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RESEARCH ON THE ACCLIMATIZATION AND BREEDING OF *SIDERITIS HYSSOPIFOLIA* L. IN ROMANIA

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ABSTRACT. *Sideritis hyssopifolia* L., perennial plant of the genus *Sideritis*, is also known as hyssop-leaved mountain ironwort. In its area of origin in northwestern Europe, it is valued as an aromatic and medicinal plant and used in the prevention and treatment of digestive ailments. It has been studied at Plant Genetic Resources Bank Buzău since 2019, being the subject of intensive acclimatization and improvement works with the purpose of obtaining new creations with distinct genotypic and biochemical characteristics. The genetic material used was from the centers of origin: Spain, Portugal, Madeira and Canary Islands, and the breeding methods were repeated individual selection followed by negative mass selection. In order to prevent the biological contamination of genotypes with foreign pollen, insulators with textile material were used. Cultivar G5, superior in terms of acclimation and genetic stability, also exhibited distinct phenotypic expressivity. At the same time, specific culture technology was developed with the aim of promoting it among farmers.

Keywords: genotype; phenotype; culture technology.

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INTRODUCTION

The genus *Sideritis* belongs to the Lamiaceae Family and includes over 150 species distributed in the tropical and temperate regions of the Northern hemisphere. Most species are found mainly in the Mediterranean area, in the south-eastern Iberian Peninsula, the Canary Islands, the Marmara and the Aegean (Güvenç *et al.*, 2005, Aslan *et al.*, 2006, Loğoğlu *et al.*, 2006). The name of genus *Sideritis* L. derives from the Greek word "sideros" (iron), as a result of the use of these plants in the treatment of wounds caused by iron weapons since ancient times (González-Burgos *et al.*, 2011). *Sideritis* species are traditionally used in gastronomy, as flavoring agents and in traditional medicine, with anti-inflammatory, antiulcer, antimicrobial, antioxidant, antispasmodic, anticonvulsant, analgesic and carminative action (González-Burgos, 2011*)*. The culture of *Sideritis* is on the 3rd place in the top of farmers' aromatic crops, in Greece, where at the same time the interest in medicinal plants purchased through e-commerce has increased after the Covid-19 pandemic wave (Skordos *et al.*, 2020). Also *Sideritis* belongs to the selected species have been approved as traditional herbal medicines, based on their longstanding medicinal use in the European Union, by the European Medicines Agency, and Herbal Monographs on them have been developed recently by the Herbal Medicinal Products Committee (Skordos *et al.*, 2020). *Sideritis hyssopifolia* L. belongs to the genus *Sideritis*. It is also called hyssop-leaved mountain ironwort but also ”hisopillo” or ”ceai de Picos”. It is a perennial plant, found on subalpine plateaus, resistant to soil and climate conditions, endemic in northwestern Europe, Portugal and northern Spain to Italy, Switzerland and France. In the northern part of Spain, characterized by wet and rocky lands, it is used intensively due to its aromatic and eupeptic properties (Rodríguez Lion, 1998). Recent studies have shown that *Sideritis hyssopifolia* L. exhibits a moderate antioxidant activity being able to be used as an accessible source of natural antioxidants, develops hypocholesterolemic effects with potential activity against atherosclerosis and decreased cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride levels (Coto *et al*, 2019). The plant is 35-40cm tall, has woody stems most often branched, with a slow growth rate. Both leaves and flowers are strongly aromatic. Leaves are dark green, narrow, opposite. The yellow flowers are arranged in up to 10 vertices/inflorescence with an average length of 1cm, bracts are wide and have serrated, strongly pointed edges. The genus *Sideritis* has been studied in Romania since 2016, in 2019 being registered in the Annual Catalog of Cultivated Plants in Romania, the first variety, the Domnesc genotype with distinct phenotypic characters, belonging to the species *Sideritis scardica.* The research undertaken so far in Romania on *S. scardica* Domnesc cultivar has been completed with real success through acclimatization to the soil and climatic conditions of our country, although in its natural growing area it is present at an altitude of 1400 m, it has been shown that it can also be grown in the field, throughout Romania (Vînătoru *et al.*, 2019). The two other species,

Sideritis syriaca and *Sideritis hyssopifolia* L., are also being studied. The latter is the subject of this article. Its specific name refers to the similarity between its leaves and the leaves of *Hyssopus officinalis* L., Lamiaceae. The infusion of its inflorescence is used as a stomachic in case of difficult digestion and as treatment for stomach pain due to its phenolic acids (chlorogenic acid) content, flavonoids and essential oils with a high content in hydroxycinnamic derivatives (Rodriguez, 1997). Pharmacological studies have demonstrated that flavonoids are what give it its digestive qualities. (Adzet *et al.*, 1990).

