

## EVALUATION OF PHENOTYPIC EXPRESSIVITY OF *SIDERITIS SCARDICA* VAR. OSSA, A NEW GENOTYPE ACCLIMATED AND BRED IN ROMANIA

Geanina NEGOȘANU<sup>1</sup>, Costel VÎNĂTORU<sup>1</sup>, Adrian PETICILĂ<sup>2</sup>, Bianca MUȘAT<sup>1</sup>,  
Camelia BRATU<sup>1</sup>, Adrian ASĂNICĂ<sup>2</sup>

<sup>1</sup>Plant Genetic Resources Bank - for Vegetables, Floricultural, Aromatic and Medicinal Plants,  
56 Nicolae Balcescu Blvd., Buzău, Romania

<sup>2</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd,  
District 1, Bucharest, Romania

Corresponding author email: geanina.neacsu11@gmail.com

### Abstract

BRGV Buzau owns a valuable collection of genotypes belonging to the *Sideritis* species, including the varieties: *scardica*, *syriaca*, *hyssopifolia*. *S. scardica* var. *Ossa*, coming from the mountain bearing the same name, has medicinal, gastronomic, pharmaceutical and ornamental value. Since 2002 it has been studied by researchers from Buzau and since 2019 it has been intensively introduced into cultivation at BRGV Buzau. The research has shown that var. *Ossa* has an increased ecological plasticity to the soil and climate conditions in Romania. Special attention was paid to the biological isolation of the genotypes, knowing that it is an entomophilous plant preferred by insects, especially bees that can easily pollute the studied genetic resource. Following the evaluation of the BRGV collection, the G2 genotype was retained, which is the subject of this article. G2 shows distinct phenotypic expressivity, genetic stability and, very importantly, a strong, specific aroma. During the research, biometric determinations and phonological observations were carried out, with average values of 708g leaf mass and 278 g flower stems, 32 cm bush height, 71 cm diameter.

**Key words:** entomophily, ecological plasticity, habitat, germplasm collection, genetic stability.

### INTRODUCTION

*Sideritis scardica* Griseb is an outcrossing diploid ( $2n=32$ ) (Esra et al., 2009) and perennial subalpine/alpine herbaceous plant, endemic to the central parts of the Balkan Peninsula (Grdiša et al., 2019). The species is distributed in southwest parts of Albania, North Macedonia, Bulgaria and Greece (Petrova et al., 2009). It is a plant of the alpine zone, occurring in dry stony meadows, mainly at altitudes of 1600-2300 m a.s.l., and only occasionally down to 500 m, a.s.l. on limestone (Strid et al., 1991; Papanikoulau et al., 1982). *Sideritis scardica* Griseb var. *Ossa* originates from the mountain of the same name, Ossa, in northern Greece (Papanikoulau et al., 1982), being a genotype with very valuable distinct phenotypic characteristics. Manel et al., 2012, suggested that genetic variation that appears to be caused by natural selection might be the results of isolation by distance, which limits gene flow among populations or the result of

secondary contact of populations that survived isolated in glacial refugia. The local population of *Sideritis scardica* var. *Ossa* is located on Mount Ossa, at 1200 meters altitude. Recently, "The Ossa - Herbal gardens" farm was established here, which organically cultivates two species of aromatic plants: *Sideritis scardica* and *Origanum vulgare* and markets the products under the name Ossa tea (Figure 1) and Ossa oregano, respectively.

In Romania, it was taken into study in 2002 by researcher Costel Vinătoru, who in 2019 obtained the first variety of *Sideritis scardica*, patented and registered in the Official Catalogue of Cultivated Plants in Romania under the name of Domnesc (Vinătoru et al., 2019). Research continued at Plant Genetic Resources Bank (PGRB) Buzau, with the aim of enriching the germplasm base of the *Sideritis* species and developing the cultivation technology specific to this perennial crop. This article presents the studies carried out on genotype G2, *Sideritis scardica* Griseb var.

Ossa, and the evaluation of its phenotypic characters with the aim of obtaining a new genotype with distinct phenotypic expressiveness acclimatized to Romanian soil and climatic conditions.



