Bulgarian Journal of Agricultural Science, 28 (No 2) 2022, 305–313

Karyological and morphological variability of *Suaeda salsa* (L.) Pall. in Bulgaria

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Abstract

Grozeva, N. & Atanassova, S. (2022). Karyological and morphological variability of *Suaeda salsa* (L.) Pall. in Bulgaria. *Bulg. J. Agric. Sci., 28 (2)*, 305–313

The karyological and morphological intrapopulation and interpopulation variability of *Suaeda salsa* populations in Bulgaria have been studied in quest of reliable characteristics to distinguish the species from the remaining members of the genus. In all studied populations a tetraploid chromosome number $2n = 36$ and a karyotype of metacentric and submetacentric chromosomes have been registered with the metacentric ones being dominant. Vegetative traits are more variable than generative ones. More pronounced is the interpopulation morphological variability, which is probably due to the different environmental conditions, and in some of the populations it also correlates with the recorded karyotype differences. Two types of stomata have been registered: anomocytic and paracytic. Data about the pollen have been presented: pantoporate, spherical, with diameter from 21.1 to 25.81 μm and pore diameter from 1.91 to 2.46 μm. The karyotype has been described for the first time and data have been published about the type of stomata and pollen morphology of *S. salsa* from Bulgarian populations.

Keywords: Salsola salsa; karyology; morphology; Bulgaria

Introduction

The genus *Suaeda* Forssk. ex Scop. is one of the relatively big genera numbering 110 species, most of them cosmopolitan (Clemants & Mosyakin, 2003). Its representatives inhabit wet and saline sands and clay. The taxonomic composition of the genus has undergone certain changes in recent decades based on phylogenetic molecular studies (Schütze et al., 2003; Kapralov et al., 2006; Lomonosova & Freitag, 2009).

So far three species from genus *Suaeda* have been reported for Bulgaria: *S. altissima* Pall., *S. maritima* (L.) Dumort., and *S. heterophylla* Bunge (Delipavlov, 1966; Kozhuharov, 1992; Delipavlov & Česchmedjiev, 2003; Assyov & Petrova, 2012). *Sueda maritima* is a polymorphic taxon with a number of varieties and subspecies (Boucaud, 1962; Bassett & Crompton, 1978). Based on the latest expert evaluations of the Bulgarian flora, *Salicornia europaea* and other annual halophyte communities, incl. the species from the genus *Suaeda* – are threatened by extinction in Bulgaria, and are therefore included in the Red Book of the Republic of Bulgaria, Vol. 3 (Tzonev & Gussev, 2011) in the category Endangered habitat (EN). Regardless of the strict environmental measures, the state of annual halophyte communities in the country, incl. these with *Suaeda* species changes quite dynamically, following the environmental changes. Undertaking adequate measures about their conservation requires studying them comprehensively.

The object of the present study is the most widely distributed species of genus *Suaeda* along the Black sea coast,

which was established in the references in Bulgarian herbaria (SOM, SO, SOA) and the materials gathered for the study, has the typical morphological features of *Suaeda salsa* (L.) Pall. (= *Suaeda maritima* var. *salsa* (L.) Moq.) as stated by Freitag & Lomonosova (2006). The objective of the study is to examine the karyological and morphological population variability of *S. salsa,* to seek relationships and interdependences between the degree of variability and the environmental conditions and to present a detailed morphological description of the species.

Materials and Methods

The karyological and morphological study includes seven populations of *S. salsa* from the Southern Black Sea Coast (Table 1).

The chromosome number has been determined from durable squash specimens of metaphase plates following the methodology of Grozeva (2007). Squash specimens have been obtained from root tips of seeds collected from the natural habitats of the species and germinated under laboratory conditions. From each population at least five metaphase plates have been measured. The chromosome type has been determined by the centromeric index I = s/s +l following the Methodology by Grif & Agapova (1986), where s is the chromosomal short arm, l – the long arm. Intrachromosomal and interchromosomal karyotype asymmetries were estimated with the coefficient of variation of chromosome length (CVcl) according to Paszko (2006) and the determination of the mean centromeric asymmetry (Mca) proposed by Peruzzi & Eroğlu (2013). The herbarium specimens of the karyologically studied plants have been deposited at the herbarium of the Bulgarian Academy of Sciences (SOM). The performed morphological analysis took into account 20 quantitative characters measured in 30 specimens of each population: 1. Stem height (mm); 2. Basal leaf length (mm); 3. Basal leaf width (mm); 4. Basal leaf length/width ratio; 5. Upper leaf lamina length (mm); 6. Upper leaf lamina width (mm); 7. Upper leaf lamina length/width ratio; 8. Flower petiole length (mm); 9. Length of perianth lobes in bisexual flower; 10. Width of perianth lobes in bisexual flower; 11. Diameter of flower; 12. Length of inflorescence; 13. Seed length (mm); 14. Seed width (mm); 15. Seed length/width ratio; 16. Thickness of seed; 17. Length of fruit; 18. Width of fruit; 19. Length/width ratio; 20. Thickness of fruit. Plant height was measured on site, the other traits – on collected herbarium speciemens. To compare variability of the various traits in the population, the variation coefficient has been used. To assess the coefficient value, the scale of Mamaev (1975) has been adopted.

