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Studies on the halophytic *Sphaerophysa salsula* (*Fabaceae*) in Armenia

Abstract

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Morphological, karyological, palynological and ecological features of *Sphaerophysa salsula* halophytic vulnerable species of the Armenian flora are investigated based on the specimens collected from Ararat salt marshes of Armenia. The expanded morphological description and illustration of this species is given. The study shows that the somatic chromosome number is 2*n*=16, with basic chromosome number x=8.

Key words: morphology, karyology, palynology, eco-physiology, vulnerable species.

Introduction

The genus *Sphaerophysa* DC. is distributed in Middle and Central Asia, from South Siberia to North China, West Caucasia and Anatolia. The genus is represented by two species in the world – *Sphaerophysa salsula* (Pall.) DC. and *S. kotschyana* Boiss. (Polhill & Raven 1981). *S. salsula* grows in the south of European Russia, Middle Asia, Kazakhstan, Iran, Afghanistan, Altay, Tuva, Mongolia, China (Gorshkova 1945), while *S. kotschyana* is endemic to Central Anatolia (Duran & al. 2010). An isolated fragment of the range of *S. salsula* is also located in the Caucasus, where it grows in the coastal zone of Dagestan and in the valleys of the middle course of the Kura River and the Arax River (Grossheim 1952; Zakharyan & Fayvush 1991). In Armenia, *S. salsula* was found in 1990 within *Juncus acutum* community in the relict salt marshes of the Ararat valley, which is the extreme western point of the species range (Zakharyan & Fayvush 1991; Gabrielyan 2007; Avetisyan 2011). *S. salsula* meets in the Ararat and Armavir provinces of Armenia, in lower mountain belt, at the altitudes of 800–900 meters above sea level, in salt marshes, in slightly salted areas, on sands, along the banks of streams. The species is included in the Red Book of Plants of Armenia (2010) under the category VU (Vulnerable species). In Armenian flora at present two sub–populations of the target species are known from Yerevan floristic region.

The extent of occurrence and the area of occupancy are less than 500 km^2. It is not included in the Annexes of CITES and that of the Bern Convention. According the Bern Convention, while *S. kotschyana* is in under absolute preservation plant list (CITES 2008). The species *S. kotschyana* is closely related to *S. salsula*. It mainly differs from *S. salsula* by stipules 2–9 mm long (not c. 2 mm), calyx 5–7 mm long (not 4–5 mm), corolla purplish-pink to violet (not red), ovary glabrous (not short appressed-hairy), seeds 3.5–4 mm long (not c. 1.5 mm) (Davis 1970).

In the Ararat valley of Armenia *S. salsula* blooms (Fig. 1a) from May to July (August), fructifies (Fig. 1b) in July-September. The plant is very ornamental in all phenological phases of its development. On the salt marshes of the Ararat valley the occurrence and abundance, as well as the status of the population are satisfactory (Akopian & al. 2018).

It is propagated by seeds and vegetatively through the rhizome. Fruits and seeds are often damaged by insects. It is eaten by cattle. It belongs to the ecological group of halo-xerophytic plants and can adapt to the increasing dryness of the habitat. The main threat to the population is grazing, ignition of vegetation, and territory reduction.

The purpose of this work is investigation of karyological and some palynological and eco-physiological features of the vulnerable halophilic species *Sphaerophysa salsula*, which is insufficiently studied in these aspects. The obtained data can contribute to the identification of the adaptation features of this species in the conditions of the Ararat valley and its protection.

Material and methods

Morphological, karyological, palynological and ecological features of the halophytic rare species *S. salsula* were investigated based on field observations and material collected from the Ararat valley of Armenia.

Fig. 1. *Sphaerophysa salsula*: a) in flowering, b) in fruiting.

S. salsula is perennial grayish plant 30–80 cm high, covered with short, adpressed, sparse hairs. Leaves odd-pinnate 4–10 cm long, with 6–10 pairs of elongated-elliptical, gray leaflets. Stipules acute, lanceolate, 2 mm long. Flowers numerous with short pedicels, collected in oblong, axillary raceme. Calyx 4–5×2–3 mm, campanulate, with 5 short, wide-triangular, sharp tooths. Corolla brick-red colored, vexillum rounded, slightly emarginate, 13–15×10 mm, wings oblong, curved, almost equal in length to the carina. Legumes 15–35×10–20 mm, inflated, oblong or almost rounded, membranous, glabrous or scattered-pubescent, indehiscent. Seeds 1.5 mm long, brownish, smooth. Rhizome long, cord-like (Akopian & al. 2018; pers. obs.).

