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EXPRESSION OF GENETIC POTENTIAL OF QUALITY INDICATORS IN CONDITION OF ECOLOGICAL AGRICULTURE UNDER THE AIM OF PROMOTING CONSERVATION AND USE OF MINT GERMPLASM RESOURCES

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INTRODUCTION

Recently, medicinal and aromatic plants have received much attention in several fields such as use of essential oil yield in agro alimentary, perfumes, pharmaceutical industries and natural cosmetic products. (Olfa Baatour et all. 2009)

The genus *Mentha* (Lamiaceae) includes 25 species and some hybrids with essential oils rich in monoterpenes, which are accumulated in glandular trichomas, especially in leaves and flowers. To this morphologic variability correspond a wide chemical diversity which is reflected in a varied number of essential oils commercially obtained (Kokkini 1992).

Currently, *Mentha piperita* is a species with high ecological plasticity, its area being spread around the world, the largest surfaces in countries with the most favorable climate. The major crops in Europe are in France, Italy, Bulgaria (approx. 10.000 ha), Serbia (5.000 ha), Poland (over 4.000 hectares) in Russia over 14.000 ha of highly productive varieties; cold climates countries (Netherlands, Germany and England) much lower surfaces are established. In Asia mint species is well represented in China, especially in the area of Shanghai. In Japan the most popular mint is *Mentha arvensis* species var. piperascens featured by high oil content and about 90% menthol).

Known as mint or peppermint, *Mentha piperita* L. is used for medicinal and food purposes (Lorenzi and Matos, 2002). Because of the divers potential use of species in areas like: food and beverage industry, cosmetics and perfumery, medicinal products, high demands for fresh and dry herb, and volatile oils on the world market (about 1000-1400 tons of volatile oil and 12000 tons of leaves) makes this culture to be constantly expanding with great interest to developing countries. In this purpose breeding is needed in order to develop new genotypes featured by quantitative and qualitative parameters, resistance to pathogen attack. Starting with 2006, a pre breeding program for mint was established at Vegetable Research and Development Station Bacau, in order to detect valuable genetic resources for development of new cultivars, suitable for cultivation in our climate conditions.

MATERIAL AND METHODS

Plant material and growing conditions for the experiment

Biological material was represented by local populations of mint. Research methods used: - individual selection in mint local populations, selection of natural mutants and individual selection in descendent.

Harvesting of mint

Harvesting was accomplished according with literature recommendation: at the beginning of flowering in order to obtain higher amounts of the essential oil content (Duriyaprapan et al. 1986) that is concentrated in the glandular trichomas of leaves and floral calyxes (Lawrence 1992).

Investigations

After the harvest, the fresh yield was weighed and then dried (at +40^o C). Random samples (2 x 200 g) were used to assess the dry matter content.

Oil content determination

For oil content determination, four replicates of 100 g of leaves samples were hydro-distilled in Clevenger apparatus (Simões and Spitzer 2003) for a 2-hour period. The oil obtained was separated of the water and dried, being then weighted for the determination of estimated yield and content per plant.

RESULTS AND DISCUSSIONS

As a result of distant hybridization and incompatible chromosomes in gamete development, peppermint is considered complete sterile. Sterility is manifested by under development of mint flower male organ (Korneva 1976) - abnormal developing stamens remain short, sometimes degenerate or even dried bud, do not produce normal pollen, while the stigma is suitable for pollination.

Deprived by the opportunity of fructification, mint (and thus all lines obtained from VRDS Bacau), accomplish multiplication in a vegetative manner by solons. They appear in the latest part of the summer, and also as strains they live one year. Stolons vegetation period does not coincide with that of aerial stems, which are forming new stolons.

