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PHENOTYPIC EXPRESSIVENESS OF NEW *SIDERITIS SCARDICA* GENOTYPES OBTAINED AT VEGETABLE RESEARCH AND DEVELOPMENT STATION BUZĂU

Geanina NEGOȘANU[1], Costel VÎNĂTORU[1], Adrian PETICILĂ[2], Elena BARCANU[1], Ovidia-Loredana AGAPIE[1], Bianca TĂNASE[1], Ion GHERASE[1]

[1]Vegetable Research and Development Station Buzău, 23 Mesteacănului Street, 120024, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: geanina.neacsu11@gmail.com

Abstract

Numerous species have been acclimatized and bred at VRDS Buzau, among which special results have recently been obtained for Sideritis scardica, also known as Mursalski chai. After completing the acclimatization stage, the valuable genotypes were subjected to intensive breeding works, obtaining six genotypes with distinct phenotypic expressiveness. During the vegetation period, biometric, phenological and biochemical observations were performed, applying the same crop technology for all studied genotypes. The results showed visible differences in terms of phenotypic expressiveness of the main characters. Regarding the weight of the inflorescence and the number of inflorescence/plant, G1 ranks first with an average value of floral mass of 790 g/plant, and G5 has the highest leaf mass value with an average value of 1198 g/plant, while G2 has the most intense fragrance on all vegetative aerial parts. All six genotypes showed genetic stability and uniformity in descent. In 2019, G1 genotype was patented and registered in the Official Catalog of Crop plant varieties from Romania under the name 'Domnesc' cultivar and used in this study as control variant.

Key words*: acclimatization, breeding, honey plants, inflorescence, Mursalski Chai.*

INTRODUCTION

Sideritis scardica is a medicinal and honey plant that belongs to the *Lamiaceae* family. Native of southeastern Europe, also called Mursalski Chai after Mount Mursalița in Bulgaria, it has been known since antiquity as Ironwort or Iron Grass, because it heales wounds caused by iron weapons. At the same time it was used for treating from simple colds to severe pulmonary, gastrointestinal, anemia disorders. In recent decades, *Sideritis scardica* has been extensively investigated for its content of phenolic compounds, terpenoids, hydrocarbons and related compounds, and essential oil composition (Kostandinova et al., 2007; Tadić et al., 2012). It has been suggested that phenolic and/or polyphenolic compounds are responsible for the antioxidant activity of the alcohol extracts, while terpene compounds from the essential oil and diterpenoids are responsible for the anti-inflammatory, analgesic, antiulcer, antibacterial, antifungal, cytotoxic, antitumor activities (Todorova and Trendafilova, 2014) and new studies made in rats suggested that it also fights against Alzheimer's disease (Hofrichter et al., 2016).

Sideritis is a species with a great diversity, its variability being expressed by many names, varieties, forms and names of varieties. It is a perennial, herbaceous, bluish-white plant with abundant hairs, which grows up to a height of 50 cm in the areas of origin, on mountain plateaus. The plant's root has a predominantly horizontal development. The opposite, obovate leaves are covered by small hairs with a double role: to protect the plant against extreme conditions in the alpine areas and to secrete a specific aroma, due to the volatile oils. Comparative studies of the volatile oils of *Sideritis scardica* Griseb from Macedonia, Albania and Greece, from dried and fresh plants, showed that the characteristic smell, for which Mursalita tea or Mountain Tea is so

appreciated, is due to various components, the best known being: monoterpene, myrtenal, hexadecanoic acid, myristicin, menthol, limonene (Stefkov G. et al., 2014). *Sideritis* plants have four distinct stamens, similar to most species in the *Lamiaceae* family, and the clearest identifying feature is the characteristic smell of the leaves (Solomou A., 2019).

