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CREATING AND EVALUATING THE NEW OCIMUM *BASILICUM* L. GENOTYPES

[Note I]

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The *Ocimum* L. genus includes about 200 species and varieties, and one of the best known is sweet basil – *Ocimum basilicum* L. [common basil, sweet basil]. This species grows in the spontaneous ora of Asia, Africa and the warm areas of North America [5]. It has been cultivated for over 1000 years. To many countries of Europe, sweet basil was brought from India and Egypt [1]. It was known in Antique Greece where it had frequently been used in the treatment of many diseases. It was even used in cases of insects and scorpion stitches. Today, the species is cultivated and used as both an aromatic and medicinal plant and as a decorative one. The plant synthesizes and accumulates in the leaf and ?ower odiferous glands essential oils with a ne balsamic fragrance, appreciated by the perfume producers. The same fragrance as well as the fact that basil contains C vitamin and carotene makes the species be used as fresh and dry weight spice.

In traditional medicine it is used in the form of infusions in the treatment of asthma, whooping cough and intestinal in ammations. The fresh juice is used in the treatment of ear swellings. The pharmaceutical preparations obtained from basil are used in case if respiratory tracts in ammatory processes, as an antispasmodic remedy as well as a tonic of the nervous system. It has been demonstrated that the *herba* has a carminative and stomachic action, it is galactosis, anti-in ammatory. Antimicrobial and antifungal properties also noticed [5]. The chemical composition, the concentration of each component substantially differs concordant with the variety, the soil, the genotype, and also with the origin and the pedo-climatic cultivation conditions. For example, *Ocimum basilicum* L. synthesizes 2 types of essential oils: 1 – the one cultivated in Europe and America with the main component methylhavicol, (—)-linalool of very good quality with a ne fragrance and without camphor. 2 – sweet basil from Africa – with the main component methylhavicol and an appreciated content of camphor [7, 8]. Therefore it is possible existence (creation) 4 chemo types Ocimum basilicum L. with maximally concentration of principal component in essential oils: linalool types; methylchavicol types; methyleugenol types and methilcianamat types 2, 3, 8]. Our investigations aim to evaluate and select the most valuable genotypes for the future created by means of hybrids and culture promoting.

MATERIALS AND METHODS

A hybrid originated genotype of *Ocimum basilicum* L. was studied. To create performing genotypes, we included in the hybridizations programs a series of proveniences from the Republic of Moldova (Slobozia-Ciobruci, Brătuleni, Chişinău, Lozovo-Străşeni), Germany (Stuttgart), France (Bordeaux) and Romania (Cluj-Napoca). Was analyzed genotypes (hybrids) which in the pedoclimatic conditions of the Republic of Moldova, with rich soils but insuf cient humidity, that produce a relatively raised production sustained by tall plants with a rich branching, long in orescences with a great number of verticilles on the oral ear, and leaves that preserve the green colour and do not fall till harvesting. The control was a genotype from Romania, which in the preceding years had a raised herba yield. So, genotype studied was next: 1-GT (1) green leaves, red ?owers; 2-GT (2) green leaves, white owers; 3- GT(2) red leaves, red ?owers; 4-GT(3) green leaves, white ?owers; 5-GT(3) green leaves, red owers; 6-GT(4) green leaves, white ?owers; 7-GT (5) green leaves, white owers; 8-GT(5) green leaves, red ?owers; 9-GT Romanian provenience, green leaves, white owers, standard. All the 9 genotypes were cultivated in 2007 on the eld of the Institute of Genetics and Plant Physiology, on a common chernozem. Superphosphate was put into the ground (P_{60}), and before seeding the lot was fertilized with $N_{30}P_{30}K_{30}$. The transplant was cultivated in the III-rd decade of May, in rows with the eda c surface of 50 x 20 cm. Maintenance included 2 mechanic weddings between the rows and 2 manual ones in the rows. In the period of July-August 2 irrigations were made.

The cultivation conditions of 2007 were characterized by drought and excessive heat. The spring precipitations were of 89.2 mm, with 21.8 mm less than the multiyear average of the period. The summer months were dry and with a water de cit of 137.5 mm compared to the registered multiyear average. The driest month was July, with only 3.6 mm precipitations, compared to multiyear data the 66.0 mm. Drought was accompanied by excessive heat especially in the period June-August. Air humidity decreased considerably. For example, in July, the average air humidity was of 43 %, with 20 % less than the multiyear average.