Figure 1. Culture of *Sideritis scardica* var. Ossa from Mt Ossa and the commercial product of "The Ossa - Herbal garden" <https://ossaherbs.gr/mountaintea.html>

## MATERIALS AND METHODS

The research works were carried out at PGRB Buzau (45°09'N, 25°5'E, 95 m) located in SE Romania, characterized by continental climate, steppe characteristic vegetation and leached chernozem soil.

The period analysed in this study was 2019-2022.

The genetic resources of *Sideritis scardica* var. Ossa come from the PGRB seed collection and purchase from the area of origin. Because of the very low germinations ratio of under 27, the seeds were purity tested in the Seed Conditioning and Analysis PGRB Laboratory and were sown in the greenhouse using 80% peat and 20% dolomite, which alkalizes the substrate creating premises for better germination. By comparison, the seedlings of *Sideritis scardica* var. Domnesc had germination between 80 and 95%, with the same type of substrate being used (Figure 2).

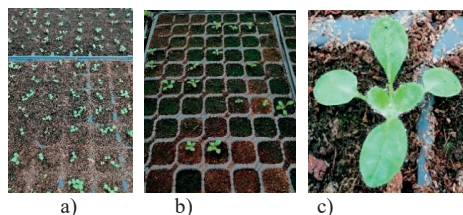


Figure 2. Seedlings of *Sideritis scardica*: a) var. Domnesc, germination approx. 80%; b) var. Ossa germination < 27%; c) seedling of var. Ossa - detail

The sowing was done in the first decade of March in protected spaces. The crop was established by direct sowing, after land modeling using the L445 tractor in aggregate with MMS 2.8 with 140 cm gauge, 94 cm canopy length, 46cm gutter and 70 cm between rows. About 60 days after sowing, the culture was established through seedlings around 10.05.2019, at the stage of 6-8 leaves and 7-8 cm in height (Figure 1).

The breeding methods used were repeated individual selection followed by negative mass selection. Phenological observations and biometric measurements were carried out according to the UPOV and IPGRI data sheets. Weighing operations were performed at PGRB laboratory on green plants and the device used was the two-decimal precision balance Kern 572-33.

## RESULTS AND DISCUSSIONS

*Sideritis scardica* Griseb var. Ossa prefers rocky soils with sandy-clay composition, pH between 6.9-8 and low nutrient content. The crop doesn't require special handling because it originally grows in mountainous, sloping, stony soils, in the high mountains (Kloukina et al., 2020). On the other hand, observations in Rhodopean locations (Bulgaria) have shown that *Sideritis scardica* inhabits dry, sunny, grassy, and stony terrains, solely on a calcareous rock base. That is why a number of its morphological characteristics relate to its adaptation to permanently scanty humidity typical of the karst massives, namely a powerful rootsystem and profuse hairiness (Yordanova & Apostolova, 2000).

During the study, we did not detect any diseases and/or pests that would cause significant damage to the crop. *Sideritis scardica* Griseb is a perennial herbaceous plant from Ossa Mountain. Through the breeding methods used, repeated individual selection followed by negative mass selection, the G2 variety presented in this article was obtained.

It should be noted that in the first two years of cultivation the two genotypes of *Sideritis scardica*, var. Ossa and var. Domnesc, are very similar in morphology and the difference lies in the development of the bush in the horizontal plane.

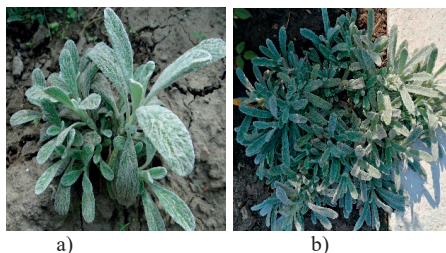


Figure 3. The two *Sideritis scardica* varieties in the second year of cultivation: a) var. Ossa; b) var. Domnesc

More precisely, the diameter of the shrub var. Ossa is between 12-15 cm, and that of var. Domnesc is between 25-35 cm (Figure 3).

