MENTHA GATTEFOSSEI MAIRE – A THREATENED MEDICINAL SPECIES CULTIVATED IN THE BOTANICAL GARDEN (I) OF ASM

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Rezumat: *Lucrarea prezintă date despre Mentha gattefossei Maire, specie medicinală rară, protejată la nivel mondial şi metodele de conservare ex-situ în Grădina Botanică (I) a AŞM. Sunt expuse rezultatele cercetărilor pe teren experimental care includ diferite procedee de înmulțire precum şi influiența spațiului de nutriție asupra creşterii şi dezvoltării plantelor. Au fost descrise aspectele fenologice ale plantelor în condițiile pedo-climatice ale Republicii Moldova. Au fost, de asemenea, înregistrați principalii parametri morfometrici şi ritmul de creştere al plantelor.*

***Cuvinte cheie*:** *Lamiaceae*, *Mentha gattefossei*, particularități biologice, metode de înmulțire, conservare.

INTRODUCTION

Lamiaceae Lindl. family contains a large number of genera and species with economical value due to their use as medicinal, aromatic and ornamental plants. *Mentha* L. genus is one of the most important members of this family considered as a source of active substances, especially essential oils. The plant genus *Mentha* L. is well known as a taxonomically complex group belongs to the mint family (*Lamiaceae* Lindl.), subfamily *Nepetoideae*, tribe *Mentheae* [11, 16]. Most *Mentha* species (about 20-25) are widely distributed and occur primarily in temperate regions of Europe and Asia, a few in the Southern Hemisphere [1, 11], and species identification is frequently difficult, since, in addition to much phenotypic plasticity and genetic variability, most of the species are capable of hybridization with each other [1, 9]. *Mentha gattefossei* Maire is one of the members of this genus, native to Morocco, and represents a potential source of valuable essential oil [6-8, 10].

Mentha gattefossei was first described in 1922 by René Charles Maire and named in honor of the chemical engineer and botanist Jean Gattefossé who remarkably contributed to the research of Moroccan flora [14].

The species is listed in the IUCN Red List of threatened species as Near Threatened [15]. This endangered species is endemic to Morocco [4, 12, 15]. It is found at five sites in the Atlas Mountains (Muddle, High Atlas and Anti Atlas) [13]. Another occurrence is noted at the edges of Ziz River

Foto 1. Samples of *Mentha gattefossei* (flowering stage, 2009)

in the Moroccan Sahara [14, 15]. The habitat area is estimated to be above 2000 km^2. The species occurs in wet meadows, at the edges of pools and rivers of chalky and flinty mountains at 1600-2100 m altitude. The size of the populations is decreasing as a result of harvesting for medicinal purposes and as a food source. The plant is intensively exploited for the extraction of essential oils at national and international level [15].

The evidence of chloroplast DNA sequences for assessing the phylogenetic relationships in *Mentha* L. genus suggests that *Mentha gattefossei* is related to *Mentha cervina* [2, 9, 16]. For this reason were established the taxonomical affiliation of *M. gattefossei* species (foto 1) on the basis of literature data and herbarium. Also were conducted studies on plant propagation, on inter row spacing and growth rhythm of the plants in the conditions of Republic of Moldova.

MATERIAL AND METHODS

The research was conducted during 2007-2013 in the experimental field of the collection of medicinal and aromatic plants. The seeds of *M. gattefossei* were received by the international exchange of seeds with the Botanical Garden from Coimbra, Portugal in 2006.

Herbarium material collected was revised on the basis of literature references. The morphology of the herbarium specimen was described and illustrated on the basis of material collected in the Collection of Medicinal plants in agreement with existing descriptions in the literature [1, 3, 5, 11, 12-14]. Voucher specimens are lodged in the Herbarium of Botanical Garden (Institute) of ASM.

The experiments aimed at seed germination were performed in January-February 2007. The seeds received by *Delectus Seminum* were sown in special substrate under greenhouse conditions and in Petri dishes in laboratory conditions.

For cutting propagation, 5-6 cm long shoots were cut from the mother plant and inferior leaves were removed. The shoots were planted in a growth substrate consisting of a mixture of sand and peat (1:1) in the greenhouse conditions during February 2010.

