

LAVENDER CULTIVATION IN BULGARIA – 21ST CENTURY DEVELOPMENTS, BREEDING CHALLENGES AND OPPORTUNITIES

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Abstract

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Bulgaria has a near century long tradition in lavender (*Lavandula angustifolia* Mill) cultivation and essential oil production. Following significant reduction of lavender plantations during the country's transition period at the end of the last century, the industrial lavender cultivation gained considerable attention and rapid expansion during the last decade. Here we present the main trends of the 21st century developments of the industrial lavender cultivation in Bulgaria. The data of parallel growing and essential oil production of the currently cultivated Bulgarian lavender varieties demonstrate the higher impact of cultivating vegetative propagated varieties instead of seed derived lavender populations. The main directions and challenges of the 21st century lavender growing in the country related to increased lavender cultivation are pointed out. The recent advances in development of lavender genomic resources, molecular markers, comparative analysis of flower volatiles and related opportunities for targeted breeding and marker assisted selection are discussed.

Keywords: *Lavandula angustifolia*, lavender varieties, Bulgaria, cultivation, essential oil, targeted breeding

Abbreviations: EST – Expressed Sequence Tag; SSR – Simple Sequence Repeat; SRAP – Sequence-Related Amplified Polymorphism; GC/MS – Gas Chromatography/ Mass Spectrometry

Introduction

Lavender is a perennial sub-shrub plant that belongs to the mint family (Lamiaceae) and is native to the Mediterranean region. The genus *Lavandula* includes above 30 flowering species, but only three of them are industrially cultivated for production of essential oil: lavender (*Lavandula angustifolia* Mill.), spike lavender (*Lavandula latifolia* L.) and lavandin - a sterile hybrid developed by crossing *L. angustifolia* × *L. latifolia* (Lis-Balchin, 2002; Lesage-Meessen et al., 2015). Lavender is cultivated worldwide in a number of countries, as some of the main lavender oil producers are Bulgaria, France, UK, China, Spain and others. During the last few years the volumes of lavender oil produced in Bulgaria steadily exceed those of France and the country becomes the world's top lavender oil producer, growing over 6000 ha of different lavender varieties.

Lavandin is mainly cultivated in France, which supplies 90% of the world production of lavandin oil. The main spike lavender cultivation and essential oil production are located in Spain. Lavender oil is considered of higher quality in comparison to lavandin oil and it is marketed at around 3–5 times higher price. Both lavender and lavandin essential oils have a wide range of applications in various industrial products including perfumes, pharmaceuticals, cosmetics, as well as, personal care and home maintenance products (Cavanagh and Wilkinson, 2005; Lesage-Meessen et al., 2015). Additionally, lavender oil has been increasingly applied in aromatherapy and integrative medicine due to its positive effect for treatment of anxiety, insomnia and hair loss (Lis-Balchin and Hart, 1999; Cavanagh and Wilkinson, 2002; Kashani et al., 2011; Sienkiewicz et al., 2011; Soltani et al., 2013). All this makes lavender oil a valuable ingredient and stimulates the expansion of lavender cultivation and essen-

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tial oil production.

In Bulgaria, the industrial cultivation involves only the common lavender, while lavandin is grown in limited areas without economic significance. Lavender cultivation has been introduced in the country for the first time during 1903 by Konstantin Malkov, the famous agronomist and founder of the agricultural science in the country. After his death the established lavender plantation were abandoned for more than a decade and lavender cultivation was re-initiated by the agronomist Konstantin Georgiev in 1925 using some of the survived lavender bushes. The first large lavender plantations were established in the areas around Kazanlak and Karlovo within the next few years using locally produced planting material and newly imported seeds from England and France (Georgiev, 1964). Since then, the lavender cultivation in Bulgaria has passed through periods of changing agricultural practices and expansion and reduction of the planted areas, which was heavily influenced by the overall country development.

Here we present the recent developments of lavender cultivation in Bulgaria, as well as, the impact of growing Bulgarian lavender varieties. The main challenges of lavender improvement and possibility for targeted breeding and application of marker assisted selection are discussed.

