

# Sideritis syriaca – A New Species Introduced into Culture in Romania

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#### RESEARCH ARTICLE

#### Abstract

*Sideritis syriaca* is an endemic plant in the mountains of Crete, well-known from the antiquity (Karousou R. et al., 1992). Also named Malotira, it has been used as an anti-inflammatory, antimicrobial, antioxidant and analgesic agent in folk medicine (Goulas V. et al. 2014). In Romania, the Domnesc variety was created in 2019 as a result of breeding and acclimatization to *S scardica*. Acclimatization, identification of suitable areas for cultivation in Romania and breeding to obtain genotypes with distinct phenotypic expressiveness that meet the international norms of the DUS test are the study's purpose. The genetic resources are from the germplasm collection of P.G.R.B. and endemic resources acquired from the area of origin and the breeding methods used are repeated individual selection followed by negative mass selection. Type E was selected from the 5 varieties obtained, based on its valuable phenotypic characteristics and good response to environmental conditions; during the three years of study, the plants were monitored, biometric measurements were performed, the culture technology was established; the observations showed an optimal evolution and development of the plants: biomass was 286 g in the first year and 1495 g in the third year. The establishment scheme of the culture was 70 cm between rows and 40 cm between plants/row. Breeding and acclimatization of the *S. syriaca* species to the climatic conditions of our country were achieved, conditions for the approval of a new variety were created, culture technology was developed.

Keywords: breeding, antioxidant, Malotira.

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#### **INTRODUCTION**

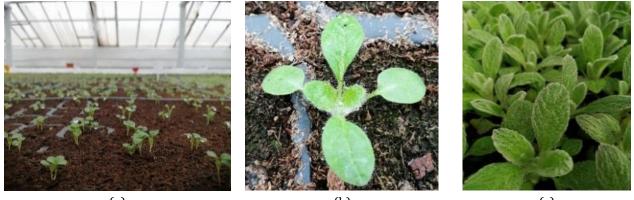
*Sideritis syriaca* L. (syn.: *S. cretica* Boiss.non L.), an East-Mediterranean species of the Lamiaceae family, belongs to the section Empedoclia, well known from the antiquity for their therapeutical properties (Karousou R., et al., 1992). *S. Syriaca* is an endemic plant in the mountains of Crete (Gikas E. et al., 2001). In folk medicine, *S. syriaca* preparations have been used as anti-inflammatory, antimicrobial, antioxidant, analgesic, antirheumatic and tonic agents, while its decoction is still used as a relief from common cold (Goulas V. et al. 2013). Their pharmacological properties have been mainly attributed to flavonoids, diterpenes and essential oils (Plioukas M. et al. 2010). In 2012, a variety of *S. scardica* was studied, successfully breeded and acclimatized in Romania by the researcher Costel Vînătoru. The Domnesc variety was obtained, a genotypically valuable creation with distinct phenotypic traits, which in 2019 was entered in The Romanian Official Catalogue of the Varieties of Plant Species (Vinatoru et al. 2019). Like the other species of the *Sideritis* genus, *S. Syriaca* is used in traditional