MATERIALS AND METHODS

The acclimatization and improvement works on the genus *Sideritis* started in 2019 and are still ongoing at Plant Genetic Resources Bank (PGRB) Romania (45°09´N, 25°5´E, 95m). PGRB is located in SE Romania, in the Danube plain, Muntenia region. The genetic resources of *S. hyssopifolia L.* were exclusively acquired from the area of origin: Spain, Portugal, Madeira and Canary Islands, where it is found on mountain plateaus at high altitudes, on rocky soils. Sowing was carried out in protected areas in the third decade of March, when the day/night temperature ratio was 8/17°C, under 12h photoperiods. The substrate used was 100% peat. In order to establish the culture 60 days after sowing, the seedlings were transplanted into the field (*Figure 1*). The G5 variety, superior in terms of acclimation and genetic stability, also exhibited distinct phenotypic expressivity. The prevention of biological contamination with foreign pollen of the genotypes was done with fabric insulators. At the same time, specific culture technology was developed with the aim of promoting it among farmers. Unlike other *Sideritis* species, *hyssopifolia L.* shows reduced hairiness, does not require increased attention to irrigation, and is not exposed to the risk of leaf rotting in case of water stagnation. The abundant flowering since the first year of cultivation shows the very good adaptability to the soil and climate conditions of our country. Hyssop, related to *Siderits hyssopifolia L.*, also from *Lamiaceae*, both similar in leaves, in Romania is harvestable only in the 2nd year.

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.

a b

Figure 1 - Aspects of the *Sideritis hyssopifolia* L. culture:
a) in the seedling phase, in pallet; b) cultivated in the field

RESULTS

Sideritis hyssopifolia L. is a perennial herbaceous plant. Through the breeding methods used, repeated individual selection followed by negative mass selection, the G5 variety presented in this article was obtained (*Figure 2*). The bush has a lignified stem from the base to the level of the secondary and even tertiary branches, which gives it resistance to low temperatures, strong winds, and large temperature fluctuations in the day/night interval. The bush is semi-erect, the plant has a height of 23-38cm and a diameter of 21-26cm; in origine areas, in Spanish rural regions like Cantabrian Mountains and the Pyrenees, *S. hyssopifolia L.* is a perennial plant with a woody base, up to 40cm and leaves lanceolate, 1-4 cm, entire, shallowly toothed or crenate (de Santayana *et al.*, 2005). *S. hyssopifolia L.* shows a first flowering wave at the beginning of summer, in June, with 8-10 inflorescences per plant on average, and a second wave in October, when the flowering will be much more abundant with 25-45 inflorescences per plant, which demonstrates a good ability to adapt to the soil and climate conditions of our country.

The main stem is lignified along its entire 10-16cm length, depending on how vigorous the plant is; the main basal stem has a diameter between 0.4-0.6cm. It branches into 12-30 secondary branches, lignified on variable lengths of 1.2-5.5cm. Secondary rods have lengths between 16-27cm and diameters between 0.12-0.17cm; the secondary ramifications can present 2-5 tertiary ramifications; usually, the secondary and tertiary stems are the ones that show inflorescences at the terminal part. Tertiary rods are between 6-9cm long and 0.07-0.11cm in diameter.

Leaves are lanceolate in shape, whole, slightly toothed or crenate, slightly pubescent, intense green, glossy, with well-marked secondary veins. The intense coloring of leaves and the lack of glandular bristles clearly differentiates *S. hyssopifolia L.* from *S. scardica* or *syriaca*, also studied at PGRB Buzau, Romania. The average length of the leaves is 3.4-5.1cm, the average width varies between 0.6-1.3cm and the thickness is 0.17-0.48mm (*Figure 3*). Both leaves and flowers give off a strong, extremely aromatic smell, very different from the other *Sideritis* species studied (*scardica, syriaca*).

Inflorescences are in the form of a dense spike with toothed bracts, long bristles and yellow flowers. Inflorescences have 5-9 vertices, with lengths varying between 4.6-7.8cm and diameters between 0.9-1.6cm. Vertices can be compact or spaced. The basal ones can be spaced 1cm apart on average, while towards the top they are compact. Flowers are light yellow (*Figure 4*). Bracts are strongly pubescent compared to the glossy, intense green leaves, without glandular hairs.