Perennial plant with a short lifespan (5-6 years on average), it reproduces by seeds. It is found in open, dry, grassy and stony meadows, on calcareous soil or eroded surfaces, but also in rocky areas, on slopes with southern exposure, at high temperatures and in dry conditions, in belts of subalpine and alpine vegetation, at altitudes of 1300-2100 m (Strid et al., 1991; Petrova et al., 2009, Bulgarian Academy of Sciences and the Ministry of Environment and Waters, 2011). There are no statistics available on the worldwide production of *Sideritis scardica*. In a working group study on medicinal and aromatic plants made in 2004, it was noticed that in Albania, Bulgaria, Greece, Macedonia, Serbia and Turkey, *S. scardica* is grown only on small areas in individual households, for personal use or for distribution in markets, health stores or drugstores. Due to its healing properties and the small quantities available, Mursalita is increasingly sought after, representing a business opportunity.

It has gained attention in recent years due to its medicinal properties. In order to maintain its healing properties, it is recommended that the cultivating area and the pedo-climatic conditions be similar to those of the natural habitat. The yield and lifespan of the culture largely depend on its care. The demand for *Sideritis* has increased because it is natural, ecological and recognized as a safe product, with high economic value (Aneva I., 2012). Therefore, for farmers in the areas of origin, it can provide added value, it can be grown in poorer areas or on degraded slopes in semi-arid areas, hence the need to expand studies to report additional information on the aromatic and medicinal importance of *Sideritis scardica*. Also, its cultivation can be used as a tool for biodiversity conservation, with beneficial effects for the environment. (Solomou A. et al., 2019). In Romania, the plant began to be studied in 2012 by the Genetics and Breeding Laboratory within V.R.D.S. Buzau, which showed a special interest in this species and studied varieties from *Sideritis syriaca*, *S. cyprea* and *S. congesta*. This is where it was introduced into the study a variety from the Bulgarian Mountains, considered authentic and highly valued for its many uses: food, medicinal, aromatic, ornamental, variety approved under the name of 'Domnesc' (Vînătoru C. et al., 2019).

MATERIALS AND METHODS

V.R.D.S. Buzau founded a Mursalski chai culture in 2015 and several genotypes with distinct phenotypes, valuable features and implicitly with high economic potential were noted in the culture. The Genetic, Breeding and Biodiversity Laboratory set out to study five of the new varieties. The Domnesc variety was a control variety and it was numbered G1, and the other five genotypes were numbered G2, G3, G4, G5 and G6.

The breeding method used was the negative mass selection for the 'Domnesc' variety, and for the other genotypes G2-G6, the repeated individual selection (an elite plant was used).

Compared to other vegetable plants, the growth rate in both young and mature plants is slightly slower.

The care works were classic: maintaining the soil humidity, destroying the weeds through mechanical and manual plows, loosening the soil and carefully avoiding bringing soil on the stem. In the case of irrigation, excess moisture has been avoided, which leads to wilting of the plants, as the very pubescent leaves easily adhere to the soil surface and can no longer come off, therefore rotting easily. During the vegetation period, phenological and biometric measurements and determinations were performed in the experimental field within the Biodiversity Improvement and The Genetic, Breeding and Biodiversity Laboratory, V.R.D.S. Buzau. Thus, the electronic balance Pioneer, Model PA413CM was used to determine the weight of the foliar and floral masses. Sensory and olfactory analyzes were also performed.

RESULTS AND DISCUSSIONS

Next, there will be presented the results of the study performed on the six genotypes of *Sideritis scardica*, obtained at VRDS Buzau: Genotype G1, which is also the control genotypes, or the 'Domnesc' variety, and the other five genotypes from G2 to G6, as follows: **Control genotype - G1**: perennial, herbaceous plant, with strongly developed root horizontally, strong appearance, rich foliage, and large number of stems with intense green to silver leaves. A characteristic of the variety is the high percentage (20%) of the branched floral stems compared to the other genotypes that have very small percentages of branched stems. The variety has large inflorescences with four edges, well outlined, compact, with light green vertices; the size of the bush is characteristic for this variety, with an average diameter of 91 cm and average height of 43cm, dimensions that give the bush a rich, strongly developed appearance, with large inflorescences that have an average length of 15 cm (Figure 1).