In all populations included in the morphological analysis, the following qualitative features have been recorded: 1. Shape and colour of stem; 2. Shape and colour of leaf lamina; 3. Type of inflorescence; 4. Colour of perianth; 5. Degree of perianth concrescence; 6. Presence of keeled perianth lobes; 7. Shape and colour of seed; 8. Colour of pericarp; 9. Indumentum. No significant interpopulation variation has been registered for these traits within the species range.

The results from the morphometric measurements have been processed statistically with the Statistica 10 software for Windows, Statsoft.

To determine the relative share of the intrapopulation and interpopulation variability, for each quantitative trait of the studied species, One-way dispersion analysis (One-Way ANOVA) has been used.

To assess the interpopulation variability for the entire set of traits Cluster analysis has been used. The Squared Mahalanobis Distance values have been used calculated by comparing each trait between the pairs of populations and not by comparing their mean values and these is quite indicative of an existing degree of similarity.

The Scanning electron-microscope method has been used for a more detailed study of the morphology of the studied populations. The examined plant parts were dried and observed directly without any preliminary physical or chemical treatment. The electron-microscope studies were conducted at the SEM analysis laboratory at the Faculty of Chemistry and Pharmacy at Sofia University "St. Kliment Ohridski".

Table 1. Studied populations of Suaeda salsa

Location, population No	2*n*	GPS coordinates, altitude (m)
1. Black Sea Coast (South), the Salt Museum in Pomorie, No. 504	36	42º 35′ N, 27º 37′ E, 10
2. Black Sea Coast (South), town of Burgas, Atanasovsko Lake along the road Burgas-Pomorie, No. 505	36	42º 39′ N, 27º 28′ E, 30
3. Black Sea coast (South), town of Pomorie, from the Pomorie Saltworks, No. 506	36	42°35′29.67″N, 27°36′57.93″E, 0
4. Black Sea coast (South), town of Burgas, Burgas Saltworks-north, No. 507	36	42°32′15.18″N, 27°29′17.77″E, 0
5. Black Sea coast (South), town of Aheloy, the saltworks near Aheloy, No. 508	36	42°37′24.63″N, 27°37′53.58″E, 0
6. Black Sea coast (South), town of Nessebar, the Old town, near to the sea, No. 513	36	42° 39′ N, 27° 44′ E, 0
7. Black Sea coast (South), town of Burgas, Burgas Saltworks-south, No. 514	36	42°31′14.37″N, 27°29′5.25″E, 0

Results and Discussion

Karyology

Tetraploid chromosome number $2n = 4x = 36$ has been found in the seven studied populations of *S. salsa* (Table 1). A total of 18 pairs of homologous chromosomes have been registered (Figures 1 and 2; Table 2). Our results confirm the numbers known for Bulgaria about populations of the species from the Black sea coast (Grozeva, 2010; 2015). Tetraploid chromosome number for *S. salsa* has been mentioned in populations from: Russia (Lomonosova et al., 2001; 2005; Lomonossova & Krasnikov, 2006; Lomonosova & Lysenko, 2010; Lomonosova & Shaulo, 2010; Lomonosova, 2012), Kazakhstan (Lomonosova et al., 2003; 2005; 2019), and the Ukraine (Lomonosova & Freitag, 2009).

Hekmat-Soar & Manafi (1982) mention for populations of the species from Iran a diploid chromosome number $2n = 18$. These data, according to Lomonosova (2011), need confirmation and most probably relate to another taxon, since all studies show that in its entire area *S. salsa* is a tetraploid with chromosome number $2n = 36$.

In 6 of the studied populations the karyotype consists of 14 pairs of metacentric and 4 pairs of submetacentric chromosomes, $2n = 28m + 8sm$, while the karyotype of the population No. 513 from the town of Nessebar shows higher symmetry and the number of metacentric chromosomes is with one pair more, $2n = 30m + 6sm$ (Table 2). The mean chromosome length varies slightly, from 1.80 to 1.94 μm. The longest chromosome is 3.10 μm, while the shortest one is 1.16 μm (Figure 1).

