Study on Essential Oil and Free Menthol Accumulation in 19 Cultivars, Populations, and Clones of Peppermint (*Mentha x piperita*)

Stanko Stanev
Research Institute for Roses, Aromatic and Medicinal Crops
49 Osvobojdenie Blv, 6100 Kazanluk
Bulgaria

Valtcho D. Zheljazkov
Department of Plant and Animal Sciences
Nova Scotia Agricultural College
PO Box 550, Truro, Nova Scotia
B2N 5E3 Canada
vjeliazkov@nsac.ns.ca

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Abstract

Bulgarian peppermint oil, also known as 'Bulgaro-Mitchum', is famous as having the finest quality. Being the main constituent of the oil, menthol is the targeted compound in the selection and breeding process. The menthol in the oil is represented in free form (menthol, isomenthol, and neomenthol), or in associated form (menthyl acetate). A two-year field experiment was conducted to study the essential oil and menthol productivity of 19 cultivars and population of peppermint. The aim of the investigation was to evaluate the available peppermint material obtained through various breeding methods with respect to further selection and breeding by using two indices: (1) intensity of essential oil synthesis (as the necessary dry herba for obtaining of 1 g of essential oil), and (2) intensity of menthol synthesis and accumulation (as the necessary dry herba for obtaining of 1 g of menthol). The highest essential oil content in dry herba was measured in two varieties: Dulgo pole (2.5 %), and Bulgarian population #2 (2.9 %). For these two forms, the necessary dry matter for obtaining 1 g essential oil are 40 and 34 g respectively. The highest menthol content was established in the oils from cultivars Zefir, Dulgo pole, and Clone 11-6-22 and Clone 80-121-33.

INTRODUCTION

Peppermint in Bulgaria is commercially grown for production of essential oil and dry leaves. Dry peppermint leaves (Folia Menthae) are used for herbal teas. Essential oil is obtained from the whole aboveground herbage (Herba Menthae) and is used in the food, cosmetics, and pharmaceutical industries. Plants wastes from the distillation are excellent forage and have been used as animal feed (Djouvinov et al., 1997; Topalov, 1962). Extracts from Bulgarian peppermint cultivars have been shown to possess significant antioxidant activity (Marinova and Yanishlieva, 1997). The Bulgarian peppermint essential oil (Bulgaro-Mitchum) obtained from Bulgarian varieties is known for its high quality (Topalov, 1962; Stojanova et al., 2000). Main constituents of Bulgarian peppermint oil are menthol and menthone, other constituents include α-pinene, β-pinene, sabinene, myrcene, limonene, cineole, α-terpinene, β-cymene, hexanol, menthyl acetate, β-caryophyllene, menthofuran, pulegone, carvone (Stojanova et al., 2000; Topalov and Zheljazkov, 1991; Zheljazkov et al., 1996). Menthol is presented as either free form (menthol, isomenthol, and neomenthol) or as menthyl acetate.

One of the objectives of the intensive breeding and selection program at the Research Institute for Roses, Aromatic and Medicinal Crops in Kazanluk, Bulgaria, is developing of new high-yielding cultivars of peppermint, suited either for essential oil or dry leaves production. Being the main constituent of the oil, menthol is the targeted compound in the selection and breeding process. Mentha x *piperita* is a complicated natural hybrid derived probably from crosses between *M. sivestris* L., *M. longifolia* L., *M. viridis* L., and *M. aquatica* L. Its flowers are sterile and so are incapable of producing seeds. However, there is high probability for developing of bud mutations on peppermint, some of them being with desirable production characteristics. In addition, there are some fertile forms of peppermint that could be used for intraspecific crosses.

The objective of this paper was to evaluate 19 cultivars, clones, and selection

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numbers of peppermint (obtained through various breeding methods) with respect to further selection and breeding by using two indices: (1) intensity of essential oil synthesis (as the necessary dry herba for obtaining of 1 g of essential oil), and (2) intensity of menthol synthesis and accumulation (as the necessary dry herba for obtaining of 1 g of menthol).

MATERIALS AND METHODS

The study was conducted for 2 years at the experimental field of the Research Institute for Roses, Aromatic and Medicinal Crops in Kazanluk, Bulgaria. Four out of the 19 cultivars, clones, and selection numbers (Tundja, Sofia, Kliment, and Zefir) are being grown in Bulgaria for production of essential oil and dry leaves. The rest of the cultivars and clones include: one population introduced from France, two clones obtained trough individual selection from local peppermint populations, and 10 clones obtained trough intraspecific cross of fertile forms of *M.* x *piperita*.

Experimental plots were 25 m^2, in 4 reps. Plants were irrigated during the vegetative period to maintain 70-80 % of FWC, and fertilized in accordance with the crops requirement and soil fertility tests. Peppermint was harvested in full flowering stage. Essential oil content in the biomass was measured using hydrodistillation in Clevenger type apparatuses. Essential oil for chemical analysis was obtained using steam distillation in semi industrial distillation units. The GC analyses were performed on Gas Chromatograph Pay Unicam, series 204, with CARBOWAX 20m capillary column.

