

RESULTS AND PERSPECTIVES IN *OCIMUM BASILICUM* (BASIL) BREEDING AT VEGETABLE RESEARCH AND DEVELOPMENT STATION BUZĂU

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Abstract

The species Ocimum basilicum originates in Asia and is now widely spread throughout the globe. In Europe, it is assumed that it was introduced in 356-323 BC by Alexandru Macedon. Although the plant has been present in the crop for a very long time, it is grown on very small areas in the household system. In Romania, very few researches have been carried out that have targeted this species, although in the world, the plant is cultivated and widely used in food as well as decorative, ornamental, medicinal, aromatic, melliferous plant. But in our country, it has been used mainly in the cult of the church as sacred plant. At V.R.D.S. Buzau research was started for breeding this species in 1996, because at that time there was no registered autochthonous variety in the Official Catalog of Crop Plants in Romania, the only commercial variety being a local "de Radovanu" population. In 2006, the researches on this species ended with the first Romanian basil variety registered in the Official Catalog of Crop Plants under the name of 'Aromat de Buzau'.

Key words: 'Aromat de Buzau', Macedon, germplasm, genotype, variety.

INTRODUCTION

The basil plant (*Ocimum basilicum*) belongs to the *Ocimum* genus which is part of the *Lamiaceae* family, a family well-known for its aromatic and medicinal species. This family contains a large number of species, over 60 of which the most representative in our country is mint (*Menthapiperita*) widely used for a long time in our country.

Sweet basil (*Ocimum basilicum*) is one of the leading herb crops, used fresh or dry (Dudai et al., 2008).

Although, in Europe, basil was brought by Alexandru Macedon for a very long time (356-323 BC), in our country, this species was cultivated on small surfaces, in the household system, being used predominantly as sacred plant in the cult of the church. Lately, the interest in aromatic and medicinal plants has increased, a special emphasis being placed on this species, which is why the research has been extended since 1996 at V.R.D.S. Buzau, with a special emphasis on the breeding of this species. The main goals of breeding: high yield

of herb, high content of essential oil, good yield of seeds. (Seidler-Lozykowska, 2001)

It should be noted that until then, in Romania, there was no variety approved for this species, but only one local population under the name "De Radovanu" was approved and registered in the Crop Plants Official Catalogue of Romania. In 2006 the first Romanian commercial variety was registered in the Crop Plants Official Catalogue of Romania under the name of 'Aromat de Buzau'.

Research has been focused on the genetic potential exploitation and use of the species.

Sweet basil is grown for culinary use for both fresh and dry consumption and as a source of essential oil and oleoresin for manufacturing perfumes, food flavors, and aromatherapy products (Wyenandt, 2010).

It is a valuable plant species with multiple uses in medicine, cosmetics and gastronomy (Burducea M., 2016; Lagunovschi et al., 2016). Basil, being an entomophilous plant, shows great variability in the phenotypic expression of the characters, imposing special measures of selection and breeding.

There are many cultivars of basil which vary in their leaf size and colour (green to dark purple), flower colour (white, red, lavender, purple), growth characteristics (shape, height, flowering time), and aroma, making this plant an increasingly popular culinary and ornamental herb (Morales, 1993). Lately, besides the many possibilities of cultivating the species, research has been developed on new crop technologies.

MATERIALS AND METHODS

Currently V.R.D.S. Buzau has a rich and valuable collection in this species, composed of over 60 genotypes in various breeding phases. Depending on the stage of breeding, genotypes were divided into three fields: the collection field, that has the genetic stabilized genotypes in which the variability of the main characters are in the normal range, appropriate to crop varieties; the work field, in which are studied the advanced families whose characters are not well stabilized, but in a large proportion, the main characters expressivity is uniform; the work field in which are studied the segregating families where are the new introduced cultivars in the germplasm collection, or the ones that present a great variability of the main characters.

The undertaken researches for inventorying the genetic stability resulted with the following dispersion of the genotypes: the collection field has a number of 12 stabilized families which are presented in this work, the work field with 17 families that have advanced bred genotypes and the work field in which are studied a number of 32 segregant families. The researches undertaken by breeding this species were achieved with difficulty due to the fact that this plant is entomophilous, its pollination being made by the wind and insects, it is preferred by bees, and for maintaining its genetic purity, safe isolators were used (divided greenhouses and textiles isolators) and in some cases, large distances between the genotypes undergone in the breeding program were applied.