The total length of the haploid chromosome set is within the range from 32.34 to 34.90 μm (Table 2). The ratio between the longest and the shortest chromosome in the population No. 504 from the town of Pomorie, Salt Museum is 2.5:1, with difference in their size 1.86 μm. The ratio between the longest and the shortest chromosome in the population No. 506, from Pomorie Saltworks is 2.3:1, with difference in the size 1.66 μm, and in No. 513 from town of Nessebar it is 1.76:1, with difference in their size 1.09 μm. In the population No. 507 from Burgas Saltworks-north the ratio between the longest and the shortest chromosome is 1.51:1, with difference in the size 1.13 μm, while in population No. 514 from Burgas Saltworks-south, the one from the town of Nessebar the ratio between the longest and the shortest chromosome is 1.92:1 with difference in their size 1.07 μm. The ratio between the longest and the shortest chromosome in the population No. 508 from the saltworks near Aheloy is 1.88:1, with difference in the size 1.17 μm, while in the No. 505 from Atanasovsko Lake along the road Burgas-Pomorie it is 2.33:1, with difference in their size 1.69 μm (Figure 2).

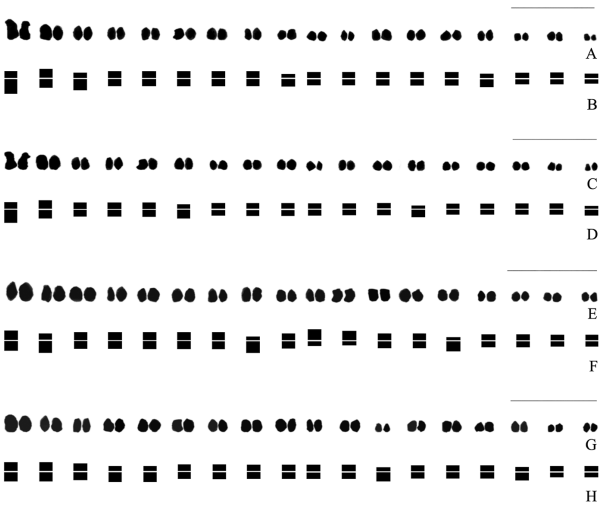

Fig. 1. *Suaeda salsa*: A-B karyogram and idiogram of the population No. 504 from the Salt Museum in Pomorie; C-D karyogram and idiogram of the population No. 506 from the Pomorie Saltworks; E-F karyogram and idiogram of the population No. 505 from Atanasovsko Lake along the road Burgas-Pomorie; G-H karyogram and idiogram of the population No. 507 from Burgas Saltworks-north

Table 2. Karyomorphometric data for the studied *Suaeda salsa* populations, chromosome size variation (μm) – short (S) and long (L), total haploid chromosome length (hcl, μm), interchromosomal asymmetry indices (A2); coefficient variation of chromosome length (CVcl), degree of asymmetry of karyotype (A), mean centromeric asymmetry (Mca)

Population	Karyotype formula	S	L	hcl	A2	CVcl	A	Mca
No. 504	$2n = 28m + 8sm$	1.24	3.10	34.90	0.04	4.23	0.01	1.32
No. 505	$2n = 28m + 8sm$	1.27	2.96	33.16	0.04	4.34	0.01	1.17
No. 506	$2n = 28m + 8sm$	1.31	2.97	33.35	0.04	4.12	0.01	1.27
No. 507	$2n = 28m + 8sm$	1.40	2.53	34.23	0.04	4.38	0.01	1.16
No. 508	$2n = 28m + 8sm$	1.33	2.50	32.49	0.04	4.46	0.01	1.43
No. 513	$2n = 30m + 6sm$	1.44	2.53	34.52	0.04	4.45	0.01	1.05
No. 514	$2n = 28m + 8sm$	1.16	2.23	32.34	0.04	4.21	0.01	1.18

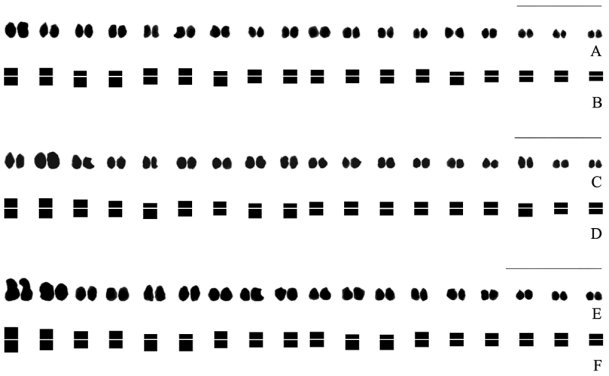

Fig. 2. *Suaeda salsa*: A-B karyogram and idiogram of the population No. 514 from Burgas Saltworks-south; C-D karyogram and idiogram of the population No. 508 from the saltworks near town of Aheloy; E-F karyogram and idiogram of the population No. 513 from town of Nessebar