RESULTS AND DISCUSSON

Essential oil content in dry *herba* of the tested cultivars, clones, and selection numbers of peppermint varied between 0.84 and 2.9 % (Table 1). The cultivars, clones, and selection numbers could be grouped in three groups depending on the essential oil content. The first group includes cv. Dulgo pole, cv. Zefir, Bulgarian populations #1&2, Selection numbers 50-P-64, and 80-121-33, having essential oil content of 2 % and higher. The second group includes selections with essential oil content of 1.5 to 1.99 % (cv Tundja (a check cultivar), Kliment, Sofia, Population from France, selection numbers 11-B22, 19-P-65, 22-P-16, and 23-P-17). The third group includes selections with essential oil content less than 1.5 % (Clone 45 and selection numbers 16-P-65, 18-P-11, 49-P-65, 50-P-16).

The intensity of essential oil accumulation varies widely among various cultivars, clones, and selection numbers. For accumulation of 1 g of essential oil 34 g to 120 g of dry herba are needed (Bulgarian population #2, and Clone 45, respectively).

The menthol content in the selections varies from 20.7 % to 66.2 % (Table 2). The average menthol content in the essential oils was 37.5 %. However, there was a large variation of menthol content between different cultivars, clones, and selection numbers.
Again, the selections can be clustered in three groups depending on the content of free menthol in the essential oil. Menthol in the essential oil of plants in the first group varies between 61.7 and 86 %. Menthol content in the essential oil of the plants in the second group varies from 41 to 60 %. And in the third group we have selections with menthol content in the oil under 40 %.

In addition to the essential oil and menthol content, we are screening and targeting other important production characteristics such as rust and verticilium wilt resistance, low content of menthone and pulegone. For instance, although with high menthol content, cv. Tundja has higher content of menthone as well (data not shown). Higher menthone content is found also in the oil from cv. Kliment (Stojanova et al., 2000). Thus, such cultivars have been traditionally used mainly for dry leaves production (Yankulov et al., 1975). Peppermint in Bulgaria is attacked by different fungus diseases such as: rust (*Puccinia menthae* Pers.), verticilium wilt (*Verticilium dahilae* Kleb.), powdery mildew (*Erysiphe cichoracearum* D.C.), anthrachnose (*Sphaceloma menthae* J.), septoriaose (*Septora menthicala* Sac. Et Let.) (Margina and Zheljazkov, 1994). Cultivar Sofia has been shown to posses' high rust resistance (Yankulov et al., 1975), which is the economically most significant disease in Bulgaria. Some of the cultivars, clones and selection numbers are good for production of dry leaves; others (with higher essential oil quality) are suitable for essential oil production.

CONCLUSIONS

The highest essential oil content was measured in cv. Dulgo pole and in Bulgarian Population #2. The highest menthol content was found in the essential oils of cv. Dulgo pole, in Bulgarian Population #2, and in Selection # 11-6-22.

The above cultivars and selection numbers may be suitable for industrial production of essential oil. Results demonstrate that the Research Institute for Roses, Aromatic and Medicinal Crops, Kazanluk have a wide selection of promising cultivars, clones, and selection number that could be further used for developing of new cultivars with improved production and quality characteristics

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Tables

Table 1. Intensity of the synthesis of essential oil in various peppermint cultivars and clones.

Clones	Essential oil content (%)	Biomass for 1 g essential oil (g)
1. Tundja	1.80	55
2. Zefir	2.22	45
3. Kliment	1.70	59
4. Sofia	1.70	59
5. Bul. population#1	2.20	46
6. Bul. Popul.#2	2.90	34
7. Clone 45	0.84	120
8. Dulgo pole	2.50	40
9. Popul. France	1.90	52
10. Sel. #11-6-22	1.80	55
11. #16-P-65	1.30	75
12. #18-P11	1.45	70
13. #19-P-65	1.80	55
14. #22-P-16	1.66	60
15. #23-P-17	1.67	60
16. #49-P-65	1.10	91
17. #50-P-16	1.10	91
18. 50-P-64	2.00	50
19. #80-121-33	2.12	47

Table 2. Intensity of the synthesis of free menthol in above ground plant parts.

Clones	Menthol content in essential oil (%)	Dry herba for 1 g menthol (g)
1. Tundja	25.3	218.8
2. Zefir	66.2	68.0
3. Kliment	36.7	160.2
4. Sofia	37.2	159.2
5. Bul. Population #1	23.3	194.4
6. Bul. Popul.#2	54.9	62.6
7. Clone 45	47.9	248.1
8. Dulgo pole	64.7	61.7
9. Popul. France	49.7	105.9
10. Sel. #11-6-22	64.5	86.1
11. #16-P-65	23.3	330.0
12. #18-P11	21.3	327.8
13. #19-P-65	35.7	147.0
14. #22-P-16	22.5	268.0
15. #23-P-17	28.1	213.2
16. #49-P-65	20.7	438.2
17. #50-P-16	25.4	358.4
18. 50-P-64	24.7	202.4
19. #80-121-33	41.3	114.1