medicine as flavoring agent in gastronomy, raw material in the pharmaceutical and cosmetic industry, but also as an ornamental and honey plant. At Plant Genetic Resources Bank (P.G.R.B.), it has been studied since 2020 and the aim is to introduce it into culture. The objectives of this work are primarily aimed at the acclimatization of S. syriaca and the identification of suitable areas for cultivation on the territory of Romania, as well as the improvement of this species with the aim of obtaining genotypes with distinct phenotypic expressiveness that meet the international norms of the DUS test (distinction, uniformity, stability). It is known that it is a Greek endemic taxon growing exclusively in the island of Kriti (Crete), wherefrom it was first described by Linnaeus (1753: 574) (Karousou R., et al., 1992). Like S. scardica, S. syriaca is particularly valuable and appreciated for its chemical composition. In S. syriaca infusions rich phenolic and flavonoid load was observed confirming its high antioxidant value. In addition, the essential oil, nutritional and mineral contents were studied (Lytra K. et al. 2021). Also, Yumrutas O. et al., examined the antiproliferative and antioxidant activities of S. syriaca in 2015 and concluded that the phenolic compounds are one of the most important classes of these phytochemicals, due to their potential antioxidant activity, with significant levels of anti-proliferation and anticancer activities. It is believed that biological effects of multiple phytochemicals, rather than a single compound, contribute to cancer prevention. Also, the results of Hofrichter J. et al. in 2016 concluded that treatment with Sideritis spp. Extracts might be a potent, well-tolerated option for treating symptoms of cognitive impairment in elderly and with regard to Alzheimer's disease by affecting its most prominent hallmarks: Aβ pathology and cognitive decline.

## **MATERIALS AND METHODS**

The study was started in 2020 at Plant Genetic Resources Bank Romania (45°09'N, 25°5'E, 95m) and is still ongoing. P.G.R.B is located in Buzau County, in SE Romania, in an area with continental climate, hot and dry summers and low rainfall in the months of June – August.

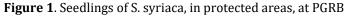
The genetic resources of *S. syriaca* involved in the study come from the germplasm collection from P.G.R.B. Buzau, as well as from endemic resources acquired from the origin area. Sowing was done in protected spaces (Figure 1, (a)) in the second decade of March, when day/night temperatures were 7/16°C, 12h photoperiod. A mixture composed of 80% peat and 20% limestone was used for the substrate, considered optimal for the establishment of the culture of *Sideritis* according to the requirements of the species from the area of origin, where it grows in alpine and subalpine areas characterized by sandy and rocky soil, low in nutrients, well drained and with alkaline or slightly alkaline reaction (Chisnicean L., 2022). Pallets of 70 cells and dimensions of 33x53cm were used (Figure 1, (b)). The culture of *S. syriaca* was established in the field 60 days after sowing (fig. 2, (c)), according to the planting scheme – 70 cm between rows and 40 cm between plants/row.



(a)



(c)



In the seedling phase, *S. syriaca* does not require abundant irrigation, as there is a danger of losing the seedlings. When transplanting, the nutrient cube must be at ground level. The soil mulching is recommended. Irrigation works require special attention due to the strongly pubescent leaves, as they can adhere to the soil surface and turn brown in the first phase, then rot. The first flowering occurs between May 15 – June 15 and the second around August 15-September 15 and it can last until October if there are no frosts. Harvesting is done when the flowers open along the entire length of the inflorescence.

The breeding methods u–ed were repeated individual selection followed by negative mass selection. Aerial parts of *S. syriaca* were used for measurements and observations and the measurements values were processed by statistical calculation using SPSS software, performing analysis of variance by ANOVA test followed by DUNCAN test with 95% confidence interval and p-value < 0.05%. Weighing operations were performed on green plants after flowering in June at P.G.R.B. laboratory and the device used was the two-decimal precision balance Kern 572-33.

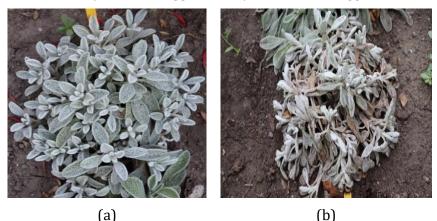
## **RESULTS AND DISCUSSIONS**

*Sideritis syriaca* is a perennial herbaceous plant. It has a fascicular root with a slightly lignified stem at the base, which gives it resistance to low temperatures, strong winds and large temperature fluctuations. The bush is erect, the stem reaches heights of 40-45 cm and up to 85-90 cm in diameter. The bush is branched and the flower stalks can be solitary, but branches are predominant. Sprouts are between 18-30 cm long.