The species shows a valuable characteristic, namely branching, which materializes in the formation of several stems and flower shanks. The bush has a lignified stem from the base to the level of the secondary and rarely tertiary branches, which gives it resistance to low temperatures, strong winds, and large temperature fluctuations in the day/night interval. The bush is erect and the port is small compared to *Sideritis scardica* var. Domnesc. In the third year of culture, the plant has a maximum height of 29-34 cm and a diameter of 68-74 cm. *S. scardica* in Slavyanka Mt. origin area has a plant diameter between 25-90 cm, with between 2-27 flowering stems each. The number of flowers in one stem varies from 12 - 84. The number of seeds in one flower is 4 (Aneva et al., 2013). The G2 genotype of *Sideritis scardica* from our study has 187-195 secondary and tertiary branches, with between 3-7 inflorescences each, in the third year crop (Figure 4).



Figure 4. *Sideritis scardica* var. Ossa, third year of culture

The basal leaves are moderate thick, oblong-lanceolate, usually crenate, mucronate, attenuate to a petiole; the length of basal leaves varies between 5.7-6.3 cm, the width between 0.9-1.2 cm, and the thickness between 1.1-1.16 mm; middle and upper stem leaves are subsessile, linear-lanceolate; their length varies between 2.8-3.7 cm, the width is between 0.3-0.7 cm and the thickness between 0.6-0.8 mm (Figure 5).



Figure 5. *Sideritis scardica* var. Ossa, detail of linear-lanceolate narrow leaf

Leaves are uniformly silver-green due to the presence of glandular hairs distributed equally on the foliage and stems. The small dimensions of the leaf apparatus in var. Ossa is reflected in the number of leaves per stem, on the one hand, which varies between 8-22, and the distance between leaves, on the other hand, which is between 4.5-5.5 cm. In *Sideritis scardica* var. Domnesc, the leaf apparatus is more developed, the distance between leaves on the stem is bigger, 6-8 cm, the number of leaves per stem is between 6-10, but leaves are double in size: leaf length 5.1-10.9 cm, width 0.7-1.3 cm and thickness 0.8-1.6 mm.

Var. Ossa inflorescences are herringbone-shaped with pointed, stellate bracts, medium bristles and yellow flowers. Vertices are dark green, small in number and spaced, which is a characteristic of this variety (Figure 6). The first two vertices are spaced at 4 cm and 3 cm respectively, narrowing the distance between vertices towards the tip of the inflorescence, where they are more compact. On average, the number of vertices in var. Ossa is between 3-11 and in var. Domnesc is between 17-21. The picture below shows the difference between the inflorescences of the two *Sideritis scardica* genotypes, var. Ossa with lax inflorescence with sparsely arranged vertices and dense glandular hairs, which gives the silver-green color, and var. Domnesc with twice the number of vertices, compactly arranged along the entire

length of the inflorescence, and less numerous glandular hairs on the surface of the bracts, which gives a deep green color.



Figure 6. *Sideritis scardica*: a) var. Ossa, lax inflorescence; b) var. Domnesc, compact inflorescence

Inflorescence is extremely fragrant, aroma is persistent, a mix of citrus and menthol. The tea obtained from the flowers of var. Ossa is extremely fragrant and golden-yellow in colour. The average weight of an inflorescence is 0.96 g compared to 2.5 g in var. Domnesc. The average number of inflorescences per bush is 290 and the average flower mass is 278 g. The average number of stems is 192 and the average weight of a stem is 3.69 g, resulting in an average leaf mass of 708 g. The average diameter of an inflorescence in var. Ossa is 1.1 cm compared to 0.9 cm in var. Domnesc, which is due to the large vertices with spaced star-shaped bracts. The first wave of flowering starts at the end of May and flowering is complete in the second week of June. The second wave of flowering lasts throughout October, in long autumn conditions the flowers can stay on the stem until frost.

### Crop technology

For optimal development, humus-carbonate soils, similar to those in the area of origin, are recommended. Acid soils destroy the root and stop the germination of the seeds. Development is optimal in the case of limestone amendments. The plant's water needs are provided by the ramifications of the root system, which penetrate deep into the cracks of the soil, conferring resistance to drought, to temperatures up to +45°C but also to extreme

minimum temperatures of -20°C, proving ecological plasticity (Vinătoru et al., 2019).

Propagation in *Sideritis scardica* var. Ossa is made by seeds. The optimal planting establishment scheme is 70 cm between rows and 40 cm between plants/row, compared to 50 cm between plants/row in var. Domnesc (Figure 7) The smaller spacing is due to the smaller height and diameter of the plant.