The values of some quantitative characters (plant height, number of branches, the length of the oral ear) were determined due to the evaluation methods of the plant cultivars of the State Commission for Crops Variety Testing.

Harvesting the aerial parts of the plants to be studied was performed in the phase of the ?oriferous corolla shake of the inferior verticiles of the oral ear.

The content in polyphenols, expressed in caffeic acid and the concentration of the avonic compounds, expressed in rutoside, was determined by GC of the mathanolic extracts.

RESULTS AND DISCUSSIONS

Known are variants of basil with intense green leaves and with white-yellow to radish ?owers [6], green or violet ?ne owers, smooth or rippled [1]. The selection of the genotypes with the features of which were quantitatively analyzed, including the phytochemical features (quantitative and qualitative), was made taking into account the color of the leaves and owers.

The values of the quantitative features, especially the plant height and the length of the in orescence, were smaller than in the previous years when the cultivation conditions were the normal ones in the Republic of Moldova. Despite this, all the genotypes reached the phase of the ?oral ear formation, all produced owers and herba. If we appreciate the resistance to drought from the point of view of the plant gabitus compared to the control, and later the drought and excessive heat were well supported by the genotypes 1-GT (1), 2-GT (2), 3-GT(2) and 7-GT (5), that formed plants of 36.7-39.5 cm high with a relative raised number of branches, long in orescences and a great number of verticiles on the oral ear (table 1). Generally, all the evaluated genotypes are interesting as sources of herba and may be cultivated for this aim.

These genotypes were analyzed to dose their content of polyphenols and avons, yet also to evaluate their biosynthetic spectrum (polyphenolic and avonoidic fractions). The same genotypes will be analyzed by GC-MS to compare the volatile fractions of each sample. The studies aim the nding of some correlations between the phenotypic expression (size of the bush, number of branches, number of leaves/stem, size of the in?orescence, number of verticiles/? owers of the oral ear, colour of the leaves and owers) and genotypic (which is written in the heredity of the species, especially from the point of view of the biosynthetic capacity). As molecular markers one may use the DNA analysis, of the enzymatic equipment, the dominant of a chemical compound (polyphenolic, avonoidic fractions, especially the dominant volatile fraction) to have a minimum variability. Analyzing the data of table 2 one can notice the variability of the polyphenolic and avinoidic content of the basil variants related to their morphological features – colour of the leaves and the owers.

Table 1. The values of some quantitative features of new *Ocimum basilicum* L. genotypes

Genotype, distinctive features	Plant height (cm)		I-st degree branching		II-nd degree branching		In orescence/ Length (cm)		Verticile/ In orescence	
	X	sX	X	sX	X	sX	X	sX	X	sX
1-GT(1)	39.5	1.5	18.3	3.9	12.0	2.7	14.4	3.1	16.1	1.2
2-GT(2)	37.8	1.2	16.9	2.8	10.4	3.0	12.8	2.3	15.2	1.6
3- GT(2)	38.1	1.4	16.3	2.5	11.9	2.1	13.9	2.9	14.0	1.5
4-GT(3)	31.9	0.9	10.9	1.8	13.1	2.4	9.6	1.7	11.8	1.1
5-GT(3)	32.8	1.1	12.1	2.0	12.9	1.9	11.8	2.9	10.6	1.2
6-GT(4)	35.7	0.8	13.3	2.6	10.8	0.9	11.4	2.5	10.4	1.3
7-GT(5)	36.7	0.9	14.1	3.1	14.9	2.1	11.5	1.8	11.0	1.1
8-GT(5)	32.6	1.0	12.7	2.8	13.0	2.4	11.6	1.9	10.9	1.3
9-GT	40.1	1.3	19.1	4.2	14.1	4.1	14.2	3.6	12.0	2.1

On the whole, the polyphenolic content of the plants with green leaves and white ?owers is greater than that of the plants with green leaves and red owers, with one exception, genotype nr.5 GT(3), the plants of which have green leaves and red owers, have a content of 0.902 g% dry material polyphenols expressed in caffeic acid. We must mention the fact that there is no direct correlation between the colour of the leaves and ?owers and the quantity of polyphenols and avones dosed in the basil plants.

Theoretically, the polyphenol quantity should be greater taking into account the biosynthetic metabolism of this type of compounds, implicitly the antocianic derivates, which are implied in changing the colors of the leaves and owers. On the whole the analyzed basil genotypes present quantities of polyphenols higher them 0.500 g% d.m. compared to the control.