The genetic material used generated several varieties, from which 5 genotypes with different phenotypic characters were studied, namely: different port in diameter and height, degree of lignification of the basal stem, leaves of various shapes and colors, petiole length, different degree of pubescence, sensitivity to environmental conditions, leaf apparatus size, number of shoots.

Next, a brief characterization of each genotype:

*S. syriaca* **A** type (Figure 2 (a)): good horizontal development, 25.3 cm diameter, 12.2 cm height, silver-green leaves, very pubescent, with very good ability to generate sprouts (43, in the first year of cultivation); the observations made on the leaves showed thickness of 0.85 mm, length of 8.88 cm, width of 1.99 cm; the average weight of a leaf is 0.28 g; it should be mentioned that due to the very high density of glandular bristles, the plant shows an increased sensitivity to excess water, being exposed to the phenomenon of leaf rot. In Figure 2 (b) the plant is affected by a series of heavy rains, with approximately 80% of the leaf apparatus destroyed.



**Figure 2**. *S. syriaca* **A** type: (a) first year of culture, 75 days after planting the seedlings; (b) the plant destroyed by excess water

*S. syriaca* **B** type (Figure 3): optimal development both horizontally and vertically with 38.7 cm and 10.9 cm diameter, respectively; it differs from the other genotypes by the intense green leaves and a low degree of pubescence (Figure 4, (a)); the leaves are opposite, elliptic elongated (Figure 3 (b)), with 8.9 cm in length, of which 3.4 cm petiole and 5.5 cm leaf blade, 1.7 cm in width, and 0.69 mm in thickness; the leaf blade profile is concave; the plant has a strong port, medium capacity to generate new sprouts, weak aroma; petiole thickness of 1.24 mm, glandular bristles at the base of the petiole of 1.04 mm; the mature leaf has an average weight of 0.25 g.



**Figure 3.** *S. syriaca* **B** type: (a) first year of culture, 75 days after planting the seedlings; (b) details: leaves

*S. syriaca* **C** type (Figure 4): optimal development in the horizontal plane with a diameter of 26.1 cm and vertical 12.9 cm; it develops a smaller number of sprouts (27) and leaves (Figure 4, (a)), but the port is higher and has the advantage of not sticking the leaves to the ground, thus reducing the risk of leaf rot; pubescence has a high level, thus giving the leaves a green-silver color; the leaves: length of 7.6 cm, of which the petiole is 3.1 cm and the leaf

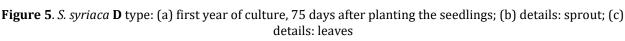
blade 4.5 cm and wider, of 2.2 cm; the mature leaf blade profile is flat (Figure 4, (b)), and the length of the glandular bristles at the base of the petiole is 4.55 mm; the average weight of the mature leaf is 0.29 g;



Figure 4. S. syriaca tip C: (a) first year of culture, 75 days after planting the seedlings; (b) details: leaves

*S. syriaca* **D** type (Figure 6): very weak development in the horizontal plane, diameter of 25.3 cm and 11.7 cm in vertical plane, a small number of sprouts (17), elongated, with poor leaf apparatus (Figure 5 (a)); leaves: small, elliptical, thin, long petiolated (Figure 5, (b)), with a length of 7.6 cm, of which the leaf limb has 4.3 cm and the petiole has 3.3 cm; the color is light green, very little pubescent (Figure 5, (c)); the thickness of the leaf is 0.31 mm; the mature leaf blade profile is flat; the length of the glandular bristles at the base of the petiole is 0.79 mm; the mature leaf has an average weight of 0.15 g;





