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Ex situ (in field) conservation of Bulgarian species from genus Mentha maintained in the collection of IPGR-Sadovo

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Abstract: The genus *Mentha* belongs to the botanical family *Lamiacea*e. In Bulgaria are spread 6 varieties and numerous natural hybrids. The species from the genus *Mentha* are been well known since ancient days as oil and honey bearing plants. That is why it is considered that most of the known nowadays species are very old. In the Institute of Plant Genetic Resources – Sadovo are maintained *in field* two *Mentha* varieties. *M. arvensis* L and *M. spicata* L. Besides the *in field* conservation of the species, *in vitro* techniques are a reliable means of reproduction and long-term storage. In the experimental work for *in vitro* conservation of microcuttings of *M. arvensis* L and *M. spicata* L in low temperatures were applied 4 different temperatures - 2ºC, 4ºC, 6ºC and 8ºC. Longest conservation period (12 months) with highest level of survived cuttings (83,50%) was observed in 4ºC. After cultivation of the newly obtained microcuttings from in vitro conservation in normal conditions (24ºC) were observed little differences between two mint varieties in shoot growth, which is due to their specific biology and the period of *in vitro* conservation under 4ºC has no influence.

Keywords: *genus Mentha, M. arvensis L., M. spicata L., in field* conservation, *in vitro* conservation, *Lamiaceae.*

Introduction

Bulgaria is very rich in medicinal plants. They have been used for centuries to treat, as well as for cosmetic purposes. Therefore conservation of wild medicinal species is very important and is one of the main priorities in the scientific activities of IPGR – Sadovo that is a part of the National programme in Plant Genetic resources (PGR) through realization of the ”Conservation, Management and Use of PGR in Bulgaria” Project. The main goal of the project is conservation of the national plant biodiversity. In the National genebank are preserved 220 medicinal plants species in long and medium term storage (Desheva, 2009), 182 species are *in field* collection and 75 in *in vitro* conditions.

The species from Lamiaceae are the most widespread in Bulgraia and comprises about 210 genera and 3500 species and subspecies (Velchev, 1989). Two species (*M. arvensis* L. and *M. spicata* L.) of genus Mentha, growin in field and in vitro conditions were chosen to carry out a comparative investigation. They are well spread in Bulgaria mainly in moist to wet grounds and most used as medicinal plants. That crop has a wide range of distribution. Found throughout Europe, Africa, Asia, Australia and North America. In Bulgaria is spread throughout the country.

The in vitro conservation of vegetative propagating plant species is carried out in two main directions: 1. Suppressing of growth by using of liquid Nitrogen азот /Engelmann, 2011/; 2. Limitation of growth rate of the explants using different factors /Reed, 1999, Gonçalves, 2007, Lata, 2010/. One of the methods for reducing the growth rate is growing in vitro cultures at low positive temperatures. Withers, 1990, develop that method through a detailed study of the methodology for in vitro storage of cells, tissues and organs. The investigations continued with application of low temperature regimens in different plant species. The range of temperatures applied is from 1 to 10º C, and the conservation period is of 3 months to 3 years. The investigations for application of low temperatures for in vitro conservation of mint are still limited /Selvakumar, 2001, Islam, 2003, Chishiti, 2006/. Shibli, 2006 found specific differences in the low temperature tolerance of different species, varieties and explant types and establishment the optimal temperature limits should be established separately for each plant species. This required a study to be done to establish the temperature range in which microcuttings from two mint varieties can keep their viability in *in vitro* conditions as long as possible.

Material and methods

The plant material for the research was taken from the collection of medicinal plants of the Institute of Plant Genetic Resources – Sadovo. The morphological data were collected at full maturation of the plants. The reported indicators are as follows: height of shoot (cm), flower`s length (mm), length of leaves (cm) and width of leaves (cm).

For carrying out of the experimental work for *in vitro* conservation of two munt varieties, M. arvensis L. and M. spicata L, were used microcuttings from both species. The explants were cultivated on nutrient media after Gamborg, 1976 with vitamins after Morel /Grenan, 1979/. The investigation was made under 4 different temperatures - 2ºC, 4ºC, 6ºC and 8ºC. Tubes with dimensions 16/160 mm, each containing 5 ml of nutrient media were used. Double cover of the cultural vessels was applied to prevent evaporation. Cultures were grown in level of light 2 000 lx with 16/8 hors photoperiod. Each option for each variety included 50 explants in 3 repetitions. The level of survival of explants /%/ was recorded each 3 months up to the 12-th month.

