (IAC), São Paulo, SP, CPQBA of Campinas University, Campinas, SP, Ceará Federal University (UFC), Fortaleza, CE, Brasília University (UnB), Brasília, DF, Paraná Agronomic Institute (IAPAR), Londrina, PR, West Paraná State University (Unioeste), Cascavel, PR. All accessions were obtained and propagated by cuttings, except accessions from Kew Garden (CM60 and CM61) and Feltrini (CM58), which were propagated by seeds.

## CONVERSATION STRATEGIES

### *Field conservation*

During the period from may/2002 to november/2003 the samples were planted in the Experimental Field of Embrapa Genetic Resourses and Biotechnology, Brasília, DF. The field is located at 15º46' south latitude and 47º55' west longitude, at an altitude of 1079 meters, and the soil in this area is a oxisoil, with clay texture. Five plants of each accession were planted in square plots (2m$^2$ each) that were irrigated throughout the year by sprinkler system irrigation.

### In vitro conservation

Activities for *in vitro* conservation under slow growth conditions of mint started in 2002. The *in vitro* slow growth conservation is very important on a mint germplasm conservation strategy. Twenty-six accessions were introduced *in vitro* for conservation. Shoot tips of mint plants were collected in the field, disinfested and disinfected using a 2% sodium hypoclorite solution for 20 minutes prior to being transfered into culture tubes containing 10 ml of basic MS medium (Murashige & Skoog, 1962) with half concentration of micro and macronutrients plus MS vitamins (10g.L$^{-1}$ myo-inositol, 50 mg.L$^{-1}$ nicotinic acid, 50 mg.L$^{-1}$ pyridoxine-HCl, 100mg.L$^{-1}$ thiamine-HCl, 200mg.L$^{-1}$ glycine and 200mg.L$^{-1}$ calcium pantotenate). They were cultivated in a growth chamber at 25±2°C, photoperiod of 12 hours and light 20±2°C, photoperiod of 12 hours and light intensity of 35ìM.m$^{-2}$.s$^{-1}$ for 10 days. The plantlets were then multiplied in the same media and culture conditions and six replicates per accession were transferred to the slow growth conservation chamber at 20±2°C, photoperiod of 12 hours and light intensity of 68ìM.m$^{-2}$.s$^{-1}$.The interval between subcultures was nine months.

### Cryopreservation

Hillary's Sweet Lemon Mint (*Mentha* sp), one accession of the field collection, was selected for the cryopreservation studies. Shoot tips and axillary buds were isolated from plants growing in the field and cultivated *in vitro* in MS medium, 25±2ºC and 12 hours light photoperiod. The protocol under development is composed of a series of four steps. On the first step (encapsulation), shoot tips and axillary buds were isolated from *in vitro* grown plants and encapsulated in Na-alginate. Encapsulation was achieved by transferring the explants to MS basal liquid medium containing 3% Na-alginate (Sigma, medium viscosity) and then dropping them in MS liquid medium containing 0.1 M $CaCl_2$ for one hour. On the second step (pretreatment), capsules containing one explant each were transferred to MS liquid medium containing 0,4M sucrose and kept for 0, 15, 30, 45 and 60 minutes at environment temperature (25±2°C), in darkness, under agitation (100 rpm) on a horizontal shaker. On the third step (vitrification), capsules were transferred to $PVS_2$ liquid solution and maintained for 0, 15, 30, 45 and 60 minutes at environment temperature (25±2°C). After each one of these steps one sample of capsules was transferred to MS solid medium for viability evaluation. Three replications were used *per* treatment assigned at random. The next step of this study includes freezing the samples in liquid nitrogen, and with that the cryopreservation protocol will be completed.

### Germplasm exchange

In 2004, cuttings of all the accessions were sent from Embrapa Genetic Resourses and Biotechnology to Campinas University (UNICAMP), Ceará Federal University (UFC), Embrapa Amazonas, Sergipe University and West Paraná States University, (UNIOESTE), for agronomic evaluation studies and subsequent chemical characterization of the essential oils, aiming at the identification of the genotypes best

