SUMMARY

of the PhD thesis:

ACCLIMATIZATION AND BREEDING OF *SIDERITIS* spp. IN ROMANIA

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In Romania, medicinal plants have been known and used since ancient times for their therapeutic, food, ornamental and honey properties. Currently, the medicinal plant sector has an under-exploited potential negatively influenced by climate change, soil erosion, partial destruction of natural habitats and ecosystems. In this context, among the solutions is the taking over of some endemic species from various geographical basins within and outside the country and "domesticating" them due to their ecological plasticity.

This PhD paper entitled "RESEARCH ON ACLIMATIZATION AND BREEDING OF *SIDERITIS* spp. IN ROMANIA" includes the results of the research on the introduction of *Sideritis* spp. procured from the areas of origin into acclimatization and improvement programs, the development of culture technology, the evaluation of biochemical composition, as well as germination and viability tests to the seeds. Among the five species studied, *S. syriaca, S. hyssopifolia* and *S. cypria* were introduced in culture **for the first time in Romania**. We believe it is necessary to address this topic in order to lay the foundations of theoretical and practical knowledge regarding the ability of *Sideritis* to use the climatic conditions of our country, and its introduction in culture with the aim of obtaining genetically stabilized new varieties, with phenotypic uniformity and distinctiveness.

The first part of this paper consists of three chapters that create the informational framework on medicinal and aromatic plants.

Chapter I, "ECONOMIC IMPORTANCE OF *SIDERITIS* spp.", includes 3 subchapters. The first one refers to the importance of using medicinal and aromatic plants and the tradition of using medicinal plants in Romania. The second subchapter is an x-ray of the directions of use: the wide spectrum of diseases for the relief of which *Sideritis* tea is used as an adjuvant, its use in the food industry as tea and as a flavoring agent, its use for pharmaceutical purposes according to the biochemical composition, its use in the cosmetic industry and its role as an ornamental and honey plant. The third subchapter summarizes the main chemical compounds found in most *Sideritis* species: terpenes, phenolic compounds and flavonoids, essential oils and minerals.

Chapter II, "CURRENT STATE OF KNOWLEDGE REGARDING *SIDERITIS* spp.", structured into 5 sub-chapters, presents the origin, distribution and taxonomy of *Sideritis* species, a brief description of the main known species, the Red List of endangered species, ways to conserve genetic resources *in situ* and *ex situ*, and current knowledge about the cultivation of *Sideritis* in countries where it is endemic, harvested uncontrolled, and where its cultivation has become a necessity: Bulgaria, Greece and Turkey.

Chapter III, "ACLIMATIZATION AND BREEDING OF *SIDERITIS* spp. IN ROMANIA", presents the research carried out in Romania so far, the experiences conducted and performance achieved by the researcher Costel Vînătoru, who introduced *Sideritis* in Romania in 2015 and succeeded four years later in getting the first Romanian variety of *S. scardica* registered in the Romanian Official Catalogue of the Varieties of Plant Species under the commercial name Domnesc.

The second part includes personal research and it is composed of 6 chapters:

Chapter IV, "PURPOSE AND OBJECTIVES OF THE RESEARCH", outlines the purpose pursued, that of introducing five *Sideritis* species in processes of breeding and acclimatization to the pedo-climatic conditions of our country and following them during three growing cycles for *S. scardica* L., *S. scardica* L. var. Ossa, *S. syriaca* L., *S. hyssopifolia* L. (2021-2023) and two growing cycles for *S. cypria* (2022-2023). The objectives were aimed at assessing, studying and monitoring the identified genotypes, establishing correlations between quantitative and qualitative descriptors through statistical analysis with implications in breeding processes, developing the cultivation technology, evaluating biochemical composition and determining germination and viability. The need for this study lies in identifying solutions for adapting to climate change, increasing biodiversity, promoting and increasing interest in the consumption of a medicinal plant with potential to prevent and treat serious conditions such as neurodegenerative diseases or cancer.

Chapter V, "LOCATION AND SETTING OF THE EXPERIMENT", describes the natural setting in Crâng Forest, a botanical forest reserve in Buzău County that hosts the Plant Genetic Resources Bank for Vegetables, Floricultural, Aromatic and Medicinal Plants Buzău, which owns the experimental field where the research was conducted. The soil characteristics, the measurements regarding the recorded temperature intervals, and the precipitation and wind patterns highlight the fact that the described area falls into zone II of favorability for aromatic and medicinal plants.