In case of traditional propagation by stolons, they were separated and divided from the roots by hand. As a propagation material was also used 4-5 cm high vegetative shoots developed from underground stolons. In early spring the plants were also propagated by division of mother plants. No chemical fertilizers were applied during experimentation. Irrigation and weeding of the plots was done as and when needed.

A field study was conducted to determine the effect of different inter row spacing on the growing rhythm of the plants during 2010-2013. Three experimental blocks were established the main factor being the inter row space (20, 30 and 40 cm).

RESULTS AND DISCUSSIONS

Mentha gattefossei is a perennial, herbaceous plant with more or less elongated, creeping, branched rhizome (foto 2). Stems simple to slightly branched, at base ascending, up to 20 centimeters high with internodes generally longer than leaves. Leaves sessile, opposite; leaf blade simple, bright green, glabrous, flat to slightly convolute, broadly linear to oblanceolate, 10-15(20) mm long and 3-4(5) mm wide, herbaceous, base rounded, margin entire or sometimes remotely crenate, apex obtuse; midrib pronounced, the secondary barely visible. Verticillasters 15-20-flowered, globose, 1,5-2(2,5) cm in diameter, few, widely spaced; floral leaves similar to stem leaves, sessile, recurved, equal or longer than verticillasters. Pedicel is 2-3 mm long. Calyx tubular, pale green, 2-lipped, 2,5-3 mm long, dotted with large, globular, golden yellow shining glands, ± conspicuously 12-veined, tube ca. 1.5-2 mm long; upper lip 3-toothed, teeth lanceolate-triangular, ca. 1 mm; lower lip 2-toothed, teeth subulate, ca. 1.5

Foto 2. *Mentha gattefossei* (voucher specimen, 2007)

Foto 3. *Mentha gattefossei* (vegetative multiplication)

mm. Corolla funnelform, whitish, ca. 4-5 mm long, glabrous; tube ca. 3 mm; lobes oblong, ca. 1.5 mm, entire, oblong with rounded tip. Stamens 4, are subequal, divaricate, erect, exserted, posterior 2 slightly longer than anterior 2; filaments glabrous. Style exserted, apex equally 2-cleft. Ovary is glabrous. Nutlets are obovoid, dry, and smooth.

The first step of the research referred to obtaining of *M. gattefossei* plants from seeds achieved through the international exchange of seeds. The seeds received by *Delectus Seminum* were sown in special substrate under greenhouse and laboratory conditions. About 70% of the seeds germinated in both experimental variants. However, a minor difference was noted. In laboratory conditions was observed a slightly higher coefficient of the seed germination. Fragile plants were kept in unheated greenhouse until next season. Being transplanted in the field they showed a very poor growth and vegetative expansion. The plants survived vegetatively, but almost half of the total number of plant did not reach the flowering stage.

Propagation of the plants in the next periods of vegetation was done exclusively by vegetative parts such as green shoots and underground stolons. The green shoots planted in a growth substrate consisting of a mixture of sand and peat (1:1) in the greenhouse conditions rooted in 14-16 days. Rooting percentage was very high. In all three experimental variants with 25 cuttings each, number of rooted cuttings ranged between 22 and 24; respectively a rooting percentage was 88-96%. After 2-3 weeks they were transferred for preliminary growth in pots or containers. (foto 3). At 7-9 cm plant height they were transplanted to experimental fields on the first decade of May. Were experienced the traditional propagation by stolons that is the most popular method of mint propagation being also the most economic.

Results of the experiments showed that inter row spacing had significant effect on growing dynamics of the plants. At the same time the analyses of the results have indicated that some morphologic parameters (leaf size, length of internodes, inflorescence diameter, etc) was not significantly affected with the variation in inter row spacing. No appreciably differences of plants height were observed with increase of planting space. But it is important to note that the plants from third experimental variant (30x40 cm) developed very well (foto 4), all individuals reached the blooming stage, demonstrating a large expansion

Foto 4. *Mentha gattefossei* (flowering plants, 2011)

and an abundant flowering. The blossom period starts from the first decade of June and lasts till the end of July. The fructification stage was noted in the first decade of August.

M. gattefossei being a species of global conservation interest it is of great importance not only from medicinal viewpoint but also for *ex situ* conservation programmes at national and international level. Further research will relate to agrotechnical cultivation methods, evaluation of volatile oil content, in terms of its quantity and quality and elaboration of the *in vitro* multiplication protocol with applications in conservation and capitalization of species.