21st century developments of lavender cultivation in Bulgaria

During the 20th century, industrial lavender cultivation became one of the well-established sectors of the Bulgarian agriculture and the lavender planted areas exceeded 7000 ha in the beginning of the 70's. During the second half of the last century nearly all the lavender cultivation was carried out by the typical for this time cooperatives. The lavender oil was distilled and exported by the state owned companies. The transition period during the 1990s in Bulgaria had a strong negative impact on

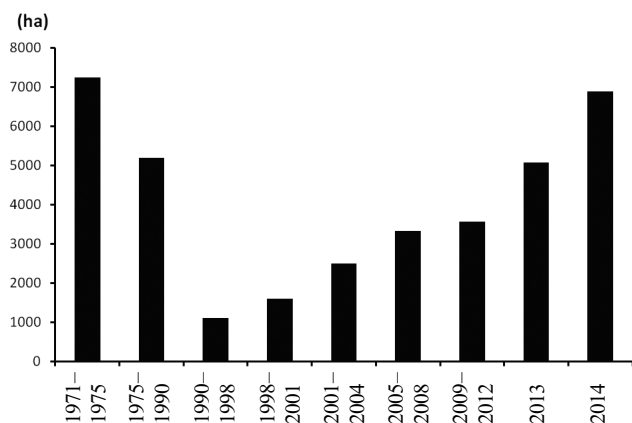


Fig. 1. Total area (ha) of lavender cultivation in Bulgaria

the lavender-growing and essential oil distillation industry. The wavering and slow implementation of the agricultural reforms during the transition period resulted in a decade without significant renovation of the existing and establishment of new lavender plantations. This led to nearly fivefold decrease of the areas of lavender plantations, reduced quality of the planting material and capacity for establishment of new plantations, Figure 1.

The stabilization and recovery of the Bulgarian economy started from the beginning of the 21st century bringing positive development and structural changes in the lavender cultivation and essential oil production. During the transition period the lavender plantations and essential oil distillation facilities became again a private property. EC Operational Programs supported by the Agriculture State Fund brought substantial new investments into the renovation and establishment of new lavender cultivation areas and oil distillation facilities. All this resulted in expansion of the country regions of lavender cultivation beyond the traditional ones. While during the last century lavender was mainly cultivated in South Bulgaria, in the regions of Stara Zagora, Plovdiv, Pazardzhik and Blagoevgrad, now it is cultivated in nearly all country regions including the North Bulgarian regions of Vidin, Veliko Turnovo, Shumen, Dobrich and Varna. Accordingly, the total areas of lavender cultivation grew rapidly during the last decade reaching 6892 ha in 2014, Figure 1. This placed Bulgaria as the world top lavender grower and essential oil producer. Lavender flower yields are heavily influenced by the environmental factors, ranging from 175 kg/da to 259 kg/da for the period 2005 – 2014 Figure 2. In spite of the year to year variations, the average yield of the harvested lavender fresh flower stalks grew during last few years reaching 259 kg/da for the 2014 campaign.

Lavender improvement and variety cultivation

The increased lavender cultivation and essential oil production in Europe during the first half of the last century stimulated

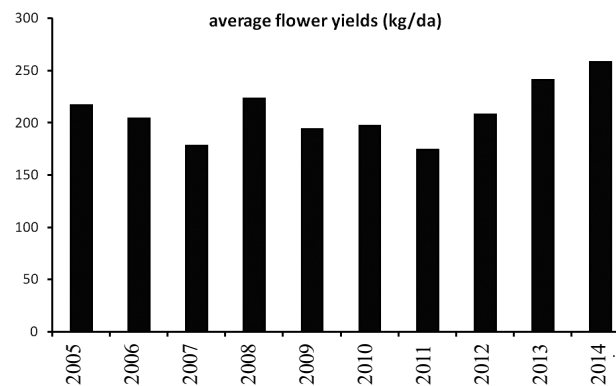


Fig. 2. Average flower yields from lavender plantations in Bulgaria during 2005–2014 period