The monitoring of climatic conditions between 2021-2023 confirmed the manifestation of global warming phenomena, which in Romania resulted in increased thermo-pluviometric contrasts. The thermal characterization revealed the absence of negative average temperatures in the winters of the last three years, which eliminated the risk of ground frost of perennial *Sideritis* crops. The climatic context of the summer of 2021 was favorable for optimal rooting and leaf rosette formation, while the onset of soil drought in July-September and lack of abundant rainfall ensured plant growth and eliminated the danger of waterlogging in the stem area, with the risk of pubescent leaf rot. The high temperatures in February-March 2022 and 2023 favored the start of growth for *Sideritis* species and the presence of strong wind gusts characteristic of Buzău area did not affect the plants due to the strong rooting and lignified stems at the base of the bushes that confer resistance to breakage. Monthly averages in April were beneficial for the formation of flowering stems. The summers of both years were characterized by a long drought and low rainfall, climatic conditions similar to those in the area of origin, which the plants exploited efficiently through the phenophases of flower stem development, maximum flowering, and seed formation and maturation.

Chapter VI, "EVALUATION OF THE GERMPLASM BASIS", is structured in 3 subchapters: Introduction, Material and Method, and Results and Discussion.

The genetic material involved in the study is represented by a number of 18 genotypes: *S. scardica* with genotypes G01, G02, G03, G04, G05 and Domnesc cultivar; *S. scardica* var. Ossa with G06, G07; *S. syriaca* with G08, G09, G10, G11, G12; *S. hyssopifolia* with G13, G14, G15, G16, G17, and S*. cypria* with G18.

The breeding methods used were: *repeated individual selection* in year I starting from a heterogeneous population in order to sum up all the valuable genotypes characterized by vigorous growth, high leaf mass, concentrated flowering for optimal harvesting, strong aroma; *negative mass selection* aimed at eliminating unsuitable forms from the crop in years II and III. The characterization of genotypes was based on 37 agro-morphological descriptors, of which 17 analyzed qualitative plant traits and 20 analyzed quantitative traits.

Results and discussions on seedling production and development showed very good percentages of sprouting except for *S. cypria* and *S. glacialis*, which did not sprout. By monitoring the phenophases of year I, from sowing to planting of seedlings, the cumulative daily rate of development was determined in order to compare the growth stages, the genotypes being classified into four categories: early (G13, G14, G15, G16, G17), medium (Domnesc cultivar, G01, G02, G03, G04, G05, G06, G07) late (G08, G09, G10, G11, G12) and very late (G18). In years II and III of cultivation, by monitoring the developmental phenophases from the emergence of flower stalks to seed harvest, the species were classified as: early (*S. syriaca*) with a minimum of 75 days, medium (*S. scardica* and *S. scardica* var. Ossa) with a total of 79 days and late (*S. hyssopifolia*) with a maximum of 83 days.

The results on the development of cultivation technology took into account the growth and development characteristics of each species. Planting distances were highest for *S. syriaca* characterized by

long and arched stems generating large plant diameters, medium for *S. scardica* with erect, vigorous flowering stems and horizontally balanced growth, followed by *S. scardica* var. Ossa with moderate vigor and smaller flowering stems, and *S. hyssopifolia* with low vigor and the smallest short, erect flowering stems.

Observations on the variability of qualitative descriptors confirmed the biodiversity within the germplasm collection and the use of the Agglomerative Hierarchical Clustering analysis (AHC) generated a dendrogram that classified the germplasm collection according to the shape and traits of the upper aerial parts of the genotypes. The four resulting clusters actually correspond to the four studied species: cluster 1- *S. scardica*, cluster 2 - *S. syriaca*, cluster 3 - *S. scardica* var. Ossa and cluster 4 - *S. hyssopifolia*.

S. scardica showed highly significant differences in vegetative mass, G05 obtaining the best yield/plant as a result of the high floral stem weight. Correlations between genotypes revealed interdependence between the number of main branches and vegetative mass. Leaf thickness was an indicator of the degree of pubescence and an important selection criterion. G01 showed highly significant differences and maximum values of this descriptor. G05 and G03 genotypes are recommended for ornamental purposes.

S. scardica var. Ossa did not reveal highly significant differences in morphological features of the leaves and stems of the two genotypes. In terms of yield, the G06 genotype recorded maximum values in 2022, while in 2023 higher values were obtained in G07 due to the increase in the number of flowering stems. The statistical analysis revealed significant differences in height and horizontal development in 2023 alone. Highly significant correlations were reported between plant diameter and vegetative mass.

S. syriaca showed highly significant differences in year III of cultivation for the vegetative mass descriptor with a maximum in G11. The yield elements showed significant differences between genotypes with the highest value in G09 in both years of cultivation, due to the higher degree of development and the large number of flower stems. In the quantitative descriptors regarding leaf and stem morphology no significant variations were reported. In 2023, the year of maximum growth, G10 height and diameter monitoring revealed highly significant differences, also highlighting the highest values in this genotype.

S. hyssopifolia showed good ability to adapt to the climatic conditions by forming flowering stems since year I. It showed small size, intense green color of the upper aerial parts due to the lack of glandular hairs, hyssop-like leaves and very strong and persistent specific aroma. Highly significant differences were recorded in the vegetative mass descriptor of flowering stems during the two years, with maximum values in G14. In year III of cultivation, G15 stood out with the highest value for height and G17 with the highest value for diameter.