The main genotypes bred belong to the following varieties: *Ocimum basilicum* f. *minimum*, characterized by a small size, small leaves and an intense scent in which we encounter the following types: *viridis* - with

green leaves, *violaceum* - with violet leaves, *viridicrispum* - with green blistered leaves, *viola crispum* - with violet blistered leaves and also *Ocimum basilicum* f. *bulatum* - with blistered and very strong serration on margin.

As the main breeding method hybridization between families was used, then segregation, positive mass selection and negative mass selection, the stabilized families were isolated and self-pollinated for 6-7 generations, in order to be realized the homozygous of the main characters.

At the Biological Research Centre "Stejarul" Piatra Neamt were analysed volatile oils from two lines of basil, respectively the control variant, 'Aromat de Buzau' and L 12 using gas chromatography method coupled with mass spectrometry GC/MS by means of Gas Agilent Technologies type 6890N chromatograph coupled with mass detector (MSD) TYPE 5975 INERT xl Mass Selective Detector. Volatile oils were obtained by hydrodynamics.

The applied crop technology was species-specific, and seed production was performed on alveolar pallets with Klamann TS 3 ground peat substrate.

The crop for all studied genotypes was set by seedlings and the planting was done using the following scheme (Figure 1).

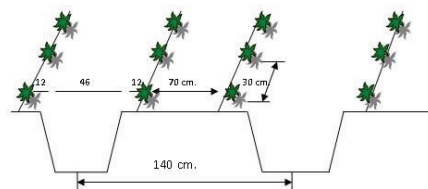


Figure 1. Planting scheme for basil

For most of the species, the crop was set using the following distances: 70 cm between rows and 30 cm between plants, an exception being L5 cultivar that has a small globular habitus, for which a smaller distance between plants was used, only 25 cm (Figure 2).



Figure 2. Basil seedlings

RESULTS AND DISCUSSIONS

A valuable germplasm of 60 genotypes was established within the research and, after their evaluation, 12 distinct phenotypic genotypes were genetically stabilized and retained for the

work field. ‘Aromat of Buzau’ basil was used as control variant in the field.

During the vegetation period biometric and phenological measurements were made and the main plant features are presented in Table 1.

Table 1. The main plant features - average values

Character/Genotype	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
Plant height (cm)	60	57	120	76,3	28	42	48	45,5	89	107	75	96
Stem height to first branch (cm)	6	2	4	7,5	0,6	1,6	2,6	2,1	4	6	4	8
Bush shape	globular	globular	globular	globular	globular	erect	erect	erect	erect	erect	globular	globular
Internodes distance	6,3	6,8	7,2	12,5	1,7	4,4	3,2	6,2	8,1	8,2	7	8,5
Bush diam. (cm)	70	65	110	80	38	38	36	39	72	76	51	68
Stem diam. (cm)	2	1,6	2,4	1,8	1,2	1,2	1,3	1	1,6	1,4	1,4	1,2
No. of main shoots	3	7	15	18	7	9	8	7	3	24	8	12
Length of main shoots (cm)	29,3	33	82	59	72,5	38,5	40	39,5	77,1	63	39	64
No. of sec. shoots (cm)	15	46	163	76	119	69	78	87	68	196	34	72
Length of sec. shoots (cm)	11	16,5	36	44	11	14,4	19,5	8	75	18	20,5	22
Leaves colour	Light green	Light green	Medium green	Green	Green	Dark purple	Dark purple	Dark purple	Medium green	Gray-dark green	Light green	Dark greysh green
Leaf blade length (cm)	7,25	19	14	6,9	2,2	7,5	7,4	8	10,6	7,5	13	5,9
Leaf blade width (cm)	3,6	11	11	2,5	1,3	4,3	3,5	4,8	5,3	3,4	12,5	2,7
Peduncle length (cm)	2,7	7,5	6,5	2,6	0,8	2,3	1,7	3,5	4,1	3,5	4,8	2,9
Leaves area (cm ²)	22 714	28 572	30.008	17 196	14 084	17 917	18 693	17 820	24 538	18 925	29 143	7102
Flowers color	White	White	White	Purple-lilac	White	Purple-lilac	Purple-white	Violet	White	White	White	White slightly anthocyanin
Inflorescence length (cm)	12	10	26	26	8,1	15	9,6	13,1	22,7	16,5	11	15
No. infl.	49	17	449	503	119	22	52	40	65	123	26	86
No. florets	8-10	7	20	12	6	13	10	9	14	9	9	11
Flavour	Typical basil	slightly sweet	cloves	anise	cloves	Sweet rosy	Slightly mint	cloves	Peppery	lemon	tasty	lemon

L1 belongs to *Ocimum basilicum* L., classic basil, with a mint flavour.