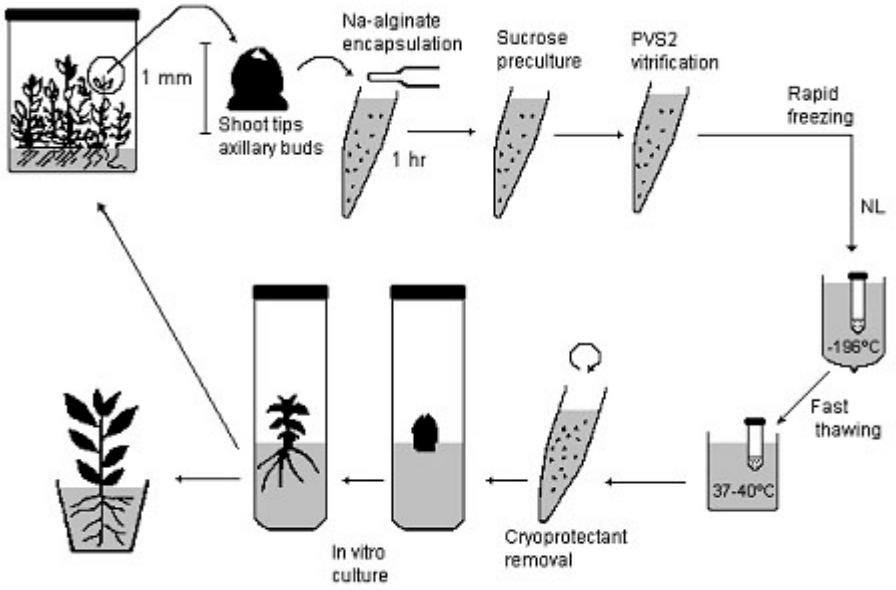


**FIGURE 1**. Diagram illustrating the different steps of the cryopreservation protocol by encapsulation-vitrification developed for Hillary's Sweet Lemon Mint (*Mentha* sp) tissues.

adapted to the local conditions. Oils extracted from these plants are being used to build an essential oils library of mint, which will in the future be presented to the Brazilian industries in the pharmaceutical, cosmetics, food, hygiene and cleaning products sectors, with the purpose of viabilizing the use of the genetic resources of this collection and of recovering mint production in Brazil.

## RESULT AND DISCUSSION

The germplasm collection includes 14 species and 67 accessions, which are maintained in the field and in the greenhouse (Table 1). Nineteen accessions are not identified yet. Twenty-seven accessions from USA have been maintained under *in vitro* conditions. The following species are included in this collection: *M. aquatica* L. (5); *M. arvensis* L.(6); *M. campestris* Schur. (1); *M. canadensis* L.(2); *Mentha* cf. × *gracilis* Sole (1); *Mentha* cf. *spicata* L. (6); *M. longifolia* L. (2); *Mentha* × *piperita* L. (12); *Mentha* × *piperita* subsp. *citrata* Ehrh. (2); *Mentha* × *villosa* Hudson (4); *M. rotundifolia* (L.) Hudson. (2); *Mentha* sp. (19); *M. suaveolens* Ehrh. (3); *M. suaveolens* Ehrh. × *M. aquatica* L. (1) and *M. sylvestris* L. (1). The accessions of this collection are available for exchange, in accordance with the Brazilian legislation regarding genetic resources. Voucher of all the accessions are being collected and deposited in the Embrapa Genetic Resources and Biotechnology herbarium (CEN), and duplicates were sent to Dr. Art Tucker for botanical identification and deposit in the Delaware State College, DE, EUA, herbarium.

The genotypes maintained *in vitro* have showed an excellent performance. In 2004, all genotypes will be maintained both in field and *in vitro* collection. The basic MS medium has showed to be efficient for mint *in vitro* conservation.

Preliminary studies for the development of a cryopreservation protocol by encapsulation-vitrification of mint have been initiated at Embrapa Genetic Resources and Biotechnology. The protocol under development is composed of a series of steps, as illustrated in Figure 1. Up to this point the effect of encapsulation in Na-alginate gel, preculture in 0.4M sucrose and vitrification using $PVS_2$ has been tested. Shoot tips and axillary buds showed high viability after each one of these treatments (Table 2). These results are very promising and since these treatments consist of a preconditioning of the tissue, preparing them to withstand exposure to liquid nitrogen, they suggest that cryopreservation of mint tissues might be possible. Cryopreservation results should be available soon. In case the protocol under development proves to be efficient, it will be tested with other accessions present in the filed, and in the future all the accessions of the mint germplasm collection will be cryopreserved.

Morphological, agronomic and chemical characterization of accessions from Purdue University cultivated at Embrapa Genetic Resources and Biotechnology showed a different behaviour for each character evaluated. Genotypes "Green Curly Mint" (*M. piperita*), "Grapefruit Mint" (*M. piperita*), "Orange Mint" (*M. aquatica*), "Lime Mint" (*M. aquatica*), "Persian Mint Field" (*M. piperita*), "Chinese Mint" (*M. canadensis*), "Eau de Cologne" (*M. piperita*) and