The karyological investigations were made on the mitotic chromosome preparations in the stage of metaphases obtained from the meristematic cells of root tips. The seeds were germinated on wet filter paper in Petri dishes in the laboratory (19°−21°C). The root tips were pretreated in 0.4% colchicines solution for 2 hours and fixed in fluid of alcohol and glacial acetic acid at 3:1 ratio for at least 2 hours at room temperature. After hydrolysis in 1N HCl for 10−15 minutes at 60°C the root tips were stained in Schiff reagent for 1.5 hours. Then the root tips were squashed on a glass slide with 45% acetic acid. The preparations were placed in butyl alcohol for 5 minutes, then in xylene for 5 minutes, and were placed in Canadian balsam. For chromosome counts, a minimum of 10 plates of chromosome preparations were examined. The determination of the number of chromosomes and the description of the species karyotype were carried out by AmScope Photomicroscope using an oil immersion objective (×100). Photographs were taken with the same microscope.

Specimens karyologically examined: Ararat province of RA, near Armash village, at an altitude of 1000 m a.s.l., 39°45’38.5’’N 44°48’23.5’’E, 02.07.22. Leg. J. Akopian, A. Ghukasyan, L. Martirosyan, A. Elbakyan. Det. J. Akopian, A. Ghukasyan; Ararat province, “Salt marshes” in the vicinity of Ararat town, at an altitude of 900–1000 m a.s.l., 39°49’28.9’’N 44°43’53.2’’E. 02.07.22. Leg. J. Akopian, A. Ghukasyan, L. Martirosyan, A. Elbakyan. Det. J. Akopian, A. Ghukasyan.

Fertility, that is the ability of pollen to carry out complete fertilization was determined by staining with acetocarmine on temporary preparations under a light microscope at a magnification of 350 times (Pausheva 1980). The flower anthers of *S. salsula* were opened with a dissecting needle and tweezers, the pollen was removed and placed on a glass slide. For determine the pollen fertility was calculated 100 pollen grains in 5 repeats, totaling 500 pollen grains from each herbarium specimen. Pollen was taken from the ERE herbarium for different years to compare pollen fertility depending also on the year of collection. Statistical processing of experimental data was carried out according to Dospekhov (1973) and Wolf (1966). For income comparison, the arithmetic mean X=Sx was calculated. The size of pollen grains was also determined, because the morphological heterogeneity of pollen can be attributed to the failures in microsporogenesis, which can lead to unsuccessful seed formation.

Specimens examined: Armenia, Ararat region, in the vicinity vil. Ararat, salt marshes, 850 m a.s.l. 01.07.1997. Leg. *E. Gabrielyan, M. Hovhannisyan, K. Tamanyan, G. Faivush* ERE 147804; Armenia, Ararat province, in the vicinity vil. Armash, fish farm area, 830 m a.s.l. 06.09.2007. Leg. *G. Nikoghosyan* ERE 162900; Armenia. prov. Ararat, pr. Eraskh, 39°44’35’’N; 34°49’41’’E, 843 m. 28.06.2005, Cunneta de la carretera, *A. Quintanar &*

al., 1721 ERE 168083. Hortus Regius Matritensis (MA) Iter Armeniacum (VI.VII.2005) *Leguminosae-Papilionoideae*; Armenia, the shore of the reservoir near the Metsamor Museum. 11.07.2005. Leg. *Gabrielyan E.Ts., Barseghyan A.M., Aghababyan M., Gabrielyan I., Mkrtchyan A.* ERE 160162; Armenia. Ararat distr., near Ararat town, saline soil, 800 m a.s.l., N 39°49'581'' E 44°43'401'' 15.06.2011, in bloom. Leg. *E. Navasardyan, I. Gabrielyan* ERE 183096.

Eco-physiological studies of water regime, the intensity of transpiration and photosynthesis were conducted by Mezhunts, Navasardyan (2010) and Salnikov, Maslov (2014). We were the first to investigate the eco-physiological characteristics (water regime, water deficiency, transpiration, and photosynthesis intensity) of *S. salsula*. All the measurements were done in the period between 11:00 and 13:00. Each measurement was done with 3 repetitions and in 3 options (7-10 shoots were chosen for each sample). The presented data are the average results of the performed analysis which were subjected to statistical development. Water deficiency was determined based on the principle of water saturation of leaves. The water deficiency was calculated by the following formula:

$$WD = \frac{\text{the water content saturating the leaf (mg) - the initial water content (mg)}}{\text{the water content saturating the leaf (mg)}} \times 100$$

100 - is for expressing percentages

The fresh sample was promptly weighed and dried in a thermostat under 150 degrees of celsius for determining the total water content.

The total water content was calculated by the following formula:

$$X = \frac{P_1 - P_2}{P_1} \times 100$$

X - is the total water content, % from the wet weight, P_1 - is the wet weight of the leaf before drying, in grams, P_2 - is the dry weight of the leaf sample, in grams, 100 - is for expressing the total water content in the leaf from the wet weight in percentages.