The period of stolons development is influenced by the planting depth. From our phenological observations we found that normally the development of stolons begin simultaneous with the first floor of the central stem branches and continues until the end of vegetation. Mint stolons do dot exhibit a period of absolute rest, so completely stop of growth processes or an end of exchange of substances was never found. Lack of rest period's leads to the possibility to start vegetation even in winter, during warmer days. In condition of lower temperature the stolons are endangered to perish. This makes it difficult and inconvenient silage stolons from the first decade of October.

In our experimental parcels, stolons were planted in a well-prepared soil layer at a depth of 3-5 cm, in first decade of October. The advantages of autumn planting contribute to an increased production and its quality per unit area. One of the most important advantages is the uniformity and vigorously of stolons, fact that confer resistance at low temperatures over the winter. The vegetation promotes early in spring, the plant grows quickly, and giving large qualitative leaves.

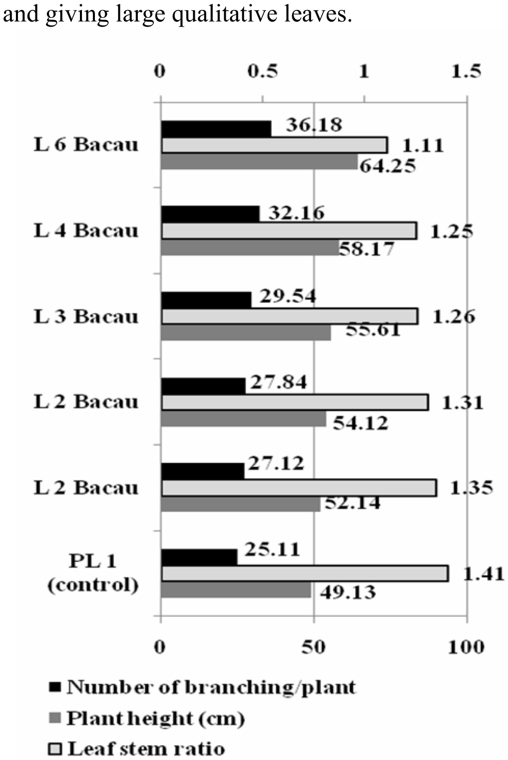

Fig. 1. Morphological investigation on mint lines

To establish our experimental field we used vigorous stolons with a length of 8-12 cm and minimum 4 viable eyes. We use a distance of 70 cm between rows, and gullies were opened with no more than 25 m in front in order to conserve water in the soil and ensure a proper contact between soil and stolons. Immediately after planting gullies were covered. They begin to vegetate in early spring when the soil temperature varied from 3 to 5^0 C. During the rest period the mint stolons can resist in condition of -10^0C to -25^0C but, lands has to be covered with a layer of 25-30 cm of snow. For a proper yield during growth and development period, a level of 18-22^0C was needed. High temperatures can harmful indirect, resulting in decreased soil moisture and air. There were applied special woks for maintaining the soil clean, free of weeds, but a portion of 7-10 cm from the plant was not mobilized, and the depth of 8-10 cm was not exceeded because it disturbs stolons development and production is diminished.

In figure 1 there are presented some investigation related with plant height (cm), number of brunches per plant and leaf stem ratio. We investigated the plant height and number of brunches per plant because of its importance on yield development and also in total volatile content oil.

It is already demonstrated the fact that entire herba contain volatile oil. The variation of plant height was between 49.13 cm and 64.25 cm.

The highest plants were the plants of L6 Bacau. The plant height can be positive correlated in our case with the total number of brunches and total yield. The total number of brunches per plant registered a variation from 25.11 to 36.18. In case of leaf steam ratio, the genotypes registered a small variation 1.11 – 1.41.

Data presented in tables 1 and figures 2 - 3 indicates the fact that lines developed at VRDS Bacau obtained superior yields comparing control variant. The highest level of green herba was registered by L6 Bacau 35650 kg ha^{-1}, representing 10450 kg ha^{-1} more than control variant.