This cultivar is patented as ‘Aromat de Buzau’. This variety of spicy basil is late, with well-defined genetic structure, and adapted to the environmental conditions of our country.

This variety is easy to recognize due to its specific flavour, and also thru other distinct characters, such as: leaves colour, shoots length, the ability of retaining its characteristics during conservation.

The plant is distinguished by mean values in terms of the expressiveness of the main characters, recording a height of 60 cm, a diameter of 70 cm and a number of 48 inflorescences.

It is so typical of the classic basil plant (Figure 3).



Figure 3. ‘Aromat de Buzau’ (L1) variety - *Ocimum basilicum* L., classic basil

L2 belongs to *Ocimum basilicum* f. *bulatum* species, a valuable cultivar, genetically stabilized, with distinct phenotypic characteristics, a globular plant, with very large leaves that have a medium length of 19 cm and an average width of 11 cm. Due to its rich foliage, light green, juicy, this cultivar is suitable for salads and other dishes.

It is included in the earliness 02 group and has a specific slightly sweet flavour, mild to low intensity. Genotype is distinguished by the smallest stem, 2 cm but the largest leaf, 19 cm with the longest petiole, 7.5 cm (Figure 4).



Figure 4. Plant detail of L2 - *Ocimum basilicum* f. *bulatum*

L3 belongs to *Ocimum basilicum* f. *viridi crispum* species, a cultivar characterized by a vigorous globular bush, that has a great foliage device, with an average foliar surface of 30 008 cm², the leaves are large, slightly corrugated and deeply toothed edges. The foliage colour is medium green and has a flavour that is specific to cloves. Because of the richness of the foliage, tender and juicy, this genotype can be used in salads and other dishes. This variety is the one with the highest port, 120 cm tall and 110 cm in diameter, and the main shoots are 82 cm high (Figure 5).



Figure 5. Plant detail of L3 - *Ocimum basilicum* f. *viridi crispum*

L4 belongs to *Ocimum basilicum* f. *thyrsiflora* species, a new cultivar obtained at V.R.D.S. Buzau by crossbreeding a variety of Thai basil with V.R.D.S Buzau's owned variety 'Aromat de Buzau'. This plant is globular and the

inflorescence is corymb type. The foliage is green, the main ribs are slightly red and the inflorescence has white-lilac flowers with burgundy-red florets. The flavour is similar to anise released by the whole foliage give more value to this cultivar, recommended for various dishes and tea. This genotype is the one with the largest number of inflorescences, 503 with a length of 26 cm. Thus, it becomes even more productive (Figure 6).

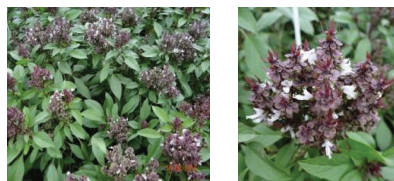


Figure 6. Plant detail of L4 - *Ocimum basilicum* f. *thyrsiflora*

L5 belongs to *Ocimum basilicum* f. *minimum* species, a new cultivar genetically stable which has a shape of a small bush. Due to this characteristic, this cultivar is intended to be grown in pots or jardinière, outdoors like terraces and also indoors. The leaves are small, with a medium length of 2.2 cm and a width of 1.3 cm. It has a strong, fine flavour, slightly peppered, in which can be found a light scent of thyme, feature that recommends it to be grown in pots, for freshened air purposes. This variety records the lowest values for the leaf (2.2 cm long), the plant height (28 cm) and the inflorescence length (8.1 cm) due to the dwarf habit (Figure 7).



Figure 7. Plant detail of L5 - *Ocimum basilicum* f. *minimum*

L6 belongs to *Ocimum basilicum* f. *violaceum* species, a cultivar that has plants shaped as a slender bush. The defining characteristic is given by the colour of the foliage which is dark purple, with elliptical leaves, no hair, with acuminate top. It presents a strong flavour, sweet with scents of rose. It can be used in

various dishes, teas, juices and due to strong anthocyanin pigmentation it can be used as a natural food colouring.