The intensity of transpiration of the leaf was determined by rapid weighing with a torzon scale. Transpiration intensity was calculated by the following formula:

$$T = \frac{(B_1 - B_2) \times 60 \times 1000}{B_1 \times t} \text{ mg/grams from wet weight, hour}$$

T_1 - is the intensity of transpiration, mg/g from wet weight, hour, B_1 - is the initial weight of the leaf, mg, B_2 – is the leaf weight after 3 minutes, 60 minutes - to express in hours,1000 mg - to express the wet weight in 1 gram, T - is the period between the initial and the final weighing in minutes.

The intensity of photosynthesis was determined by the change in the dry weight of the leaf sample. The amount of dry material synthesized during photosynthesis is determined by the difference between the initial and the final measurements of the dry weight. The intensity of photosynthesis in mg/dm2, per hour, is expressed by dividing the amount of synthesized dry material by the time period between the measurements. The intensity of photosynthesis is measured by the following formula:

$$P = \frac{P_2 - P_1 \times 1 \times 100}{23.55} \text{ mg/dm}^2\text{, hour}$$

The content of photosynthetic pigments (chlorophylls a and b, carotenoids) was determined by a modified method based on the use of an organic solvent of dimethyl sulfoxide, which allows obtaining stable extracts necessary for performing extralaboratory studies (Mezhunts, Navasardyan 2010). The measurement was carried out on a spectrophotometer (SF-16), and the quantitative accounting of chlorophylls a, b and carotenoids was carried out according to the Mackini-Arnon and Wettstein formulas (Shlyk 1971); chlorophyll a=12.7E663-2.69E645; chlorophyll b = 22.9E645-4.68E663; sum of carotenoids = 4.695E440.5-0.268 (a + b), where E is the spectrophotometer reading.

Results and discussion

The studied samples of the species *S. salsula* were collected from the Yerevan floristic region. According to the literature data, only the diploid cytorace is characteristic for *S. salsula* with 2*n*=16 (Reveal & Spellenberg 1976; Nazarova 1997). The first report on chromosome count for the genus *Sphaerophysa* is believed for species *S. salsula*, that was introduced into the United States from central Asia about 50 years ago (Reveal & Spellenberg 1976).

Our material revealed a diploid cytorace for this species 2*n*=2x=16 (Fig. 1a), with basic chromosome number x=8. The karyotype of *S. salsula* is asymmetric, consisting of 5 pairs of submetacentric and 3 pairs of metacentric chromosomes (Fig. 1b). Karyotype formula is: 2*n*=16=10SM+6M.

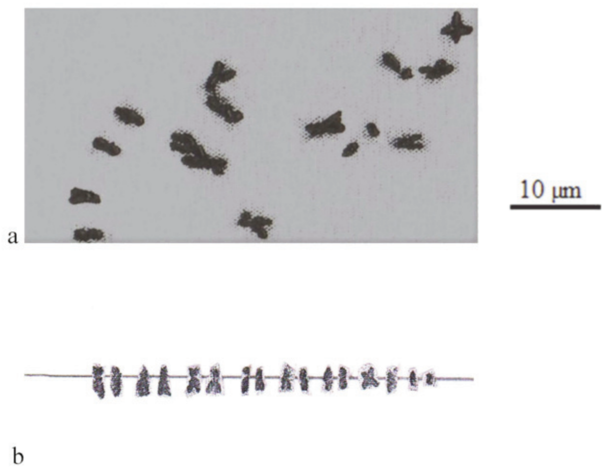

Fig. 2. a. Mitotic metaphase plate of *Sphaerophysa salsula*; b. Karyotype of *S. salsula*.

It is interesting to note that for species *S. kotschyana* from the vicinity of Tuz Lake (Turkey) a diploid cytorace with B-chromosomes was determined: 2*n*=14+0-2B, with basic chromosome number x=7 (Duran & al. 2010).

Fertility, that is, the ability of pollen to carry out complete fertilization, is a relatively constant value. The painted pollen grains were recorded as fertile and viable, and unpainted or very poorly colored pollen grains recorded as sterile or non-viable.

Pollen grain size measurements were carried out in order to identify morphological heterogeneity of pollen. If the size of pollen grains varied from 12.3 to 27.0 µm, the pollen fertility was high, almost unchanged, and ranged from 94.6 to 98.2, regardless of the year of collection from 1977 to 2011, which suggests that the difference in the pollen grains size will not affect the process of fertilization in the future (Tabl.1, Fig. 3).

Table 1.Pollen fertility of *Sphaerophysa salsula*.