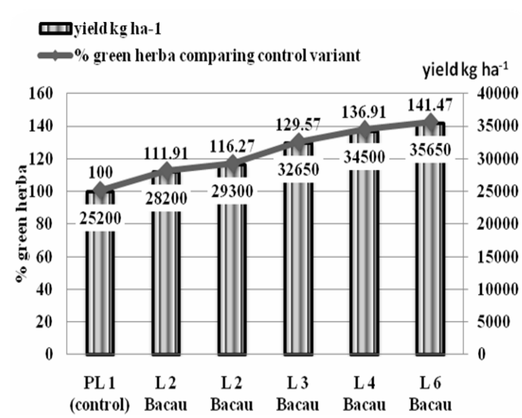

Fig. 2. The content of herba ha^{-1}

The differences between investigated genotypes are genetically explained, taking into account the fact that our experimental fields were placed in similar climate condition, and same technology was applied.

The highest volatile oil yield was provided by L6 Bacau. All genotypes provided superior oil yields, comparing control variant.

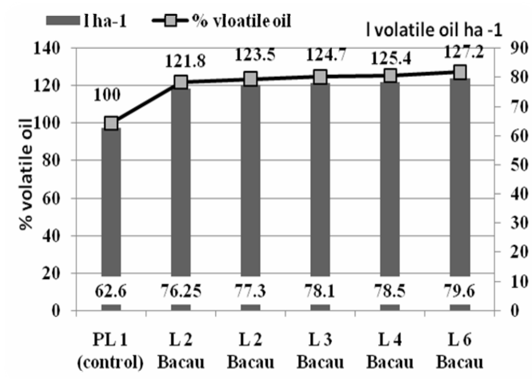

Fig. 3. The content of volatile oil l ha $^{-1}$

Photo 1. Clevenger apparatus (source: http://homepages.ihug.com.au/~panopus/lab/labequip.htm)

Table 1. Synthesis of production results

Genotype	Green herba yield		Difference on control kg ha^{-1}	Significant difference against the control
	kg ha^{-1}	%		
PL 1 (Mt)	25200	100.00	-	-
L 2 Bacau	28200	111.91	3000	**
L 2 Bacau	29300	116.27	4100	***
L 3 Bacau	32650	129.57	7450	***
L 4 Bacau	34500	136.91	9300	***
L 6 Bacau	35650	141.47	10450	***

DL 5 % = 2950 kg /ha
DL 1 % = 3100 kg /ha
DL 0,1% = 3500 kg /ha

In order to detect the quantitative volatile oil content we used a hydrodistillation method, by Clevenger glass apparatus, present in photo 1. The oil was extracted using as biological material whole plant located above the ground, thanks to its rich oil content. Regarding entire plant volatile content oil, our results confirm other previous result that highlight the importance of light in volatile oil content development. All parts of mint steams required a good exposure to light. The practice proved that if the mint culture is conducted as a biennale or perineal, the risk to appear defoliation facilitates the installation of fungal diseases (especially rust) and finally the production of volatile oil is reduced.

Whatever the destination of production was, harvesting was done only in condition of fine weather, without fog or excessive humidity (usually between 10 am and 5 pm o 'clock).

The optimal harvest time was established according with destination, as follows:

- to obtain the optimal leaves yield - harvest at early flowering 5-10%,
- to obtain the optimal volatile oil yield - harvest when flowering was over 50%.

Medicinal uses of mint

Mints (*Mentha* spp.) are famous aromatic and medicinal herb that are used since ages in traditional and folk medicines for its antimicrobial and antioxidant properties. In our region the most popular use of mint is related with digestive discomfort, the mint tea being applied in order to combat the unpleasant effects of: diarrhea, cramping, flatulence, vomiting. Mints contain volatile components, flavonoids, organic acids, quinones, such as for the digestive system, central nervous system, respiratory system (Stafford et all. 2005, Lucchesi et all. 2004). It was used in antimicrobial, anti-inflammatory or anesthesia (Hussain et all. 2010).