For observation of growth temp of the explants microcuttings from both mint species were cultivated in new nutrient media in 24ºC. The length of shoots /mm/ was recorded each 10 days for a period of 30 days. Microcuttings from the same mint varieties, obtained from plants grown without conservation were used as control.

Results and discussion

Biological features of cultivated mint. Mint has increased demand to soil conditions. Successfully can be grown on alluvial, black, gray forest, meadow drained soils. Heavy clay and light sandy soils are unsuitable for mint.

Mint is very demanding to light. Does not tolerate compressed crops or shading. M. spicata is a plant of the long day, and M. arvensis – of the short.

Mint is not especially demanding to heat. It can withstand temperatures up to minus 10°C and in the presence of snow even to minus 18 ° C. The development of plants begins slowly at air temperatures of 2-3°C. The optimum for the growth and development of the mint is 19-20°C. Depending on average day temperatures the vegetation period of the mint from germination to flowering is 80 -100 days. During that period mint needs temperature sum from 1500 to 1600°C. Negative affect on plants have low atmospheric humidity in combination with air temperatures above 35°C.

Moisture requirements. Mint is moisture-loving plant, demanding to soil and atmospheric humidity during the entire growing season. The highest yields of essential oil, and dried leaves are produced when soil moisture is 80-90% of the PPW. Although the mint is very plastic culture it must be grown under irrigation. Therefore, depending on rainfall during vegetation 4 to 7 irrigations are a must. Excessive soil moisture, however, lowers the yield of the shoot biomass and the quality of oil in it, due to diseases or death of some of the plants.

In the analysis of the morphological features of the two examined mint species it was stated that they vary quite significantly in morphological terms. The results from the morphological study are shown in fig 1.

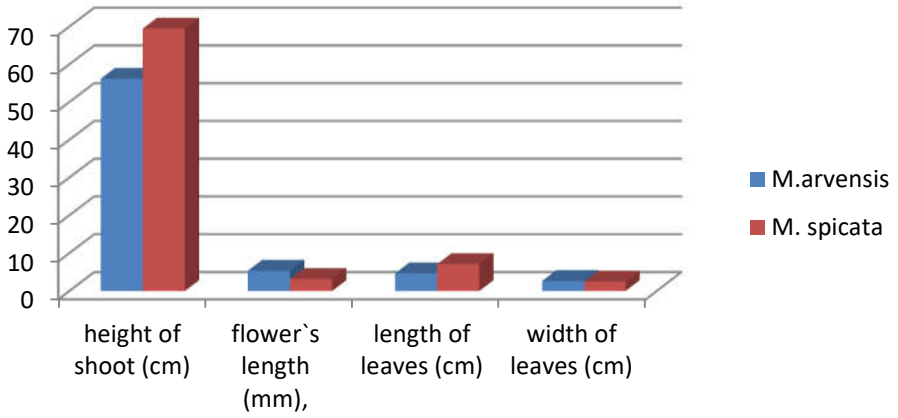

Fig.1. Comparative morphological study of M. arvensis and M. spicata

Mentha arvensis L. is an erect herbacious perennial plant that grows 20 - 80 cm tall on square, hairy stems. It is rhizomatous and has opposite leaves that are attached to the stem with very short stalks (fig.2). It has a very fragrant minty aroma.

The leaves are 2 - 8 cm long and 0.6 - 4 cm wide. They are lance to oval shaped, taper to a point, and have sharply toothed margins. The flowers are whorled around the upper leaf axils. They are 4 - 7 mm long, irregular, and range from white to pink and violet. They have longer stamens than petals. They bloom from June to August. The fruits are brown nutlets. There are four per flower and they enlarge and remain on the plant during the winter.

In Bulgaria *M. arvensis* grows naturally in wet lands from low to mountain elevations up to 1800 meters above sea level.

Mentha spicata L. (fig.3) is a herbaceous, rhizomatous, perennial plant growing 30–100 cm tall, with variably hairless to hairy stems and foliage, and a wide-spreading underground rhizome. The leaves are 5–9 cm long and 1.5–3 cm broad, with a serrated margin. Spearmint produces flowers in slender spikes, each flower pink or white, 2.5–3 mm long and broad. The fruits are red, brown to black, rigid or smooth. Used as a spice. Its leaves are used in soups, broths and stews of beans in preparation puree of spinach in stews of lamb and sheep meat stuffing for lamb in boiled and stewed meat - pork or beef. Due to its specific and strong flavor it must be carefully combined with other spices because they can "suppress" the taste.