S. cypria showed extremely slow growth during the two years, with moderately significant differences between quantitative parameters. The species was characterized by a very pronounced silvery-white color due to an extremely high level of pubescence, which also gives a pronounced leaf thickness.

Chapter VII, "ASSESSMENT OF BIOCHEMICAL COMPOSITION", presents information on the variability in the content of chemical compounds depending on the species, the time of harvesting and the freshness of the flowering stems. Measurements were carried out in the Sensory Analysis Laboratory of the Research Center for Studies of Food Quality of Agricultural Products, part of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The dry matter content was determined by the gravimetric method but was not relevant, the total polyphenol content was determined by the Folin-Ciocâlteu spectrophotometric method, the antioxidant activity of DPPH radical scavenging was performed using the DPPH assay, and the essential oil content of the leaves and flowers of the studied species was determined by GC-MS analysis.

The total polyphenol content showed the highest values in *S. hyssopifolia* in both years of cultivation, while *S. scardica* and *S. syriaca* showed slightly lower values.

The antioxidant capacity assessment revealed no significant differences between the analyzed species in 2022, with the maximum value recorded in *S. hyssopifolia* and close values in *S. syriaca* and *S. scardica*. In 2023, *S. scardica* showed the strongest antioxidant activity, followed at a very small interval by *S. hyssopifolia*, while *S. syriaca* had the lowest antioxidant level. The analysis of correlations between the total polyphenol content and the antioxidant activity revealed moderately positive values in 2023 in *S. syriaca*, which was harvested at maximum

flowering stage, and strongly positive values in *S. hyssopifolia*, which showed very good potential to capitalize on the existing pedo-climatic, technological and nutritional conditions.

The determination of the composition and concentrations of essential oils in *S. hyssopifolia* showed a clearly superior configuration compared to the other two species, confirmed by the constant presence of two very valuable molecular compounds: alpha-bisabolol and alpha-pinene with very high weights, exceeding half of the total weight.

The large variation in the chemical composition of the three species *S. scardica, S. syriaca* and *S. hyssopifolia* can be explained by the different climatic conditions during the two years of cultivation, the response of the plants to abiotic stress factors and hence the different degree of acclimatization.

Chapter VIII, "DETERMINATION OF THE VIABILITY AND GERMINATIVE CAPACITY IN *SIDERITIS* spp.", established the level of germination, the possibility of the existence of seed dormancy in *S. scardica, S. scardica* var. Ossa, *S. syriaca* and *S. hyssopifolia* in 2023 and their vitality over time, and in *S. scardica* in 2019, 2020, 2021, and 2023. Identical conditions of temperature, humidity and light in the germination chamber were used to determine germination with two methods – TP (Topper Paper) and BP (Bellow Paper) - except that in method 2 the samples were moistened with gibberellic acid solution to eliminate the dormancy state. The tetrazolium test for red staining of structures showing respiratory activity was used to test viability.

S. scardica var. Ossa showed the highest germination percentage and *S. hyssopifolia* the lowest. By treating the seeds with gibberellic acid to help germination, *S. hyssopifolia* reacted best, which makes it possible to have seed dormancy that can be broken under pretreatment conditions. *S. scardica* had adequate and consistent germination in all test methods performed. In viability tests *S. scardica* var. Ossa showed the highest value, closely followed by *S. scardica* and *S. syriaca*. *S. scardica* var. Ossa showed the highest germination potential but also the risk of blockage due to dormancy. This explains the poor germination under field conditions where a large number of plants showed low vigor and could not survive under unsuitable conditions of external factors (high temperatures, drought).

Chapter IX, "GENERAL CONCLUSIONS AND RECOMMENDATIONS", includes the general conclusions drawn from the experiment.

All *Sideritis* species studied were found to be of food, medicinal, honey and ornamental interest. In terms of food, *S. scardica* var. Ossa has the most pleasant and strong flavor. From a medicinal perspective, *S. hyssopifolia* has the highest concentration of antioxidants and essential oils beneficial to health. It has also shown the best acclimatization potential, which is why it was proposed for approval in 2024 under the provisional name 'Enescu'.

For cultivation in the field we recommend following the developed cultivation technology. The crop can be maintained for a maximum of 6 years, with maximum results in year III of cultivation. The absence of diseases and pests during the study recommends successful cultivation in an organic system of the *Sideritis* spp.

We draw attention to the G05 genotype, which proved to be the most productive, given the importance of production for medicinal plants. In terms of germination, it is optimal to use seeds up to three years old.

Given that the majority of the studied *Sideritis* spp. adapted very well to the climatic conditions in the south-eastern part of Romania and their economic importance, it is recommended to continue the research in order to approve new varieties and use them as sources of genetic material for large-scale cultivation in Romania, as therapeutic medicinal plant.