Figure 1. Control genotype G1 or 'Domnesc' variety

Genotype G2: it is also called 'Ossa', after the Greek mountain town it comes from; it is characterized by a very strong aroma with pleasant slightly mentholated accents, being the most fragrant genotype; the appearance is smaller both in diameter and height compared to G1 (71 cm and 32 cm, respectively - average values), with inflorescences with dark green, silvery, arranged stellate and spaced vertices, and the spike is not dense but sparse and airy; another noteworthy feature of the G2 genotype is that branched stems with 3 or more branches predominate (Figure 2).

Figure 2. Genotype G2 or 'Ossa' variety

Genotype G3: it is characterized by a stuffed appearance, with short inflorescences, with a small number of compact vertices rounded towards the top, which give the plant a blunt, unfinished look. The bush has medium dimensions, with weak foliage, rarely arranged leaves, being the genotype with the most spaced leaves; also to be noted is the total lack of branched stems, the plants having only solitary stems. The plant's fragrance is medium to intense, with early flowering. In the first week of June, the plants are fully flowered, the bushes are practically covered by bees (Figure 3).

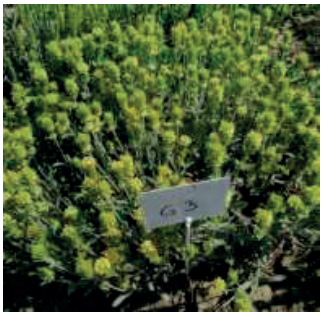

Figure 3. Genotype G3

Genotype G4: distinguished by the large expanse of the bush, without excelling in height; thus, the average value of the diameter is 101 cm, with an average height of only 50cm; the solitary stems predominate and the branched ones are in small numbers, on average 16/bush. It has long stems with rich foliage, the largest number of leaves per stem (10); long, sharp leaves, large inflorescences (average value of 16 cm), with star-shaped, very sharp and light green vertices (Figure 4).

Figure 4. Genotype G4

Genotype G5: the plant has medium size and is characterized by: poor foliage, with small and narrow leaves, inflorescences colored in honey yellow; it has the largest inflorescences (average value of 13 cm) and very pubescent, spaced vertices. The spike is flexible, semi-erect, due to its very long length. The aroma is medium to weak (Figure 5).

Figure 5. Genotype G5

Genotype G6: it has a pleasant appearance, with spherical, globular bushes, dark green

Figure 6. Genotype G6

foliage, predominating solitary stems; vertices are intense green and the inflorescences are regular, truncated in size, compact, with a wider base and sharp towards the top, which gives the bush a spectacular appearance; this is the most beautiful of the 5 genotypes studied. It should be noted that the strong aroma is given by the yellow flowers, the bushes being covered almost entirely by bees during the flowering period, in mid-June (Figure 6).

Next, for an even more relevant presentation of the 5 genotypes and the control variety, in Table 1 below, are found the main quantitative characteristics of Mursalski bushes, namely the height and diameter of the bush, the length and width of the leaves, the distance between leaves/stem, number of leaves/stem, length of the stem and inflorescences, number of vertices/inflorescence and diameter of inflorescences.

Table 1. Calculation of average values, standard deviation and coefficient of variatio§n of quantitative traits

Variable	G1	G2	G3	G4	G5	G6	cv%
Plant height (cm)	43.2 ±9	32.20 ±9	40.60 ±6.06	50.30 ±8,5	53.10 ±7.7	39.5 ±4.40	17.25
Bush diameter (cm)	91.1 ±8.7	71.05 ±9.35	96.30 ±3.35	101.2 ±1.8	84.39 ±5.89	66.20 ±1.80	6.05
Leaf length (cm)	7.67 ±2.87	4.20 ±1.71	6.10 ±0.92	5.43 ±0.76	5.67 ±2.51	6.20 ±2.05	30.68
Leaf width (cm)	1.47 ±0.21	0.60 ±0.1	1.03 ±0.05	1.13 ±0.31	0.97 ±0.12	1.57 ±0.15	13.88
Distance between leaves/stem (cm)	7.13 ±1.00	5.00 ±0.50	10.00 ±0.93	7.33 ±1.53	7.37 ±1.15	7.6± 0.87	13.46
Number of leaves/stem	8±2	14.67 ±7.02	6.17 ±0.12	10.00 ±2	8±2	6±2	28.65
Stem length) (cm)	38 ±9.85	31.67 ±10.1	32.33 ±1.53	41.00 ±3	31.00 ±7.55	19.17 ±8.61	21.04
Inflorescence length (cm)	15±4	5.87 ±3.59	9±3.61	16.00 ±1	13.00 ±7.94	10.67 ±3.01	33.29
Vertices number/inflorescence	19.67 ±1.15	7.33 ±4.04	7.33 ±1.53	11.33 ±4.04	11.67 ±6.03	16.00 ±5.57	30.49
Inflorescence diameter (cm)	0.9 ± 0.1	1±0.1	1.8± 0.1	0.9 ± 0.1	1.7 ± 0.1	1.70 ±0.1	7.50