CONCLUSIONS

1. This study provided information about endangered *Mentha gattefossei* Maire species and its *ex-situ* conservation perspective in the Botanical Garden (Institute) of ASM.
2. The primary results of investigations showed that the most suitable nutrition space for *M. gattefossei* is 30x40 cm; the plants develop vigorous branched stems that reach 25-30 cm in height. The most successful method of propagation is by stolons, otherwise this is the most popular and economic method of mint multiplication.
3. The plants positively responded to climatic and soil conditions of the Republic of Moldova; vegetation period takes 136-155 days, flowering stage lasts 55-60 days. They undergo a complete ontogenetic cycle, which demonstrates high adaptive potential and *ex-situ* conservation perspective.

REFERENCES

1. Ball, P.W. *Mentha* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S. and Webb, B.A. (Eds), Flora Europaea, Cambridge: Cambridge University Press, 1972, Vol. 3, p. 183-186.
2. Bunsawat J., Elliott N. E., Hertweck K. L. et al. Phylogenetics of *Mentha* (*Lamiaceae*): Evidence from Chloroplast DNA Sequences. // Systematic Botany, 2004, vol. 29, nr. 4, p. 959-964.
3. Catalogue Semences and Plants CNPMAI. Issus de l'Agriculture Biologique, 2013. www.cnpmai.net
4. Fennane M. and Tattou Ibn M. Catalogues des plantes vasculaires rares, menacées ou endemique du Maroc. Palermo: Bocconea 8. 1998, p. 5-343.
5. Fennane M. and Tattou Ibn M. Flore vasculaire du Maroc. Inventaire et chorologie. //Trav. Inst. Sci. Univ. Mohamed V, Ser. Bot., 2005, vol. 37, p. 259.
6. Fujita Y., Fujita S. I., Essential oil of *Mentha pulegium* and *Mentha gattefossei* viewed from the standpoint of comparative biochemistry. // Nippon Kagaku Zasshi, 1967, vol. 88, p. 767-769.
7. Fujita S. I., Nakano T., Fujita Y. Studies on the essential oils of the genus *Mentha*. Part VIII. A biochemical study of the essential oils of *Mentha gattefossei* Maire. // Agric Chem Soc Jap J., 1972, vol. 46, nr. 8, p. 393-397.
8. Fujita S. I., Moriyoshi K. Essential Oil of *Mentha gattefossei* Maire // Nippon Kagakkai Koen Yokoshu, 2001, vol. 79, nr. 2, p. 1366.
9. Harley R. M. and Brighton C. A. Chromosome numbers in the genus *Mentha* L. // J. Linn. Soc. Bot., 1977, nr. 74, p. 71-96.
10. Holeman M., Berrada M., Bellakhdar J. et al. Analysis of the essential oil of *Mentha gattefossei* Maire. // Parfums, Cosmetiques, Aromes, 1984, nr. 59, p. 61-62.
11. Menitski, Ju.L. *Mentha* L. In: Fedorov, An.A. (Ed.), Flora Partis Europaea U.S.S.R., 1978, Leningrad: Nauka, Vol. 3, p. 204-207.
12. Oualidi J. E., Khamar H., Fennane M. et al. Check-list des endémiques et Spécimens types de la flore vasculaire de l'Afrique du Nord. // Document de l' Institut Scientifique, 2012, nr. 25, 192 p.
13. Ouyahya A. *Mentha*. In: Fennane M., Tattou M. Ibn, Ouyahya A., Oualidi J. E. Flore Pratique du Maroc. Manuel de détermination des plantes vasculaires. Vol. 2: *Angiospermae* (*Leguminosae – Lentibulariaceae*). //Inst. Scien., Ser. Botanique, 2007, vol. 38, p. 485.
14. Maire René Contributions a l'etude de la Flore de l'Afrique du Nord. // Bulletin de la Société d'histoire naturelle de l'Afrique du Nord, 1922, band 13, nr. 2, p. 37-44.
15. Rhazi L., Grillas P. and Rhazi M. *Mentha gattefossei*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2 www.iucnredlist.org
16. Wagstaff, S. J. A phylogenetic interpretation of pollen morphology in tribe *Mentheae* (*Labiatae*). In: R. M. Harley and T. Reynolds (eds.), Advances in *Labiatae* Science, Kew: Royal Botanic Gardens, 1992, p. 113-124.