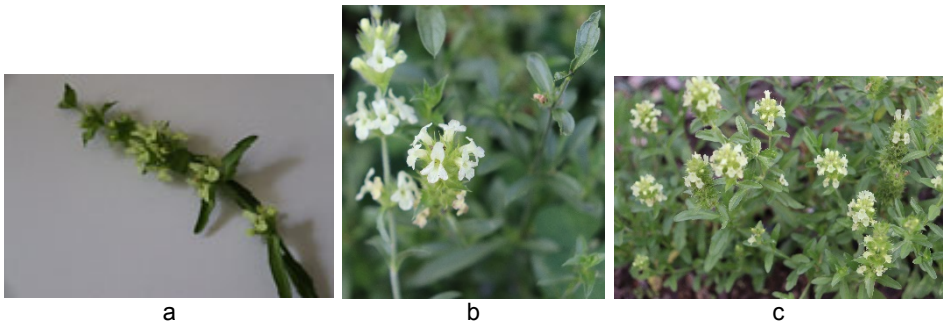
Since the first year of cultivation, obtained seeds will be subjected to germination tests. On average, 109.47g of vegetable mass containing leaves and inflorescences were obtained from 10 plants of *S. hyssopifolia L.* in the first year of cultivation. The samples collected in the first decade of October for laboratory determinations will be the subject of a future article (*Figure 5*).

a b

Figure 2 - *Sideritis hyssopifolia* L., variety G5 - (a) inflorescences; (b) leaf formation

a b

Figure 3 - *Sideritis hyssopifolia* L., variety G5 - (a) inflorescences; (b) leaf formation



a b c

Figure 4 - Inflorescence details of *S. hyssopifolia* L.: (a) – with spaced vertices; (b) and (c) short and compact inflorescences

Figure 5 - Plant mass of *S. hyssopifolia* L. harvested for the purpose of determining the chemical composition of leaves and flowers

Crop technology

As far as the culture technology is concerned, both the innovative character and the importance and usefulness of this study should be underlined, especially due to the fact that Romania is the first country in south-eastern Europe where the acclimatization and improvement of the *S. hyssopifolia L.* species has been studied. In Spain, the country of origin, the researchers have recognized that is need to know more about the reproductive biology of these species in order to make a more responsible mining, and using technical and non-harvest periods to facilitate the regrowth as well as criteria for management of habitat in which these plants grow, in order to further its use (Herrero and Martìn-Lobera, 2012).

The used scheme for establishing the culture was: 70cm between roes and 30cm between plants/row because: the small sizes of the plants do not require larger distances between plants, facilitating the execution of technological works for culture maintenance; faster harvesting of the inflorescences as a result of the erect growth of flower stems through the mutual support of bushes; the maintenance works were the usual ones, namely: filling the gaps after planting, fighting weeds with two manual and three mechanical weeders and supplying the crop with water using a watering rate of 200-250m3/ha. It should be mentioned that compared to *S. hyssopifolia L.* where a plant spacing of 30 cm was established, *S. syriaca* was planted at a spacing of 40 cm and *S. scardica* at 50 cm, due to the larger size of the plants.

Values in *Table 1* represent: MD – the arithmetic average of characteristics studied in variety G5; SD – standard deviation; CV – coefficient of variability, expressed as %. It can be noted that CV% ≤ 10 in the first two columns, hence plant height and diameter showed a small variability, while CV% = 10 – 20 represents an average variability for characteristics of leaves and inflorescences.

DISCUSSION

The work reveals the unique nature of the study, by introducing a new species of *Sideritis* into the breding and acclimatization program of P.G.R.B Buzau together with *S. scardica*, var. Domnesc, *S. scardica* var. Ossa and *S. syriaca*. Recently *S. hyssopifolia L.* has been introduced in the study and has shown vigour and adaptability to environmental conditions since the first year. Aggressive harvesting for commercial purposes through the practice of rural tourism in the centers of origin in Cantabria, Spain, and the intensive use in the preparation of teas and digestive drinks, as a domestic brand, have endangered species of the genus *Sideritis*.

Table 1 - Calculation of variability coefficients of G5 – *S. hyssopifolia L.*

***S. hyssopifolia* studied character**	**Canopy diameter (cm)**	**Plant height (cm)**	**Leaf length (cm)**	**Leaf width (cm)**	**Inflores-cences length (cm)**	**Inflores-cences diameter (cm)**	**Vertices number**
MD	23.42	37.14	4.59	0.812	6.92	1.24	7.6
SD	1.46	2.68	0.47	0.08	1.16	0.19	1.14
CV	6.24%	7.22%	10.15%	10.39%	16.7%	14.93%	15.00%

At the same time, scientists drew attention to the fact that no scientific research was developed to allow the introduction of this species into culture. So far in there have been only few cultivation attempts by plant enthusiasts in private gardens in the area (de Santayana et al., 2005).

Another interesting challenge for the research of *S. hyssopifolia L.* species is given by its chemical composition and its beneficial effects on health. In this regard, S. hyssopifolia was the subject of research carried out by the French company Laboratoires Clarins on inflorescences harvested from the French Alps at an altitude of 1300m, in two stages: July 2016 and August 2017. Two of the diterpenoid compounds isolated and identified as a result of the chemical investigation of *S. hyssopifolia L.* exhibit excellent anti-inflammatory capabilities, according to biological studies. Further biological tests will be performed to find potential cosmetic applications (Aimond et al., 2020). For these reasons, *S. hyssopifolia L.* was studied for the first time in Romania in 2019 and the premises were created for the promotion of this species among growers. The P.G.R.B germplasm base is enriched with a valuable species from a medicinal, ornamental and gastronomic point of view.