Table 1
Flower yield and essential oil content and yield of six Bulgarian lavender varieties and seed population

Parameters	Lavender varieties and seed population						
	Hemus	Druzha	Sevtopolis	Yubileina	Raya	Hebar	Seed population
Flower yields (kg/da)	560 ± 54	637 ± 61	625 ± 62	554 ± 52	673 ± 66	755 ± 71	588 ± 51
Content of essential oil (% of the flower weight)	1.6 ± 0.2	1.8 ± 0.4	2.0 ± 0.5	2.2 ± 0.2	2.6 ± 0.4	2.3 ± 0.5	0.9 ± 0.2
Yield of essential oil (kg/da)	9.0 ± 1.0	11.5 ± 1.3	12.5 ± 1.5	12.2 ± 1.2	17.5 ± 1.7	17.4 ± 1.9	5.3 ± 0.6

The presented data refer the three years period 2005–2007 and were collected from 3–5 year old lavender plantations, located in the experimental field of IRAP. The lavender plants were cultivated according to technology of growing commonly use in Bulgaria

Table 2
Ranges of relative contents of main compounds of essential oils distilled from fresh floral stalks of six Bulgarian lavender varieties and seed population

Compound (range for Bulgarian lavender oil according to ISO3515:2002 specification)	Ranges of FID pick area percentages of lavender oil compounds (%)						
	Hemus	Druzha	Sevtopolis	Jubileina	Raya	Hebar	Seed population
Linalyl acetate (30–42%)	26.7–37.9	30.9–35.3	25.6–28.4	28.6–31.3	31.3–33.6	28.4–35.4	28.4–32.0
Linalool (22–34%)	30.5–39.8	31.3–33.9	22.2–31.6	24.0–29.5	33.6–35.6	27.2–28.7	30.2–32.3
Lavandulyl acetate (2–5%)	2.3–3.0	1.0–1.3	4.4–4.8	4.0–5.3	4.2–4.7	3.1–3.7	4.2–5.1
Lavandulol (> 0.3%)	0.7–1.1	0.3–2.9	0.4–1.8	0.1–1.1	0.4–1.2	0.2–1.3	0.3–0.9
Camphor (< 0.6%)	0.2–0.5	0.2–0.3	0.2–0.5	0.1–0.3	0.2–0.4	0.3–0.5	0.2–0.4
Terpinen-4-ol (2–5%)	0.2–4.3	2.5–2.9	0.3–1.8	1.7–8.8	0.4–2.8	0.2–3.3	1.5–3.2
Eucalyptol (< 2%)	2.8–3.2	0.2–1.7	1.4–5.5	0.4–0.5	1.3–2.3	0.8–3.8	1.3–1.7
cis-Ocimene (3–9%)	1.7–2.2	1.7–5.9	0.2–1.9	2.7–7.2	1.9–3.0	2.1–6.9	2.9–3.8
trans-Ocimene (2–5%)	1.0–1.7	1.3–4.9	1.0–2.9	1.6–5.4	0.2–2.4	1.9–5.7	1.6–2.4

The relative content of each compound correspond to FID pick area percentage from GC-FID chromatograms. The presented data refer the range of relative contents of each compound determined for essential oils produced from 3–5 year old lavender plantations sampled for three years period 2005–2007

the initiation of lavender improvement in France and the former Soviet Union (Russia, Ukraine and Moldova). The improvement programs targeted a wide range of lavender characteristics including flower yield, essential oil content and quality, content of linalyl acetate and cold tolerance. Initially the lavender improvement involved mainly testing, selection and propagation of best performing seed-derived plants. Later the breeders studied the effect of heterosis following hybridization between different varieties and breeding lines, as well as applying experimental polyploidy, chemical and radiation mutagenesis (Louzina, 1968; Romanenko, 1973; Singh et al., 1989).