This genotype is distinguished by its very strong violet-black foliage (Figure 8).

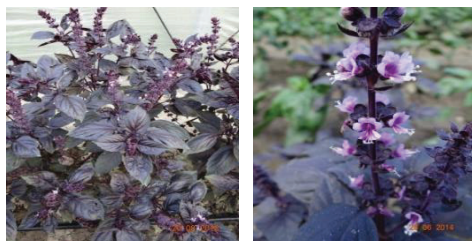


Figure 8. Plant detail of L6 - *Ocimum basilicum* f. *violaceum*

L7 belongs to *Ocimum basilicum* f. *violaceum* species, a new cultivar that has an erect shape, with great dark-purple colour foliage.

The shape of the leaves is oval-elongated, serrated and slightly wrinkled, a feature that gives distinctness to this cultivar.

The flowers are purple, discreet flavour is slightly sweet with scents of mint.

This genotype recorded the smallest bush diameter of only 36 cm (Figure 9).



Figure 9. Plant detail of L7 - *Ocimum basilicum* f. *violaceum*

L8 belongs to the *Ocimum basilicum* f. *violaceum crispum* variety, a cultivar that has an erect bush shape, the leaves are blistered, serrated on the margin, *crispum* type, a distinct characteristic of this family.

The flowers are purple and the flavour is specific, discrete, resembling with the flavour of cloves.

This plant has a long growing season, it blooms late and it is included in the 03 earliness group. This genotype recorded the smallest stalk diameter of only 1 cm but also the smallest 2120 cm² foliar surface (Figure 10).



Figure 10. Plant detail of L8 - *Ocimum basilicum* f. *violaceum crispum*

L9 belongs to *Ocimum basilicum* L. var *genovese* species, a cultivar that has vigorous plants, with an average height of 89 cm and a diameter of 72 cm. Its medium green foliage has convex, tender, juicy leaves.

The flowers are white, with a length of 22.7 cm with a total number of 14 florets.

The flavour released by this cultivar is strong, peppery. The cultivar shows only 3 main shoots, the lowest number of main shoots being recorded among the 12 genotypes studied (Figure 11).



Figure 11. Plant detail of L9 - *Ocimum basilicum* L. var *genovese*

L10 belongs to the *Ocimum basilicum* f. *citriodorum* species, a cultivar characterized by an erect tall bush, with an average height of 107 cm and a diameter of 76 cm.

The foliage is rich, with a silver-green color, with a slightly pointed leaves that has both glandular hairs on the stalk and on the main and secondary ribs.

The color of the flowers is white, and the strong asset of this variety is its flavour that resembles with the lemon aroma, feature that makes it suitable for flavoring drinks and various dishes.

L10 has recorded the biggest number of main shoots, 24 and 194 secondary shoots, characterized by a rich vegetative mass (Figure 12).



Figure 12. Plant detail of L10 - *Ocimum basilicum* f. *citriodorum*

L11 belongs to the *Ocimum basilicum* f. *bulatum* species, characterized by a globular bush, an average height of 75 cm.

It has rich foliage with large leaves, slightly blistered, a length of 13 cm and a width of 12.5 cm.

Leaves are juicy and fleshy, suitable for salads. Their flowers are white and the distance between florets is of 1.9 cm.

The plant presents a discreet flavour, specific to this species. This genotype is distinguished by the highest value of 12.5 cm leaf width (Figure 13).



Figure 13. Plant detail of L11 - *Ocimum basilicum* f. *bulatum*

The research was completed in 2018 with the approval of a new variety of basil, listed in the Official Catalogue of Crop Plants in Romania by the name of Macedon.

The researches undertaken at SCDL Buzau have demonstrated that this cultivar can be cultivated successfully in protected areas, in open field and in pots and jardinière, indoors. The strongest asset of this cultivar is given by the specific intense aroma of lemon.

The plant has a globular shape, medium size, with a height of 96 cm the median diameter of the bush is 68 cm, having a number of 12 main shoots and a number of 72 secondary shoots. The stem at the base is woody and as it evolves, growing turns from green to brown.

The height of the stem to the first branch is 8 cm and has a diameter at the base of 9.5 mm the main shoots have an average length of 64 cm and the secondary ones of 22 cm.