***Sphaerophysa salsula*, ERE Nº**	**Pollen grain size, µm**	**Pollen fertility percentage**	
		Range	**Average fertility, %**
147804	15.2–26.3	95–99	97.4±0.7
168083	14.8–22.3	98–99	98.2±0.2
160162	15.2–27.0	95–100	98.0±0.8
162900	17.6–25.4	96–100	97.8±0.9
183096	12.3–22.1	94– 96	94.6±0.5

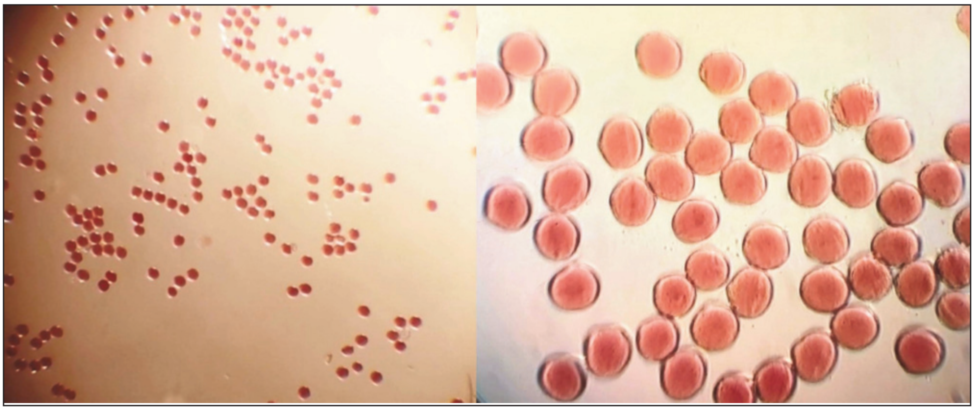

Fig. 3. *Sphaerophysa salsula* pollen fertility, ERE 147804.

To identify the adaptive features of *S. salsula* to the arid saline habitats of the Ararat valley, eco-physiological studies were carried out. It is obvious that the study of water regime is the most important at studying halophyte plants, since water is the environment where all the main biochemical reactions that determine the vital activity of plants take place. Most of the plants absorb highly mineralized water with difficulty because of the insufficient osmotic pressure in plant cells. Moreover, the degree of accumulation of salts by plants depends on both: the peculiarities of species and the amount of salts in the soil. Under the natural conditions of high salt content in soil and groundwater the water content in plant organs will be much lower than under conditions of normal moisture. The indicators of the water regime, the intensity of transpiration and photosynthesis of *S. salsula* are shown in Table 2.

Table 2. Indicators of the water regime, the intensity of transpiration and photosynthesis of *Sphaerophysa salsula.*

Indicators of water regime (raw weight)				**Transpiration and photosynthesis intensity**	
Total water %	**Free water, %**	**Bound water, %**	**Water deficit, %**	**Photosynthesis, mg/dm^2, per hour**	**Transpiration mg/wet weight, per hour**
52.33	23.71	28.62	16.85	2.15	200.0

In a complex change in the water regime caused by external factors, shifts in the intensity of transpiration are important, since it, together with the intensity of water inflow, determines the water balance of plants. Under conditions of soil salinity, the intensity of transpiration depends not only on the water content in the soil and the ability of plants to assimilate it by the root system, but also on the content of water-soluble salts in the leaves, which create an increased osmotic pressure, on the hydrophilicity of the plasma, etc. As for the change in the intensity of photosynthesis, it is obvious that the intensity of its physiological processes aimed at the fastest implementation of the reproductive cycle is determined (Rozentsvet & al. 2013).

The absorption and transformation of solar energy during photosynthesis is carried out by photosynthetic pigments of plants, in particular, chlorophyll "a" and "b" and carotenoids. To assess the state of the photosynthetic apparatus of *S. salsula*, the content of these pigments in them was studied, which is a very important internal factor in plant adaptation to unfavorable environmental conditions (Table 3).

The results of the study show that *S. salsula* plants are well adapted to conditions of Ararat valley habitats with a high content of mineral salts in soil and water, arid climate with high summer temperatures and water high evaporation from the soil surface, with changes in the groundwater level during the growing season. The content of pig-

Table 3. Chlorophyll and carotenoids content in *Sphaerophysa salsula.*

Chlorophyll “a”, mg/g	**Chlorophyll “b”, mg/g**	**Chlorophyll “a+b”, mg/g**	**Chlorophyll “a/b”, mg/g**	**Carotenoids, mg/g**
24.82±0.48	14.44±0.56	39.26	1.70	16.90±2.95

ments in *S. salsula* indicates the intensity of physiological processes associated with the life activity of this plant.

The results of our studies show that *S. salsula*, despite a different adaptation strategy, is well adapted to conditions with a high content of mineral salts in soil and water, an arid climate with high summer temperatures and high evaporation of water from the soil surface, with a change in groundwater levels during the growing season.

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