In Bulgaria, lavender improvement started in the 1950's

(Dimitrova, 1959) and significantly expanded during the next four decades following the activities of the breeders from the Institute of Roses and Aromatic Plants (IRAP), Kazanlak. The studies involved clonal selection, intraspecific hybridization, gamma-irradiation, chemical mutagenesis and resulted in development of more than ten highly productive lavender varieties producing high quality essential oil (Dimitrova, 1959; Topalov, 1962; Topalov, 1969; Raev and Boyadzhieva, 1988; Staikov and Boyadzhieva, 1989). During the last decade, the researchers from IRAP continued to study clones and seed offspring of the country's most cultivated lavender varieties and to develop new varieties (Stanev 2007, 2010). Currently, lavender cultiva-

tion in the country involves mainly seven varieties developed during the last century namely: Hemus, Druzhba, Karlovo, Sevtopolis, Yubileina, Raya and Hebar. The varieties are planted in all lavender cultivations regions. A steady increase of the areas planted with the varieties Hemus and Sevtopolist is observed during the last few years.

The cultivation of selected lavender varieties, instead of population of seed derived lavender plants or propagated old lavender populations, resulted in an increase of both lavender flower and essential oil yields. The data presented in Table 1 demonstrate the impact of growing vegetative propagated lavender varieties in comparison to the seed derived lavender population. The results of the study show 6% to 30% increase of the flower yield for four of the tested varieties and between twofold to above threefold increase of the essential oil yield in comparison to the lavender seed population. The last is due mainly to the significant, above two fold, increase of the essential oil content in the inflorescence of the tested varieties. The further comparison of the essential oil composition distilled from inflorescence material of the studied varieties shows that the relative content of nearly all compounds lies well within the ranges specified by the international ISO3515:2002 standard for high quality Bulgarian lavender oil of population and clonal lavender, Table 2. The closer observation shows relatively large variations of the essential oil composition of the cultivated Bulgarian lavender varieties, which makes possible the production of quality blends from varietal essential oils. The described data clearly demonstrate the advantages of growing selected superior lavender varieties, well adapted to the local environmental and microclimate conditions.

It is worth noted that Bulgarian lavender varieties, are also cultivated and are a subject of additional studies in other countries, as well. For example, lavender cultivation in Romania was initiated during the last century using Bulgarian varieties and var. Karlovo became predominantly cultivated in this country for a long period (Pohrib and Nistor, 2012). Bulgarian lavender varieties are also cultivated in Turkey. A recent study involving lavender varieties planted in Isparta region Turkey, showed that the Bulgarian Raya and Vera varieties surpass the other tested varieties in qualitative and quantitative traits (Kara and Baydar, 2013). In another study, the varieties Druzhba, Hemus and Raya were shown to have high enantiomeric purity of linalool and linalyl acetate (Baser et al., 2005). Most of the Bulgarian varieties are also characteristic with high adaptability to unfavorable environmental conditions and two of them, namely Hemus and Raya show elevated yield stability under cultivation at suboptimal conditions (Stanev, 2010). Bulgarian varieties have also been used as a base for selection and improvement in geographically remote countries, for example CIMAP Regional Centre, Kashmir, India, where var. Karlovo was initially introduced

for cultivation in 1978 and later used in improvement program (Singh et al., 1989).

Directions and challenges of lavender cultivation

The increased lavender cultivation in Bulgaria during the 21st century resulted in changes of the applied agricultural and flower harvesting practices, which requires implementation of specific actions to provide further sustainable development of lavender growing and essential oil production in the country and keeping its position as world top producer and supplier of quality lavender oil. Some of the main directions and developments of the 21st century lavender cultivation in Bulgaria and related challenges include:

Mechanization of lavender growing and processing. The increased total area of cultivation and enlargement of the lavender plantations led to a steady increase and presently nearly complete mechanization of the lavender agricultural practices including tilling and flower harvesting. Accordingly, there are increasing demands of growing lavender varieties with improved plant architecture, which facilitates machine tillage and flower harvesting. Desired features of such varieties are the erected growth habit and long solid flower stalks not lying down on the ground, which makes possible the efficient machine tilling and flower harvesting without significant damages of the plants.