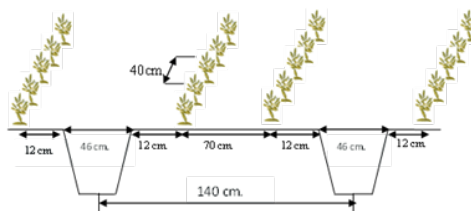


Figure 7. *Sideritis scardica* var. Ossa - scheme culture: 70 cm between rows and 40 cm between plants/row

A similar establishment scheme was used in an experiment of the Genetic Resources Bank in Thessaloniki with the *Sideritis syriaca* species. Planting took place during mid-autumn after the first rains in well-drained soils of the BBGK and the planting density was 0.70-1 m between lines X 0.50-0.60 m on lines (Kloukina et al., 2020).

Regarding the *Sideritis scardica* var. Ossa it can be observed in Table 1 the growth evolution of the G2 genotype in the first three years crop: average plant height between 13-33 cm, canopy diameter range 17-58 cm, with a third year spectacular growth; also, the number of sprouts from 4 to 44; no flowering for the first year, 56 inflorescences/plant in year 2 and 95 in year 3;

converted into vegetative mass were obtained from 114 g/plant in the first year to almost 600 g/plant in the second year and 749 g/plant in the third year crop. After harvesting, the flowering stems are dried in specially designed areas, away from sunlight and draughts; the stems are tied in bunches that weigh about 400 g in green. The plant dehydration percentage was between 70-75%. In the graphic representation (Figure 8) it can observe both the evolution of the vegetative mass and the final results obtained after drying and storage.

Table 1. Biometric measurements regarding vegetative mass in Ossa cultivar

G2 type	Canopy diameter (cm)	Plant height (cm)	Number of sprouts	Number of leaves / sprout	Number of leaves/ plant	Number of inflorescences	Number of flowers stems	Green vegetative mass (g)	Dry vegetative mass (g)
Year1	17.3±0.67 <sup>c</sup>	13.10±1.41 <sup>bc</sup>	4.4±0.89 <sup>a</sup>	4.4±1.21 <sup>a</sup>	19.7±5.43 <sup>a</sup>	0,03±0.89 <sup>c</sup>	0.04±0.55 <sup>c</sup>	114±11.10 <sup>c</sup>	27.89±4.23 <sup>c</sup>
Year2	39.88±1.49 <sup>a</sup>	23.46±1.95 <sup>b</sup>	37.2±1.3 <sup>b</sup>	4.8±1.1 <sup>a</sup>	183.5±21.3 <sup>b</sup>	56.6±7.13 <sup>b</sup>	35.3±0.35 <sup>b</sup>	598.3±15.4 <sup>b</sup>	161.14±13.21 <sup>b</sup>
Year3	71.11±5.47 <sup>a</sup>	32.55±2.19 <sup>a</sup>	44.6±1.88 <sup>a</sup>	6.5±1.12 <sup>a</sup>	264.7±35.14 <sup>a</sup>	95.4±4.31 <sup>a</sup>	79.9±8.98 <sup>a</sup>	708.51±8.76 <sup>c</sup>	201.9±11.25 <sup>a</sup>

\*Letters represent Duncan test results with 95% confidence interval and  $p < 0.05\%$ .

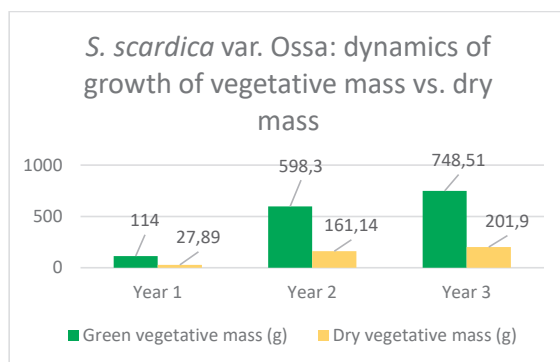


Figure 8. Graphic representation of the evolution of vegetative mass vs. dry mass