On the main stalk a number of 16 inflorescences are found and the length of the bloom of the inflorescence is on average 15 cm. The diameter of a single floret is 2.2 mm.

The inflorescence has a diameter of 7.5 mm and the distance between the florets of the inflorescence is 2.8 cm.

The diameter of a floret is 1.6 cm and the height is 7.3 mm. The number of flowers in florets is 6. The colour of the flowers is white with light anthocyanin pigmentation.

The leaves are light green with a shade of grey. The leaf has an average length of 5.9 cm and a width of 2.7 cm.

The length of the petiole is 2.9 cm, the thickness of the leaf is 0.4 mm. The thickness of the petiole is 1.3 mm, the leaf weight is 0.26 g.

On the whole plant, but more accentuated at the nodes and branches, there are glandular hairs that are responsible for secreting the specific lemon fragrance.

The plant has a high production potential, a single bush weighs 560 g, but production can increase if the young shoots are planted. Multiplication can be done by seeds, but also by sprouting.

It should be noted that the species generally has good seedlings and propagation capacity. Between 3-5 days the cuttings emit roots depending on temperature and the phenophase of the harvest.

The seeds are round, slightly oval, black with light hard tegument. There are 664 seeds per gram and MMB (1000 seed weight) is 1569 g (Figure 14).



Figure 14. Plant detail of L12, 'Macedon' variety

Table 2. Chemical composition of volatile oil in *Ocimum citriodorum* (%)

Compounds	S1	S1-C
β- myrcene	0.30	0.23
Eucalyptol	2.77	3.04
Trans-β-ocimene	0.69	0.47
Linalool	38.47	40.40
Camphor	1.03	1.18
Estragole	45.89	41.09
β- elemene	1.27	1.05
Caryophyllene	0.60	0.45
Germacren D	2.29	1.28
Elixene	0.47	0.35
δ- guaiene	1.99	1.31
ι- cadinene	1.26	1.11

S1- sample extract; S1-C-sample extract control

Following the analyses carried out, for the aromatic varieties of Buzau and ‘Macedon’ respectively, the chemical compounds of the volatile oils, which are presented in table 3 for *Ocimum citriodorum* (‘Macedon’) and 4 for *Ocimum basilicum*, (‘Aromat de Buzau’).

This genotype is marked by the study of biochemical analyses, with a high geranial content of 22.7% and carveol-17.7%. The results are consistent with the literature (Grayer, R., 1996).

Table 3. Chemical composition of volatile oil in *Ocimum basilicum* (%)

Compounds	S1	S1-C
Sulcatone	1.61	1.31
Cis-β-ocimene	1.03	0.51
Linalool	6.18	0.39
Cis-verbenol	1.13	1.31
Estragole	8.11	0.40
Nerol	9.65	11.04
Carveol	17.67	30.62
Neral	3.42	1.32
Geranial	22.67	37.50
β-caryophyllene	7.08	3.53
Trans-α-Bergamotene	2.22	1.12
α- caryophyllene	1.24	0.51
Cis-β-Farnesene	1.22	0.52
Germacren D	2.65	0.59
β- Bisabolene	6.61	3.31

S1- sample extract; S1-C-sample extract control

The volatile (essential) oils obtained by the hydrodynamics of the floral spice are characterized by a high content of estragon-45.9% and linalool-38.5%, the results are in line with those in the literature (Chalchat & Ozcan, 2008).

Both varieties are distinguished by a complex biochemical content of volatile oils that can be successfully used both for consumption and for industrial processing. ‘Aromat de Buzau’ has a balanced aroma from all points of view, being a classical basil and ‘Macedon’ comes with novelty within this species, adding value through its unique and special aroma of lemon, the aroma released by all its vegetative organs.

CONCLUSIONS

Twelve genetically stabilized genotypes were obtained, of which two were ‘Aromat de Buzau’ and ‘Macedon’ variety, a variety with a specific lemon flavour.

Biochemical analyses highlight the multiple possibilities of use the varieties and the qualities of volatile oils obtained from them.

The studies undertaken will be capitalized in the future by proposing the homologation of the genotypes that have been improved in the experience and that can meet the requirements of the DUS test (distinctness, uniformity, stability).

Along with new basil varieties, market studies have been carried out and the demands of consumers and growers and the demand for seeds and seedlings for the two new creations have increased significantly from one year to the next.

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