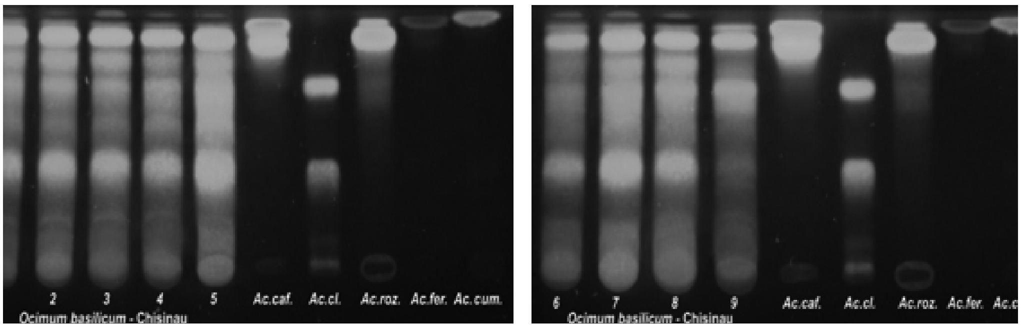
Table 2. The content of polyphenols and avons of the new *Ocimum basilicum* L. genotypes.

Genotype, distinctive features	Polyphenols Caffeic acid (g% d.m.)	Flavones Rutoside (g% d.m.)
1- GT(1) green leaves, red owers	0.602	0.773
2-GT(2) green leaves, white owers	0.716	0.715
3- GT(2) red leaves, red owers	0.596	0.700
4-GT (3) green leaves, white owers	0.711	0.825
5-GT (3) green leaves, red owers	0.902	0.819
6-GT (4) green leaves, white owers	0.513	0.615
7-GT(5) green leaves, white owers	0.577	0.503
8-GT (5) green leaves, red owers	0.548	0.419
9-GT rom., green leaves, white owers, control	0.477	0.322

The content of the avonoidic derivates is variable and is not strictly correlated with the colour of the leaves and the owers. The same genotypes 4-GT (3) and 5-GT (3), which has plants with green leaves, have the highest values related to the colour of the ?owers, so that the plants with white owers have a content of 0.825 g% dray material, and the ones with red owers of 0.819 g% dry material. We must mention that the genotypes 4-GT (3) and 5-GT (3) originate from the same hybrid combination which in F_1 had green leaves and red owers, and in F_2 segregated into plants with white ?owers and plants with red owers. We may also conclude that the genotypes 2-GT (2) and 3-GT (2), selected from another segregated hybrid combination, are different not only from the point of view of the colour of the leaves and owers, but also from the point of view of the polyphenol content, which in the case of genotype 2-GT (2) is higher than that of genotype 3-GT(2). The lowest value of this parameter belongs to the control that contains a quantity of only 0.419 g% of avonoidic derivates expressed in rutoside. The methanolic extracts of *Ocimum basilicum* L. were phytochemical analyzed from the qualitative point of new, the chromatographic aspect being shown in g. I and II.

Due to the used standards, for the polyphenolic compounds we identi ed coffeec, chlorogenic and cumaric acids, and for the ?avones, we identi ed cvercetol and kemferol.

In the following stage we shall analyze the basil genotypes for their contents in volatile oils and the volatile fractions of the oil. Comparing the data (TLC, HPLC + GC-MS) will allow the selection of the genotype/chemo type with the highest biosynthetic capacity.

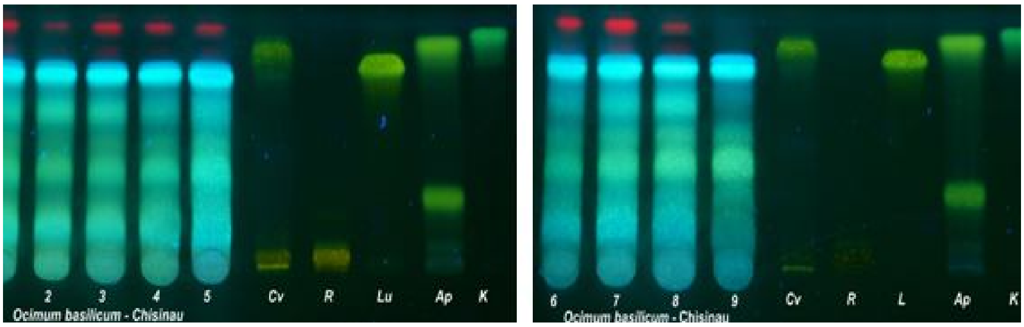


a **b**

Fig. I, a,b. The chromatograms of the polyphenolic compounds of the new *Ocimum basilicum* genotypes.