CONCLUSION

1. *S. hyssopifolia L.* was acclimatized by the PGRB Buzau researchers team, obtaining a valuable G5 variety that shows genetic stability in the offspring, as well as valuable phenotypic and biochemical characteristics;

2. The culture technology for this species was developed;

3. In the future, PGRB Buzau will provide growers with both seeds and seedlings for testing.

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REFERENCES

Güvenç, A.; Houghton, P.J.; Duman, H.; Coşkun, M.; Şahin, P. Antioxidant activity studies on selected *Sideritis*. species native to Turkey. *Pharmaceutical Biology*. **2005**, 43, 173-177. https://doi.org/10.1080/13880200590919528.

Aslan, İ.; Kılıç, T.; Gören, A.C.; Topçu, G. Toxicity of acetone extract of *Sideritis trojana* and 7-epicandicandiol, 7-epicandicandiol diacetate and 18-acetylsideroxol against stored pests *Acanthoscelides obtectus* (Say), *Sitophilus granarius* (L.) and *Ephestia kuehniella* (Zell.). *Industrial crops and products*. **2006**, 23, 171-176. https://doi.org/10.1016/j.indcrop.2005.05.0006.

Loğoğlu, E.; Arslan, S.; Öktemer, A.; Şakõyan, İ. Biological activities of some natural compounds from *Sideritis sipylea* Boiss. *Phytotherapy Research*. **2006**, 20, 294-297. https://doi.org/10.1002/ptr.1855.

González-Burgos, E.; Carretero, M.E.; Gómez-Serranillos, M.P. *Sideritis* spp.: Uses, chemical composition and pharmacological activities - A review.

Journal of Ethnopharmacology. **2011**, 135, 209-225. https://doi.org/10.1016/j.jep.2011.03.014.

Skordos, D.; Michalis, E.; Ragkos, A. Analysis of the External Environment of a Vertical Dried Herbs Business and the Role of E-Commerce Tools. Proceedings of the 9th International Conference on Information and Communication Technologies in Agriculture, Food and Environment (HAICTA 2020), 24-27 September 2020, Thessaloniki, Greece, CEUR Workshop Proceedings, https://ceur-ws.org/Vol-2761/HAICTA_2020_paper1.pdf.

Ganos, C. G., Gortzi, O., Chinou, E. B., Calapai, G., Chinou, I. B. Antimicrobial Properties of Selected Native Greek Aromatic Plants: An Ethnopharmacological Overview. Promising Antimicrobials from Natural Products, **2022**, 91-117. https://link.springer.com/chapter/10.1007/978-3-030-83504-0_6.

Coto, E.; Fernandez, N.; Garcia, J.J.; Diez, M.J.; Sahagun, A.M.; Sierra, M. Assessment of the antioxidant/ hypolipidemic relationship of *Sideritis hyssopifolia* in an experimental animal model. *Molecules*. **2019**, 24, 2049. https://doi.org/10.3390/molecules24112049.

Vînătoru, C.; Mușat, B., Bratu C. Mursalski chai. in special treatise on vegetables (in Romanian), ALPHA MDN Publishing House, Buzau, Romania, **2019**, pp. 680-688.

Rodríguez Lion, M. L. *Aislamiento e identificación de compuestos polifenólicos en Sideritis hyssopifolia subsp. hyssopifolia L: contribución a su estudio farmacológico*. Doctoral dissertation, Tesis doctoral. Facultad de Farmacia, Universidad de Alcalá, **1997**.

Adzet, T.; Canigueral, S.; Ibánez, C.; Monasterio, I.; Vila, R. Phytochemical investigation of *Sideritis-hyssopifolia* var *pyrenaica*. *Journal of Essential Oil Research*, **1990**, 2, 151-153. https://doi.org/10.1080/10412905.1990.9697848.

Herrero, B.; Martìn-Lobera, C. Threatened medicinal plants in Castilla y León (Spain). *Global Advanced Research Journal of Medicinal Plants*. **2012**, 1, 1-6.

de Santayana, M.; Blanco, E.; Morales, R. Plants known as té in Spain: An ethno-pharmaco-botanical review. *Journal of Ethnopharmacology*. **2005**, 98, 1-19. https://doi.org/10.1016/j.jep.2004.11.003.

Aimond, A.; Calabro, K.; Audoin, C. Cytotoxic and anti-inflammatory effects of *ent*-kaurane derivatives isolated from the Alpine plant *Sideritis hyssopifolia*. *Molecules*. **2020**, 25, 589. https://doi.org/10.3390/molecules25030589.

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