*S. syriaca* **E** type (Figure 6): very good development in horizontal plane (Figure 6, (a)), diameter of 38.7 cm and 10.7 cm in vertical plane, a large number of sprouts (26), very vigorous, with rich foliage; leaves are elliptical, very pubescent, with a velvety appearance; the length is 10.7 cm, of which the leaf blade has 5.9 cm and the petiole has 4.8 cm; the color is light green silver (Figure 6, (b)); the thickness of the leaf is 0.74 mm; the mature leaf blade profile is very slightly convex (Figure 6, (c)); the length of the glandular bristles at the base of the petiole is 1.76 – 4.42 mm on the surface of the leaf blade; the average weight of a mature leaf is 0.53 g;



Figure 6. *S. syriaca* E type (a) first year of culture, 75 days after planting the seedlings; (b) details: sprout; (c) details: leaves

Following the observations made, the variety of *S. syriaca* type E was selected for the acclimatization and improvement process. The image below (Figure 7) shows the 5 types of leaves, belonging to the types named: A, B, C, D, E.



Figure 7. S. syriaca, the five types of leaves: A, B, C, D si E; (a) front side; (b) back side

Type E variety introduced into culture and studied during 2020-2022 is presented. The reasons why type E was chosen: good development of the bush in the first year, a large number of sprouts and implicitly a rich vegetal mass, very high pubescence, resistant to climatic factors (heavy rains) due to the erect habit of the bush.

*S. syriaca* **E** type: in the first year of crop only a very small number of flower stalks are formed (1-2/bush) and towards the end of the summer, and the harvested seeds have a very low germination capacity; the plant develops mostly in the horizontal plane through the appearance of new sprouts, positioned both centrally and peripherally; the diameter of the bush varies between a minimum of 23cm and a maximum of 41cm, and the height between 8.5 – 14.3 cm; the bush consists of 25-30 sprouts, (Figure 8) which have an average height of 12 cm and 30 leaves/sprout; the number of leaves on the bush varies between 650 – 850.



Figure 8. Details of *S. syriaca* in the first year of culture

The leaves are opposite, elliptic-narrow, extremely velvety, covered with glandular hairs present both on the limb and on the petiole (Figure 9, (a, b)); the mature leaf: length between 5.3 - 5.9 cm, width varies between 1.7 - 2 cm and thickness vary between 0.49 - 0.58 mm; the petiole is short, 1.8 - 2 cm; the color of leaves is green-silver (Figure 9, c), the stem is not yet lignified at the basal part.

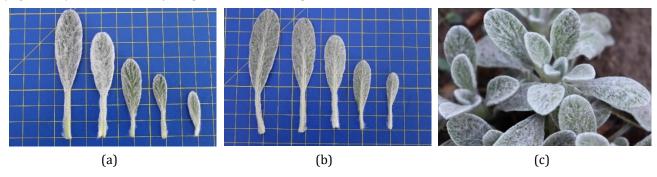
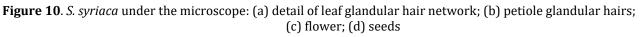


Figure 9. S. syriaca leaves, 1st year of culture; (a) – (b) double sided and (c) bush detail

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The glandular bristles at the level of the petiole are long and very dense (Figure 10, (b)), with dimensions between 0.79 - 1.95 mm at the base of the petiole and between 1.3 - 2.60 mm on the leaf surface; the average weight of a leaf is 0.53 g; Figure 10 shows details captured under the Leica EZ4 W microscope: leaf, petiole, flower and seeds of *S. syriaca*. The abundance of glandular bristles is observed.





#### In the second year of crop

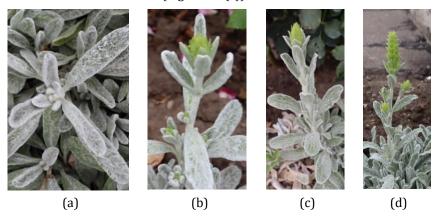
Starting with the second year of crop, the plant develops both horizontally and vertically. The flowering rate increases as a result of the large number of flower stalks formed. The diameter is between 6 - 57 cm, the height of the bush between 9 - 32 cm and the length of the flower stems has 2 - 41 cm.