Lavender varieties better adapted to the local environmental conditions. The expansion of the total lavender areas during the last decade in Bulgaria is related with increasing cultivation outside the traditional areas of growing lavender in the country. Often the local soil characteristics and/or climate conditions in the new regions of lavender cultivation are not optimal for the currently available and cultivated varieties, which results in large variations of flower and essential oil yields, as well as lower lavender oil quality in some „bad” years. This requires additional parallel growing and testing of the existing pool of varieties and superior breeding lines and selection of the best performing for the new regions, or initiation of new lavender improvement program focused on specific regions. The testing and improvement work has to consider both adaptability of the tested varieties and breeding lines and stability of a number of growth parameters, flower and essential oil yields and essential oil quality (Stanev 2010).

Flower yield, essential oil content and quality. The further improvement production parameters and increase of flower and essential oil yields are a permanent task for lavender producers in the country. However, their pursuit is largely complicated by the demand the improvement to be combined with preservation of the high lavender oil quality. The complex genetic background of these parameters requires further research on genes and alleles related to biosynthesis of lavender essential oil compounds and employment of the gained information in marker

assisted selection of new superior varieties.

New products driven lavender cultivation. So far the lavender cultivation in Bulgaria was directed mainly to essential oil production through steam distillation and direct export of the lavender oil. During the last decade there has been an increased interest and demands for development of new innovative lavender products, as well as wider application of other extraction technologies like solvent or supercritical carbon dioxide extractions. Although the currently available lavender varieties provide quality flower material for application of modern extraction technologies and new product development, it could be speculated that there is a need for additional lavender improvement and development of new varieties better suited to particular extraction technology and/or innovative product manufacture.

Certified planting material and quality check. The results of parallel cultivation of lavender varieties and seed population presented above clearly indicate, that the use of homogenous, vegetative propagated planting material for renovation or establishment of new lavender plantations significantly increase both flower and essential oil yields. The structural changes in the Bulgarian agriculture during the transition period were also related to changes in the model of production and farmers supply with lavender planting material. The centralized production and distribution of lavender planting material in the country by IRAP during the second half of the last century is presently substituted with free market offering of planting material produced by a number of small producers. This raises the questions about the proper maintenance of the collections of lavender varieties, breeding lines and lavender genetic resources, as well as the restoration of the last century operating system for production of pre-basic, basic and standard propagation materials. Additionally, at present there are no well-established practices and service for quality check of the lavender planting material in the country. This makes possible the offering and cultivation of low quality seed derived planting material or such of mixed varieties. The expected increased utilization of a larger number of varieties and lines adapted to local environmental conditions in the future will lead to increased demands of certified vegetative propagated planting material and affordable quality check services.

Lavender genomic resources and opportunities for marker assisted lavender improvement

The increased complexity of the lavender improvement tasks pointed above requires future applications of targeted breeding rather than current testing and selection of better performing mutant clones or plants from seed derived populations. The implementation of lavender targeted breeding programs requires additional development of molecular markers suited