The measurements show significant differences between the six genotypes studied. The highest plant height was registered at genotype G5 - 53.1 cm and the lowest at genotype G2 - 32.2 cm. The largest diameter was measured at genotype G4 - 101.2 cm and the smallest at genotype G6 - 66.2 cm. Regarding the length and width of the leaf, the smallest values are found at genotype G2 - length 4.2 cm, width 0.6cm, and the highest values at genotype G1 - length 7.67 cm and genotype G6 - width 1.57 cm. Regarding the length of the stem and the inflorescence, genotype G1 presents the highest values - 38.9 cm (stem) and 15 cm (inflorescence) respectively, and the smallest values were registered at genotype G2 - 5.87 cm stem length and 5.87 cm inflorescence length. The smallest number of vertices in the

inflorescences can be found at genotype G2 and G3, namely 7, and genotype G1 has the highest value - 19, while the smallest diameters of the inflorescences are found at the genotypes G1 and G4 - 0.9 cm; genotype G3 has the largest diameter - 1.9 cm. Regarding the coefficient of variability (cv%) it can be assessed that high values of the coefficient of variability were registered for the following quantitative characteristics: inflorescence length 33.29%, leaf length 30.68%, number of vertices in the inflorescence 30.49%. The lowest values of the coefficient of variability were obtained at the diameter of the bush - 6.05%, the diameter of the inflorescences - 7.50% and the distance between leaves/stem - 13.46%.

Next, several series of quantitative determinations were performed on the six distinct genotypes of *Sideritis scardica* to assess productivity, such as: number of solitary and branched stems, number of solitary and branched inflorescences, related percentage calculation.

As it can be seen in Figure 7, genotype G3 has only solitary stems, and the highest number of solitary inflorescences is 280 compared to the other genotypes.

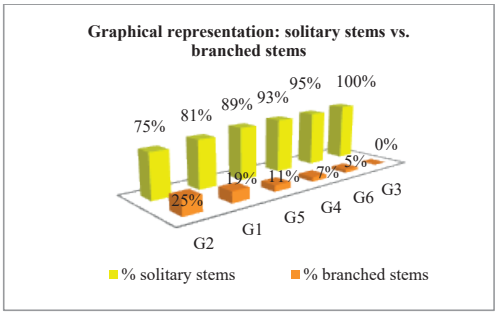

Figure 7. Graphic representation of solitary and branched stems

Genotype G2 has the lowest number of solitary stems - 143, with a percentage of 74.48%. Regarding the branched stems, we find the highest value in genotype G2, with 147 inflorescences, and the highest percentage 25.52%, while genotype G3, as we mentioned, does not have solitary stems. Next, the comparative analysis of the number of solitary and branched stems was transposed graphically.

The six distinct genotypes were subjected to quantitative measurements in order to determine the foliar and floral masses. These observations were performed both in the experimental field, to determine the number of stems / plant, and in the laboratory. Thus, based on the weight determinations of the stems (average value) and the number of stems / plant, the leaf mass/plant was calculated. More specifically, the highest leaf mass was found at genotype G5 - 1198.40 g/plant, and the lowest value was found at genotype G4 - only 505.55 g/plant. These values are represented in Figure 8.