Fig. 2. Mentha arvensis L. in field collection of IPGR

Fig. 3. Mentha spicata L. in field collection of IPGR

The comparative results from the influence of different temperatures on the level of survival and duration of in vitro storage of explants of *M. arvensis* L. show that highest level of survival /100% / is reached in 6ºC and 8ºC, but that level is constant up to the 6-th month (table 1). After that the survival of the explants is lower and in 120th month it is 73% in 6ºC and 48% in 8ºC. That is because the higher temperatures stimulate faster growth of shoots, exhaustion of the nutrient media and death of the plants. High level of survival /83,5%/ for longest period /12 months/ is observed under 4ºC. In 2ºC explants are successfully preserved for 12 months, but the survival is only 38%.

Nearly same are the results for *M. spicata* L., which shows that the temperature is the basic factor for the duration of the storage and the genotype has almost no influence.

Table 1. Level of survival of mint explants in low temperatures /%/

Temperature	Duration of storage			
	3 months	6 months	9 months	12 months
***M. arvensis* L.**				
2ºC	38,00	38,00	38,00	38,00
4ºC	83,50	83,50	83,50	83,50
6ºC	100,00	100,00	92,00	73,00
8ºC	100,00	100,00	85,00	48,00
***M. spicata* L.**				
2ºC	40,00	40,00	40,00	40,00
4ºC	81,50	81,50	81,50	81,50
6ºC	100,00	100,00	94,00	75,50
8ºC	100,00	100,00	82,50	49,50

Following the rate of growth of shoots of microcuttings, received after segmentation of plants after the conservation period in 4ºC and after cultivation in 24ºC was established that there are no significant differences in growth rate in both mint species (fig.4). The average shoot length after 30-days cultivation of *M. arvensis* explants was 82,12 mm and for *M. spicata* - 72,08 mm. The shoot length of the control plants was almost the same - 78,39 mm. The variation of 10,04 mm between both species is unsignificant.

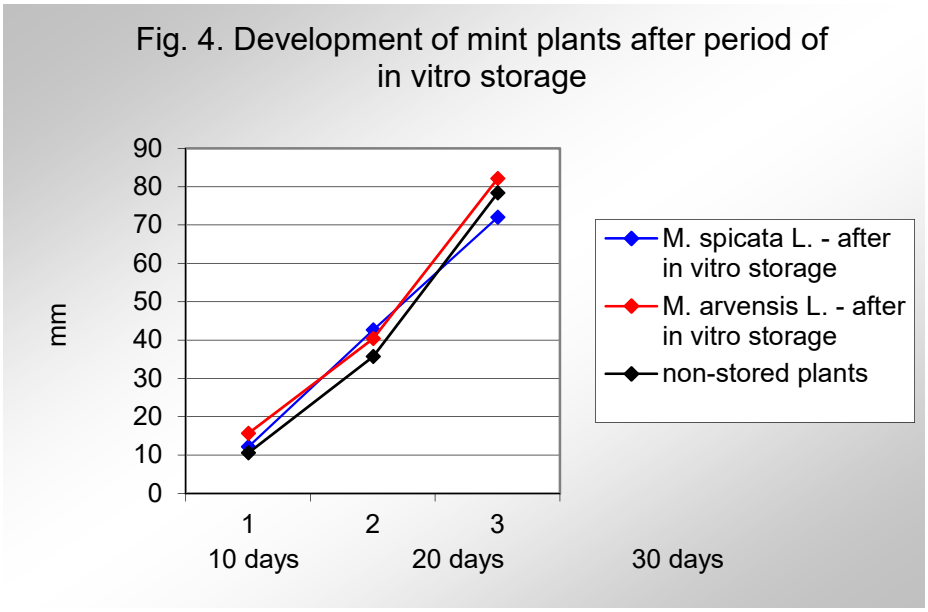

Fig. 4. Development of mint plants after period of in vitro storage

Fig. 5 and 6 show genotype specification, not suppressing result from the storage. So low temperatures applied in in vitro storage does not have any influence on the regeneration processes of explants when transferred to normal conditions after the storage period.

Fig.5. In vitro plants of M. arvensis L. stored and non stored explants

Fig.6 In vitro plants of M. spicata L. stored and non stored explants

Conclusions

The comparative morphological investigation of *M. arvensis* L. and *M. spicata* showed that there is insignificant variation and the indices are stable.

Both mint varieties /*M. arvensis* L. and *M. spicata*/ can be successfully stored in vitro under low positive temperatures and the longest period for conservation /12 months/ with highest level of survival /83,5%/ is obtained in 4ºC.

In vitro conservation of mint explants in low positive temperatures do not influence on the shoot growth rate when transferred in normal temperature /24ºC/.

The climatic conditions in Sadovo region meets the demands of mint varieties for cultivation.

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