Figure 11. S. syriaca leaves, second year of crop, front side-back side

There are two weak waves of flowering, the first in May and June and the second in August and September. The length leaf blade between 8.4 - 9.3 cm, width between 1.7 - 2.1 cm, thickness between 1.3 - 1.54 mm, petiole length between 4.8 - 5.9 cm; the weight of a leaf varies between 0.6 - 0.98 g (Figure 11).

Floral stems are branched, with short inflorescences, lengths between 6.8 - 9.42 cm; the number of vertices varies between 5 - 11, the bracts are cordiform, very pointed, green-silver color, strongly pubescent, with lengths between 1.16 - 1.18 cm and the width between at least 0.6 - 1.2 cm; on average, they exceed the flowers by 3 mm; glandular bristles: length between 1.1 - 1.72 mm at petiole level; the floral bud first appears on the floral stem (Figure 12 (a)), later the stem elongates, the inflorescence develops, the whorls become visible (Figure 12 (b)), in the next stage it branches into 3-6 floral stems (Figure 12 (c)).



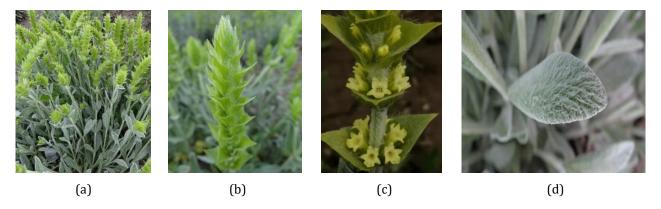
**Figure 12**. *S. syriaca*, second year of crop, the evolution of the floral stea) flower bud phase; (b) flower bud at 2cm; (c) flower bud at 4cm; (d) branched floral stem

During the development of the inflorescence, the whorls are compact; to the mature ones, the first two or three whorls are spaced: 1.51 cm between the first two whorls, 0.91 cm between whorls 2 and 3 and 0.89 cm between whorls 3 and 4, the rest being compact; the weight of an inflorescence is between 0.8 - 1.56 g and of a flower stem between 4.1 - 10.9 g, weighing performed on the green plant.

#### In the third year of crop

The plant is in the maximum development phase, horizontally and vertically with an erect, compact port (Figure 13, a). The bush diameter is between 67 – 81 cm and the height vary between 39 – 46 cm. The basal leaves are well developed, petiolated, large, opposite, obovate, slightly fleshy, the thickness of the leaf is between 1.37 - 1.78 mm, very velvety, ash gray in color due to the accentuated pilosity (Figure 13, d). The basal leaves are between 6.5 - 8.5 cm long and 2.05 - 3.2 cm wide. The median leaves are between 5.4 - 8.7 cm and widths of 1.7 cm on average, and the apical ones are small, with sizes between 2.7 - 3.8 cm and an average width of 1.4 cm. The leaves are covered with glandular hairs which protect the plant in extreme conditions and secrete compounds that give the specific aroma, a combination between menthol and citrus. The weight of a mature basal leaf varies between 0.96 - 1.03 g;

The inflorescences, with an average length of 15 cm, are formed by the vertices grouped in a dense spike (Figure 13, b); the number of vertices in the inflorescence is between 12 and 17, they are very close along the entire length of the inflorescence, which will translate into a very good yield for floral biomass; the bracts are between 12-20 mm long and exceed the flowers (Figure 13, (c)); the determination of the green weight of the stem was 4.35 g and that of the inflorescence was 2.5 g; the diameter of the inflorescence is 0.7 cm at the base, 0.9 cm median and 0.3 cm apical. The corolla has a lemon-yellow color and the calyx is tubular - campanulate, the flowers are small, grouped in number of 6 per whorl.



**Figure 13**. *S. syriaca* 3<sup>rd</sup> year of crop: (a) detail of the plant in the third year of crob) inflorescence detail; (c) detail of the whorls with flowers and bracts; (d) mature leaf detail.