for marker assisted selection, as well as efficient procedure for routine comparative analysis of lavender flower volatiles. In spite of the economic importance of lavender and other genus *Lavandula* members, genomic resources and molecular markers suitable for characterization of lavender genetic resources and targeted breeding started to be developed only during the last decade. The reported characterization of lavender leaf and flower EST sets by Lane et al. provides a solid ground for development of lavender as a model system to study molecular regulation of essential oil biosynthesis supported by adequate genomic resources (Lane et al. 2010). An essential extension of this research was the development of EST-SSR markers and successful testing of their *Lavandula* species transferability, which is an important step towards development of efficient molecular markers for genetic resources assessment and targeted breeding (Adal et al. 2015). Molecular markers for *Lavandula* species were reported by other authors as well, but they were used in biodiversity and phylogenetic studies and are with limited applications for targeted breeding (Chunshan et al. 2009; Bräuchler et al. 2010). Our recent studies for searching of affordable genome wide markers demonstrated that SRAP markers could be efficiently used for genetic diversity assessment and also have potential for targeted breeding applications (our unpublished work). Another important step in the development of efficient lavender genomic resources was the reported characterization a number of key genes involved in lavender essential oil biosynthesis (Landmann et al., 2007; Demissie et al., 2011; Landmann et al., 2011; Demissie et al., 2012; Sarker et al., 2012; Sarker et al., 2013; Jullien et al., 2014; Benabdelkader et al., 2015; Sarker and Mahmoud, 2015). The expression studies of several lavender terpene synthases suggest they have complex spatial and temporal expression profiles, but terpene synthase activities in lavender inflorescence appear to be generally regulated at transcriptional level (Tholl, 2006; Guitton et al., 2010; Lane et al., 2010). The above makes realistic to employ the available and further generated knowledge about lavender essential oil genes for implementation of a targeted breeding program in order to: /a/ retain the lavender essential oil quality in parallel to improving of other agronomic traits or /b/ to carry out targeted metabolic engineering of essential oil biosynthesis for production of specific lavender essential oils. The available gene sequence data make possible the ready identification of gene-/allele- specific SNP markers in the available lavender varieties and breeding lines. For example, the direct sequencing of PCR amplified genomic DNA fragments of a trans-alpha-bergamotene synthase gene from seven Bulgarian lavender varieties results in identification of a set of SNPs which distinguish all tested varieties and could be used for analysis of SNP allelic configurations of this gene in plants from segregating populations, (our unpublished work). Putting

together the three types of markers, EST-SSR, SRAP and gene specific SNP, offer a solid base for initiation of efficient targeted breeding programs using marker assisted selection.

Employing efficient procedure for comparative analysis of flower volatiles of larger number of lavender lines is the other essential factor for a successful targeted breeding program. So far the testing and selection of lavender lines and varieties involves analysis of the lavender essential oil rather than direct analysis of flower volatiles (Laurence, 1988; Raev and Boyadzhieva, 1988; Staikov and Boyadzhieva, 1989; Baser et al., 2005; Muñoz-Bertomeu et al., 2007; Kara and Baydar, 2013). Although the essential oil analysis provides data of direct practical application and allows assessment of the lavender oil minor compounds, the composition of the lavender oil largely depends and varies with the distillation parameters, flower harvest and processing practices (Angioni et al., 2006; Cassel et al., 2009; Zheljzkov et al., 2013; Hassiotis et al., 2014; Dušková et al., 2016). This significantly hampers the use of lavender oil analysis for a precise comparison of the volatiles composition of the tested lavender lines. Additionally, the routine application of lavender oil analysis is complicated by the need to process in parallel a larger number of samples related to characterization of the tested populations. Our first attempt to develop routine procedure for analysis of lavender flower volatiles, employing milling and extraction of frozen lavender inflorescences, shows high variations of the compound abundances in replica analyses suggesting it can't be used for comparative analysis (Zagorcheva et al., 2013). Recently we successfully tested a simple flower extraction procedure followed by direct GC-MS analysis of extracts (our unpublished work). The procedure is well suited for routine comparative analysis of a large number of lavender plants, characterization of lavender populations or to study the effect of various environmental and agriculture factors.

Conclusion

The data presented in this review displays the rapid increase of lavender cultivation in Bulgaria during the 21st century, which resulted in the country becoming the world top producer and supplier of lavender oil. The data of parallel cultivation and essential oil extraction demonstrates the higher impact of growing Bulgarian lavender varieties instead of seed derived lavender populations. The increased lavender cultivation resulted in changes of the lavender growing and flower harvesting practices, suggesting new direction and challenges of lavender improvement. This poses elevated demands on application of lavender targeted breeding programs employing marker assisted selection and a larger scale comparative analysis of lavender flower volatiles. The recent developments in lavender genomic

and molecular marker resources provide solid ground for deeper characterization of the available genetic resources and initiation of targeted breeding programs directed towards the main challenges of lavender improvements.

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