Legend: ?g. I,a: 1-GT(1) green leaves, red ?owers; 2-GT(2) green leaves, white owers; 3-GT(2) red leaves, red ?owers; 4-GT(3) green leaves, white owers; 5-GT(4) green leaves, red owers. Ac.caf.- caffeic acid, Ac.cl.- chlorogenic acid, Ac.roz.- rosmarinic acid; Ac.fer.- ferulic acid; Ac.cum.- cumarinic acid.

Fig. I,b: 6-GT(4) green leaves, white ?owers; 7-GT(5) green leaves, white owers; 8-GT (5) green leaves, red ?owers; 9-GT rom, green leaves, white owers, control. Ac.caf.- caffeic acid, Ac.cl.- chlorogenic acid, Ac.roz.- rosmarinic acid; Ac.fer.- ferulic acid; Ac.cum.- cumarinic acid.



a b

Fig.II,a,b. The chromatograms of the avonoidic compounds of the new *Ocimum basilicum* L. genotypes.

Legend: ?g.II,a: 1-GT(1) green leaves, red ?owers; 2-GT(2) green leaves, white owers; 3-GT(2) red leaves, red ?owers; 4-GT(3) green leaves, white owers; 5-GT(4) green leaves, red owers. Cv-cvercetol; R-rutin; L-luteolin; Ap-apigenin; K-kemferol.

Fig. II,b: 6-GT(4) green leaves, white ?owers; 7-GT(5) green leaves, white owers; 8-GT (5) green leaves, red ?owers; 9-GT rom, green leaves, white owers, control. Cv-cvercetol; R-rutin; L-luteolin; Ap-apigenin; K-kemferol.

The data of the scienti c literature [4] mentions that in Guinea they found 3 variants of Ocimum basilicum L. species, a variety with white owers, a variety with violet ?owers and a variety with big light green owers. The selection criterion was the chemical composition of the volatile oils, of over 380 prelevated samples from 145 locations.

The ef ciency of the essential oils of basil extraction varies from a region to the other and there were identi ed four chemotypes of the Ocimum basilicum L. species. The main components to determine the chemotypes were: methylchavicol, linalool, methyl cynamat and camphor.

CONCLUSIONS

1. The *Ocimum basilicum* L. evaluated hybrids genotypes present an interest as initial material for breeding and to obtain new cultivars.
2. By means of hybridation one can create new genotypes with a raised content of polyphenols and ?avones which differ due to their colour of the leaves and owers.
3. The polyphenolic (0.902 g%s.u.) and avonoidic (0,819 g% dry material) contents are higher in genotype 5-GT (3) with green leaves and red owers.
4. The content of the polyphenolic and avonoidic derivates is variable and is not strictly correlated with the colour of the leaves and owers.

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ZOOLOGIA

СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА СУММАРНЫХ ЛЕГКО РАСТВОРИМЫХ БЕЛКОВ У ПОДВИДОВ *CARASSIUS AURATUS* GIBELIO И *CARASSIUS AURATUS* AURATUS ВИДА *CARASSIUS AURATUS* (L)

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Введение

Вид *Carassius auratus*, на основе морфологических признаков был классифицирован на 7 подвидов: *C. auratus gibelio* – широко распространен в Европе, России, Китае и Японии; *C. auratus langsdor , C. auratus auratus, C.auratus subsp., C. auratus burgeri, C. auratus grandoculis, C. auratus cuvieri* – обитают только на территории Китая и Японии. Последние два подвида являются эндемиками озера Бива [1, 2].

Первой отличительной особенностью представителей данного вида является полиплоидное происхождение, так как современная диплоидная форма с набором хромосом 2n = 100 является производной от древней тетраплоидой.

Вторая отличительная особенность - присутствие у всех подвидов (кроме *C. auratus auratus*), как диплоидных, так и триплоидных популяций, а у подвидов *C. auratus langsdor* и *C. auratus grandoculis* обнаружены тетраплоидные формы. Это явление необычно, так как не характерно для позвоночных животных [3, 4, 5].

Третья отличительная особенность характеризуется наличием естественного гиногенеза - редкого типа полового размножения, характерного для три- и тетраплоидных популяций *Carassius auratus* (L), при котором осеменение необходимо, но ядерный аппарат, проникшего в яйцеклетку сперматозоида