Next, the quantitative measurements were performed in order to determine the average floral mass for each genotype. Therefore, genotype G1 is the most productive, with a weight of the floral mass of 790 g/plant, and genotype G2 is the least productive, with 278.4 g/plant.

For a better understanding of the quantitative characteristics, the values of the foliar and floral mass determined for each genotype are represented in Figure 8 below.

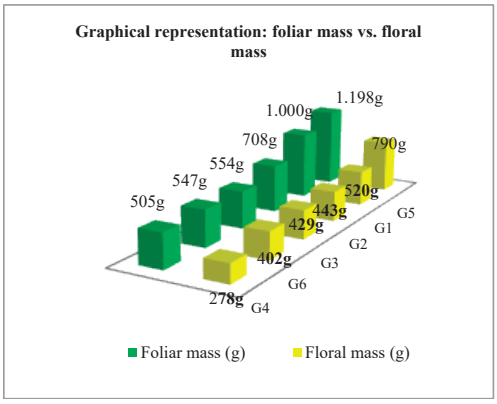

Figure 8. Graphical representation of foliar and floral mass for G1-G6

A relevant feature for *Sideritis scardica* is the fragrance released by leaves and flowers due to the presence of volatile oils. Therefore, the qualitative assessment was performed by carefully examining the fragrance of each genotype, the analysis was olfactory and was performed personally. At the same time, this evaluation was compared with the presence of bees on the bushes at the time of flowering (Figure 9). It was found that the most aromatic genotype with menthol and citrus accents is

genotype G2, also called 'Ossa'. This is, in fact, the genotype with the most pubescent foliage; on the leaves there are glandular brushes that secrete volatile oils. It is followed by genotypes G6 and G3 with intense flavors. Genotype G1 has medium intensity and geotypes G5 and G4 have weak flavors. It should be mentioned that *Sideritis scardica* is a valuable honey plant, which blooms in stages between May 15 and June 15. If the inflorescences are harvested early in May, in favorable weather conditions (late frosts), a second flowering occurs starting with September, which lasts until the arrival of frost.

Figure 9. Bee on an inflorescence of *Sideritis scardica*

CONCLUSIONS

The first conclusion is that we managed at V.R.D.S. Buzau to successfully acclimatize the *Sideritis scardica* species approved under the name of 'Domnesc' variety, genotype G1 in this case. The researches were finalized achieving six different *Sideritis scardica* varieties, each one having distinct phenotypic expressions.

G1 - 'Domnesc' variety (control genotype), has a strong appearance of the bush, vigor, and the largest floral mass – 790 g/plant due to large inflorescences and the largest number of vertices - 19.

G2 - also called 'Ossa', is the genotype with the strongest fragrance and the most pronounced foliar hairiness, although it has the smallest floral mass - 278.40 g and the smallest inflorescences and leaves. It is also the genotype that stands out with the highest percentage of branched stems - 25%.

G3 - this genotype with medium floral and foliar masses has an intense fragrance and is the only genotype with 100% solitary stems.

G4 - genotype with a special appearance, the largest diameter of the bush - 101 cm, large inflorescences, and light fragrance.

G5 - this genotype has the largest leaf mass, 1198.4 g/plant and the largest inflorescences - 16 cm. The fragrance is light.

G6 - this genotype has a strong fragrance and a spectacular appearance of the bush, with dark green leaves and large truncated cone shaped inflorescences, deep yellow flowers and light green vertices.

Consequently, as a result of the valuable characteristics they present, all five genotypes studied will be the subject of intensive breeding works, some of which will be proposed for approval, as these genotypes can form new cultivars.

It is also necessary to emphasize the importance of honey plant features, due to the fact the flowering in stages for a long time offers bees an abundance of nectar and pollen, at a time when other honey plants are in decline.

Lately it is more and more sought after and can be a business opportunity, both in terms of selling seeds and selling tea, leaves and flowers. V.R.D.S. Buzau sells both seeds and seedlings of *Sideritis scardica*, the 'Domnesc' variety (genotype G1), thus making them available to farmers and amateur horticulturists, who set up crops or use them in arranging private gardens.

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