**TABLE 1**. Mint Germplasm Bank maintained at field collection, Embrapa Genetic Resources and Biotechnology, Brasília, DF

| ACCESSION BRA | LOCAL CONTROL NUMBER | COMMON NAME | SCIENTIFIC NAME[1] | ORIGIN[2] |
|---|---|---|---|---|
| 000221 | CM 1 | Lime Mint | *Mentha aquatica* L. | Purdue University – USA[3] |
| 000230 | CM 2 | Apple Mint | *Mentha ×villosa* Hudson | Purdue University – USA |
| 000248 | CM 3 | Chocolate Mint | *Mentha x piperita* L. | Purdue University – USA |
| 000256 | CM 4 | Pineapple Mint | *Mentha suaveolens* Ehrh. | Purdue University – USA |
| 000264 | CM 5 | Chinese Mint | *Mentha canadensis* L. | Purdue University – USA |
| 000272 | CM 6 | Chewing Gum Mint | *Mentha piperita* L. | Purdue University – USA |
| 000281 | CM 7 | Grapefruit Mint | *Mentha piperita* L. | Purdue University – USA |
| 000299 | CM 8 | Eau De Cologne | *Mentha piperita* L. | Purdue University – USA |
| 000302 | CM 9 | Variegated Peppermint | *Mentha piperita* L. | Purdue University – USA |
| 000311 | CM 10 | Hillary's Sweet Lemon Mint | *Mentha suaveolens* Ehrh. x *M. aquatica* | Purdue University – USA |
| 000329 | CM 11 | Green Curly Mint | *Mentha piperita* L. | Purdue University – USA |
| 000337 | CM 13 | Orange Mint | *Mentha aquatica* L. | Purdue University – USA |
| 000345 | CM 16 | Persian Mint Field | *Mentha piperita* L. | Purdue University – USA |
| 000353 | CM 17 | Menthol Mint Gh | *Mentha spicata* L. | Purdue University – USA |
| 000361 | CM 18 | Common Mint Gh | *Mentha aquatica* L. | Purdue University – USA |
| 000370 | CM 19 | Lavander Mint | *Mentha aquatica* L. | Purdue University – USA |
| 000388 | CM 20 | Japanese Field Mint | *Mentha canadensis* L. | Purdue University – USA |
| 000396 | CM 22 | Bergamot | *Mentha x gracilis* Sole | Purdue University – USA |
| 000400 | CM 23 | Peppermint | *Mentha x piperita* L. | Purdue University – USA |
| 000418 | CM 24 | Ginger Mint | *Mentha arvensis* L. | Purdue University – USA |
| 000426 | CM 25 | Large Leaf Spearmint | *Mentha spicata* L. | Purdue University – USA |
| 000434 | CM 26 | Banana Mint | *Mentha arvensis* L. | Purdue University – USA |
| 000442 | CM 27 | Himalayan Silver Mint | *Mentha longifolia* (L.) Huds. | Purdue University – USA |
| 000451 | CM 28 | Egyptian Mint | *Mentha ×villosa* Hudson | Purdue University – USA |
| 000469 | CM 29 | Hortelã Caseira | *Mentha spicata* L. | Distrito Federal |
| 000477 | CM 30 | Menta Do Uruguai | *Mentha* sp. | Uruguai |
| 000485 | CM 31 | EMATER 1 | *Mentha spicata* L. | UnB |
| 000493 | CM 32 | Ciudad De Leste | *Mentha piperita* L. | UnB |
| 000507 | CM 33 | UnB1 | *Mentha spicata* L. | UnB |
| 000515 | CM 34 | Emater 2 | *Mentha sylvestris* L. | UnB |
| 000523 | CM 35 | Dourados 1 | *Mentha rotundifolia* (L.) Huds. | UnB |
| 000531 | CM 36 | Dourados 2 | *Mentha arvensis* L. | UnB |
| 000540 | CM 37 | Emater 3 | *Mentha campestris* Schur | UnB |
| 000558 | CM 38 | Kibe | *Mentha ×villosa* | UnB |

[1] CM = Mint Collection

[2]Plant tentative identification according to Dr. Art Tucker, Dellaware State University, USA

[3] IAC = Instituto Agronômico de Campinas; UnB = Universidade de Brasília; CPQBA/UNICAMP = Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrárias/Universidade de Campinas; UFC = Universidade Federal do Ceará; UNESP = Universidade Estadual de São Paulo, Campus Botucatu; UNIOESTE= Universidade do Oeste do Paraná.

[4] Genotypes only maintained *in vitro* conditions

**TABLE 2.** Viability of Hillary's Sweet Lemon Mint (*Mentha* sp) explants after each step of the cryopreservation protocol that have been carried out to date. Shoot tips and axillary buds of mint were isolated from plant obtained *in vitro*.

| Duration(min.) | Viability (%) | | |
|---|---|---|---|
| | Encapsulation | Sucrose pretreatment (0.4M) | Vitrification($PVS_2$) |
| 0 | 100 | 80 | 80 |
| 15 | 100 | 93 | 100 |
| 30 | 100 | 87 | 93 |
| 45 | 100 | 100 | 93 |
| 60 | 100 | 100 | 100 |

* These values are means of three replications.