Herbalists consider peppermint as an astringent, antiseptic, antipruritic, antiemetic, carminative, vermifuge, diaphoretic, analgesic (Gardiner, 2000)

Food industry - Peppermint is widely used in food industry to flavor liqueurs and spirits, candy, and for flavoring menthol cigarettes, chewing gum. Increasingly used as raw material for soft drinks and syrups was registered in the last period. Volatile oil extracted from leaves and flowers can be used as flavoring of ice cream. Using fresh and dried leaves a tea can be prepared. Beside its refreshing effect, the mint tea is successfully applied in respiratory affections, as coughs, bronchitis.

Home use - Mint is used to flavor salads, potato, omelets, meats, sauces.

Other uses - In cosmetics thanks to its tannin content and volatile oil, mint has antiseborrheic, antiseptic and calming properties.

Waste from producing essential oil, contains up to 18% crude protein and 49% fat and can be used as feed. Mint is also an important honey plant.

CONCLUSIONS

Peppermint is part of a rich biodiversity and has the potential to play an important role thanks to its multiple potential uses: culinary, medicinal, cosmetics and perfumery. The obtained ecological yield, with minimum environmental inputs demonstrates mint suitability to cultivation in organic system. High-yielding mint lines were developed, according with market request.

ABSTRACT

This study represents an attempt to evaluate the continuing need for services in order to ensure the diversification of germplasm resources, in order to promote the multiple potential use as culinary, medicinal, honey, decorative. Our focus was on *Mentha piperita* species cultivated in ecological culture system. In our study we made observations and investigations regarding the applied technology in order to ensure a proper herba and volatile oil yield. The study presents also a review of potential use of species.

REFERENCES

1. DURIYAPRAPAN S.B., BRITTEN E.J., BASFORD K.E., 1986 - The effect of temperature on growth, oil yield and oil quality of Japanese mint. Annals of Botany, 58(5): 729-736;
2. GARDINER P., 2000 – Peppermint 2000 Longwood Herbal Task Force: http//www.mcp.edu/herbal/;
3. HUSSAIN A.I., ANWAR F., NIGAM P.S., ASHRAF M., GILANI A.H., 2010 - Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. J Sci Food Agric 90: 1827-1836;
4. KORNEVA E.I., 1976 - Results of back crosses of steroid-containing species. Genetika 12: 23-29;
5. KOKKINI S., 1992 - Essential oils as taxonomic markers in *Mentha*. In: Harley RM, Reynolds T. (Eds). Advances in Labiatae Science**.** Kew: Royal Botanic Gardens; p. 325-334;
6. LAWRENCE B.M., 1992 - Chemical components of Labiatae oils and their exploitation. In: Harley RM, Reynolds T. (Eds). Advances in Labiatae Science. Kew: Royal Botanic Gardens; p.399-436;
7. LORENZI H., MATOS F.J.A., 2002 - Plantas Medicinais do Brasil: Nativas e Exóticas. Instituto lantarum, Nova Odessa;
8. LUCCHESI M.E., CHEMAT F., SMADJA J., 2004 - Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydrodistillation. J Chromatogr A. 1043: 323-327;
9. OLFA BAATOUR R., KADDOUR W., AIDI WANNES M., LACHAAL MARZOUK B., 2009 - Salt Effects on the Growth, Mineral Nutrition, Essential oil Yield and Composition of Marjoram (*Origanum majorana*). Acta Physiol Plant, 10: 0374-4;
10. SIMÕES C.M., SPITZER V., 2003 - Óleos voláteis. In: Simões, C.M. (Org.). Farmacognosia da planta ao medicamento. 5 ed. Porto Alegre/ Florianópolis: UFRGS/UFSC; p. 467-495;
11. STAFFORD G.I., JAGER A.K., VAN STADEN J., 2005 - Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. J Ethnopharmacol; 100: 210-215.

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