#### **Crop technology**

Land preparation was done in autumn, using the L445 tractor in aggregate with MMS 2.8. The works performed were: land clearing, ploughing, soil disking (discuire) and land shaped; the used scheme for establishing the culture was: 70 cm between rows and 40 cm between plants/row (Figure 14) because: facilitating the execution of technological works for the maintenance of culture; faster harvesting of the inflorescences as a result of the erect growth of the flower stems through the mutual support of the bushes;

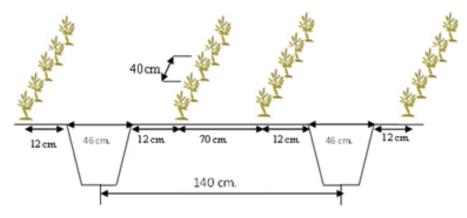


Figure 14. Crop establishment scheme

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 200-250 m3/ ha. Torrential rains during the summer can cause about 14% losses of *S. syriaca*, which is why it is recommended to have a stock - reserve of plants in pots used to replace empty ones.

The harvesting of the flower stalks is carried out starting with the second year, in the two periods related to the two waves of flowering. Bunches of about 400 g green plant are prepared. The flowers are harvested very early in the morning both to avoid the high temperatures during the day but also due to the absence of bees in that time interval, *S. syriaca* being a mellifera plant just like the other species of the *Sideritis* genus. The storage of plant material is carried out in dedicated, well-covered spaces (sheds or halls), protected from air currents or strong sunlight.

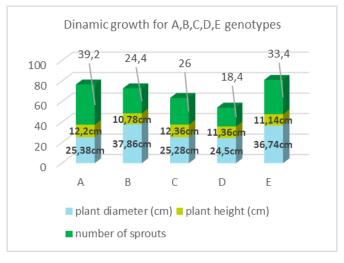
The observations and biometric determinations performed on the five distinct genotypes of *S. syriaca* are shown in Table 1. Canopy diameter, plant height, number of sprouts, leaf sizes and vegetative mass (green and dry determinations) are different in values. The plant height varies from 10.78 cm to 12.36 cm and the canopy dimeter between 24.5 cm and 37.86 cm.

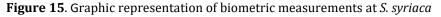
<i>S. syriaca</i> Genotypes	Canopy diameter (cm)	Plant Height (cm)	Number of sprouts	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)	Green leaf weight (g)	5	Loss on drying
Α	25.38±0.90 <sup>a</sup>	12.2±0.35 <sup>b</sup>	39.2±2.17 <sup>d</sup>	8.32±0.38bc	1.92±0.09°	0.08±0.01 <sup>c</sup>	$0.25 \pm 0.03^{b}$	$0.06 \pm 0.01^{b}$	75.25%
В	37.86±2.39 <sup>b</sup>	10.78±0.54ª	24.4±3.78 <sup>b</sup>	8.5±0.71°	1.73±0.04 <sup>b</sup>	$0.07 \pm 0.01^{b}$	$0.24 \pm 0.01^{b}$	$0.06 \pm 0.01^{b}$	75.87%
С	25.28±0.94 <sup>a</sup>	12.36±0.54 <sup>b</sup>	26±2.45 <sup>b</sup>	7.66±0.29 <sup>ab</sup>	2.03±0.16 <sup>c</sup>	0.08±0.01 <sup>c</sup>	$0.28 \pm 0.02^{b}$	$0.07 \pm 0.00^{b}$	74.27%
D	24.5±0.73 <sup>a</sup>	11.36±0.55ª	18.4±2.07ª	7.24±0.4 <sup>a</sup>	1.37±0.05ª	$0.03 \pm 0.00^{a}$	$0.18 \pm 0.02^{a}$	$0.03 \pm 0.01^{a}$	83.73%
Е	36.74±1.61 <sup>b</sup>	11.14±0.64ª	33.4±4.04 <sup>c</sup>	8.92±0.69°	1.93±0.05°	0.08±0.01 <sup>c</sup>	0.49±0.03 <sup>c</sup>	0.13±0.01 <sup>c</sup>	71.99%

Table 1. Dynamic growth for the five genotypes (mean ± S.E.) in the first-year crop at S. syriaca

Note: Different letters between cultivars denote significant differences (Duncan test, p < 0.05).