"Hillary's Sweet Lemon Mint" (*M. suaveolens* x *M. aquatica*) showed a higher dry and fresh weight. Genotype "Hillary's Sweet Lemon" showed the highest values for whole plant fresh and dry weight, and leaves dry weight, with 23,7%, 24,1% and 15,5% higher when compared to the second genotype. The genotype "Japanese Field Mint" presented the highest content of essential oil (4,17%) and genotypes "Chinese Mint", "Grapefruit Mint", "Persian Mint Field" and "Eau de Cologne" showed the highest production of essential oil per hectare, 75,0 L/ha, 67,1 L/ha, 53,6 L/ha e 50,5 L/ha, respectively (Grisi, 2003). The major constituents of mint essential oils detected were 1,8-cineole, carvone, limonene, linalool, linalyl acetate, menthol, menthone, menthyl acetate, and piperitenone oxide. The genotypes showed between 20 ("Bergamot" – *M. gracilis*) and 66 ("Green Curly Mint" – *M. piperita*) essential oil constituents. Some genotypes were found to have essential oils with a high content of a particular constituent, like piperitone oxide (74,4% in "Pineapple Mint" – *M. suavelens*), carvone (72,5% in "Chinese Mint" – *M. canadensis*) and linalol (67,9% in "Ginger Mint" – *M. arvensis*) (Gracindo, et al, 2004).

**ACKNOWLEDGEMENT**

We would like to thank Dr. P. Melillo/CPQBA; Dr. R. Inneco/UFC; Dr. L. Ming/Unesp; Dr. J. Kleber/ UnB; Dr. M. Czepak/Unioeste; Dr. J.Simon/Purdue University; Dr. M.B. Perecin; and Royal Botanical Garden, Kew, UK for kindly supplying the germplasm collection. We also thank Dr. Arthur O. Tucker for the botanical identification of the samples.

**REFERENCE**

CLARK, G. S. Menthol. **Perfumer & Flavorist,** v. 23, n.5, 33 – 46, 1998.

CZEPAK, M. P. Produção de Óleo Bruto e Mentol Cristalizável em Oito Frequências de Colheita da Menta (*Mentha arvensis* L.). In: MING, L.C. (coord.), SCHEFFER, M. C., JÚNIOR CORRÊA, C., BARROS, I. B. I., MATTOS, J. K. A. **Plantas Medicinais Aromáticas e Condimentares:** Avanços na pesquisa agronômica. v.2. Botucatu: UNESP,1998. p.53-80.

GRACINDO, L. A. M. B.; GRISI, M. C. M.; SILVA, D. B.; ALVES, R. B. N.; BIZZO, H. R.; VIEIRA, R. F. Chemical characterization of Mint (*Mentha* spp.) germplasm at Federal District, Brazil. In: III INTERNATIONAL SYMPOSIUM: BREEDING RESEARCH ON MEDICINAL AND AROMATIC PLANTS; II LATIN AMERICAN SYMPOSIUM ON THE PRODUCTION OF MEDICINAL AND AROMATIC PLANTS AND CONDIMENTS. Campinas, SP, Brazil: **Abstracts**, 2004. p. A01-5.

GRISI, M. C. M. **Avaliação de Genótipos de Menta (*Mentha* spp) nas Condições do Distrito Federal, Brasil.** Brasília: Universidade de Brasília (Monografia de conclusão do curso de Agronomia), 2003. 27p.

HARLEY, R. M. and BRINHTON, C. A. Chromosome numbers in the genus *Mentha* L. **Botanic J. Linn. Soc**. V. 74, p. 71 – 96. 1977.

KOKKINI, S. Essential oils as taxonomic markers in *Mentha*. In: ADVANCES IN LABIATE SCIENCE. p. 325 – 334. 1992.

MAIA, N. B. Efeito da Nutrição Mineral na Qualidade do Óleo Essencial da Menta (*Mentha arvensis* L.) Cultivada em Solução Nutritiva. In: MING, L.C. (coord.), SCHEFFER, M. C., JÚNIOR CORRÊA, C., BARROS, I. B. I., MATTOS, J. K. **Plantas Medicinais Aromáticas e Condimentares:** Avanços na pesquisa agronômica. v.2. Botucatu: UNESP,1998. p. 81-95.

MURASHIGE, T; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures, **Physiol. Plant**., v.15, p.473-497, 1962.