Figure 9. *Sideritis scardica* var. Ossa - plant details

Several species of the genus *Sideritis* have been studied for the first time in Romania at PGRB Buzau, such as: *S. scardica*, var. Domnesc, *S. scardica* var. Ossa, *S. syriaca*, *S. hyssopifolia*. This paper is focused on *S. scardica* var. Ossa due to its aromatic and medicinal potential. Breeding and acclimatization of this genotype found only on Mt. Ossa has demonstrated that it shows increased resistance to the climatic conditions of our country, is not a demanding plant and does not require special conditions for care and cultivation. On the contrary, irrigation below normal flow will positively

influence the concentration of essential oils. The aerial parts of these plants are used to prepare very aromatic tea. In addition, var. Ossa is also an ornamental and melliferous plant (Figure 9). Being a perennial plant, it is recommended for decorating gardens, especially rockeries, as the silvery-green colouring of the leaves, the yellow herringbone-shaped inflorescences add value to the yard. Its strong aroma, a mix of citrus and menthol, attracts bees all day long because *Sideritis* spp. are highly appreciated as a honey plant, providing pollen and nectar starting early summer until frost. During the two flowering waves, May-June and September-October, the plant is practically completely covered by bees. For this reason, harvest is recommended in the early hours of the morning followed by storing the plants in well-ventilated spaces, protected from sunlight.

## CONCLUSIONS

*Sideritis scardica* var. Ossa was acclimatized by the PGRB Buzau researchers team, obtaining a valuable G2 variety that shows genetic stability in the offspring, as well as

valuable phenotypic and biochemical characteristic;

The cultivar can be successfully cultivated in an ecological system, being appreciated as food, ornamental, honey plant, but especially as a medicinal plant due to the active principles in its biochemical composition.

The culture technology for this species was developed. In the future, PGRB Buzau will provide growers with both seeds and seedlings for testing.

## REFERENCES

- Aneva, I., Zhelev, P., Evstatieva, L., & Dimitrov, D. (2013). The ecological and floristic characteristics of populations of *Sideritisscardica* Griseb. in Slavyanka mountain. *Bulgarian Journal of Agricultural Science*, 19(2), 211-217.
- Esra, M., Duman, H. & Ünal, F. Karyological studies on section Empedoclia of *Sideritis* (Lamiaceae) from Turkey. *Caryologia* 62, 180–197 (2009).
- Grdiša, M., Radosavljević, I., Liber, Z., Stefkov, G., Ralli, P., Chatzopoulou, P. S., ... & Šatović, Z. (2019). Divergent selection and genetic structure of *Sideritisscardica* populations from southern Balkan Peninsula as revealed by AFLP fingerprinting. *Scientific Reports*, 9(1), 12767.
- Kloukina, C., Tomou, E. M., Krigas, N., Sarropoulou, V., Madesis, P., Maloupa, E., & Skaltsa, H. (2020). Non-polar secondary metabolites and essential oil of ex situ propagated and cultivated *Sideritissyriaca* L. subsp. *syriaca* (Lamiaceae) with consolidated identity (DNA Barcoding): Towards a potential new industrial crop. *Industrial Crops and Products*, 158, 112957.
- Manel, S., Gugerli, F., Thuiller, W., Alvarez, N., Legendre, P., Holderegger, R., & IntraBioDiv Consortium. Broad-scale adaptive genetic variation in alpine plants is driven by temperature and precipitation. *Mol Ecol* 21, 3729–3738 (2012).
- Papanikolaou, K., Kokkini, S. A taxonomic revision of *Sideritis* L. Section Empedoclia (Rafin) Bentham (Labiatae) in Greece in *Aromatic Plants: Basic and Applied Aspects* (ed. Margaris, N.) 101–128 (MartinusNijhoff, 1982).
- Petrova, A. & Vladimirov, V. Red List of Bulgarian Vascular. *Plants. Phytol Balcan* 15, 63–94 (2009).
- Strid, A., Tan, K. Mountain Flora of Greece, Volume 2. (eds Strid, A. & Tan, K.) 89–90 (Edinburgh University Press, 199)
- Vinătoru C., Musat B., Bratu C. (2019). *Treaty on Special Vegetables*. Buzau, RO. Alpha MDN Publisher.
- Yordanova, M., & Apostolova, I. (2000). Estimation of the status of representative populations of *Sideritisscardica* Griseb. in the Rhodopi Mts. *Phytologia Balcanica*, 6(1), 43-57.
- <https://ossaherbs.gr/mountaintea.html>