The A type has the maximum number of sprouts 39.2. The E type has the average length of the leaf 8.92 cm, the average width 1.93 cm and the average thickness 0.08 cm. The green leaf weight varies between 0.18 g and 0.49 g and the dry leaf weight ranges from 0.03 to 0.13 g. The loss on drying varies between 71.99% - 83.73% (Figure 15).





During the vegetation period biometric determinations were performed for E type genotype (Table 2). The plant diameter varied from 27.04 - 82.44 cm and the height ranged from 10.74 - 37.76 cm. Regarding the number of sprouts the 3<sup>rd</sup> year has recorded the highest value 67.8. The average number of leaves per sprout had a dynamic growth from 12.1 in the first year of crop to 67.8 in the 3<sup>rd</sup> year, which meant an increase in the number of plant leaves by 3201 and 1495 g vegetative green mass/ plant. After drying there remained 412.77 g/plant.

<b>E</b> 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	27,04±2,10 <sup>c</sup>	10,74±1,77°	12±1,58°	28,2±3,11 <sup>c</sup>	338,2±56,02°	0,6±0,89°	0,4±0,55 <sup>c</sup>	286±13,30 <sup>c</sup>	78,96±3,67°
Year2	52,54±4,28 <sup>b</sup>	24,86±5,85 <sup>b</sup>	39,2±1,48 <sup>b</sup>	42,6±2,07 <sup>b</sup>	1711,2±112,59 <sup>b</sup>	20,2±1,64 <sup>b</sup>	3,6±0,55 <sup>b</sup>	877±57,47 <sup>b</sup>	242,14±15,87 <sup>b</sup>
Year3	82,44±6,70ª	37,76±3,13ª	67,8±1,92ª	47,2±1,48 <sup>a</sup>	3201±158,27 <sup>a</sup>	203,4±5,55ª	180,8±3,19ª	1495±7,11ª	412,77±1,96 <sup>a</sup>

Table 2. E type: dynamic growth during the years of study (mean ± S.E.)

Note: Different letters between cultivars denote significant differences (Duncan test, p < 0.05).

The aerial parts of the plant were harvested and weighed green and then dry, during of 2020-2022. It can be observed in Figure 16 both the dynamic growth of the vegetative mass as well as the losses recorded through drying.

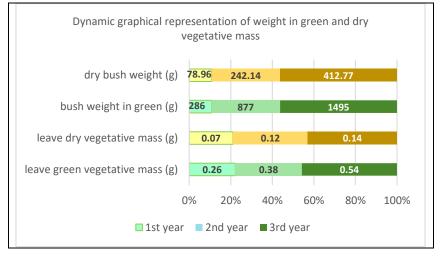


Figure 16. Graphic representation weighing in green and dry vegetative mass

## CONCLUSIONS

Evaluation of the germplasm base regarding the identification of genotypes with distinct phenotypic expressiveness that meet the international rules of the DUS test. *S. syriaca* has been acclimatized, obtaining the first improved genotype that successfully passed the DUS test and is to be proposed in 2023 for testing, homologation and registered. P.G.R.B. provides for growers' seeds and seedlings for testing, as well as information on culture technology.

**Author Contributions:** G.N. Wrote the paper, conceived and designed the analysis; C.B. collected the data; B.M. contributed data or analysis tools; A.P. Performed the analysis; C.V. and A.A. supervised the work.

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#### **Conflicts of Interest**

The authors declare that they do not have any conflict of interest.

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