Analysis of the interchromosomal and intrachromosomal karyotype asymmetry indices showed values from of 4.12 to 4.46 for CVcl and from 1.05 to 1.43 for Mca (Table 2).

Morphology

In all seven studied populations vegetative traits have higher degree of variability than the generative ones (Table 3).

The most variable in all populations are the following traits: length of inflorescence (V=24.51-47.33%); stem height (V=17.84-41.11%); lower leaf lamina length (V=12.68-21.92%) and lower leaf lamina length/width ratio (V=12.68-20.46%) (Table 3).

The most conservative are the following traits: seed length (V=4.87-8.11%); fruit length/width ratio (V=4.36-7.16%).

The lowest variation coefficients for the fruit length and width have been found in population No. 513 from the saltworks near Aheloy. In population No. 505 from Atanasovsko Lake and population No. 507 from Burgas Saltworks-South

Table 3. Mean (first line) and coefficient of variation in percentage (second line) of *Suaeda salsa* populations for each of the 20 observed characters, percentage of the interpopulation variation in the overall morphological variation for each character SSb (%)

Population No Character No	504	505	506	507	508	513	514	SSb	
1	X	318.93	146.63	151.52	244.71	239.17	300.17	111.77	82.18
	VC	25.04	18.45	22.26	17.84	20.51	30.69	41.1	
2	X	13.54	13.88	12.69	16.13	20.57	12.07	10.78	65.83
	VC	21.92	21.92	18.55	15.36	12.68	12.86	20.22	
3	X	0.97	1	0.97	1	1	0.75	1	73.12
	VC	4.82	0	4.67	0	2.04	12.01	3.44	
4	X	13.92	11.78	13.08	16.13	20.58	16.37	10.73	64.22
	VC	20.33	13.42	20.18	14.34	12.68	20.46	20.13	
5	X	2.48	2.09	3.54	2.85	3.2	3.34	2.38	64.79
	VC	10.52	9.08	14.64	15.38	13.36	14.39	15.27	
6	X	0.54	0.74	0.75	0.93	0.91	0.51	0.85	73.52
	VC	9.22	6.74	6.78	8.63	6.01	11.2	5.98	
7	X	4.62	3.48	4.74	3.07	3.51	3.81	3.81	68.45
	VC	9.31	10.48	16.67	19.21	11.12	11.81	12.81	
8	X	0	0	0	0	0	0	0	
	VC	0	0	0	0	0	0	0	
9	X	1.06	0.92	0.98	0.83	0.81	1.31	0.77	64.34
	VC	9.78	12.57	11.03	13.12	11.71	4.18	9.56	
10	X	0.49	0.59	0.49	0.58	0.59	0.66	0.58	71.12
	VC	14.53	5.17	14.46	9.09	8.65	13.42	7.46	
11	X	1.01	0.94	1.08	0.9	0.88	1.29	0.98	62.48
	VC	3.98	16.71	10.26	12.49	12.61	5.52	8.91	
12	X	159.6	134.8	115.27	150.07	84.8	181.83	87.43	87.65
	VC	35.51	25.09	24.51	27.43	26.51	37.82	47.33	
13	X	1.08	1.1	1.13	0.98	1.26	1.17	1.06	58.47

Table 3.Continued

	VC	6.45	6.75	8.11	8.39	4.87	7.15	6.36	
14	X	0.97	1.02	0.86	0.86	1.03	1.01	1	55.78
	VC	7.12	6.17	13.16	13.76	5.2	8.06	8.06	
15	X	1.1	1.1	1.27	1.13	1.23	1.17	1.06	57.22
	VC	5.41	12.72	10.61	7.08	5.72	10.37	10.38	
16	X	0.32	0.31	0.31	0.29	0.28	0.37	0.4	60.47
	VC	17.21	4.3	9.74	16.57	14.53	12.5	4.02	
17	X	1.26	1.25	1.24	1.39	1.54	1.54	1.22	62.33
	VC	7.07	11.26	14.09	11.15	6.31	2.92	6.23	
18	X	1.1	1.15	1.04	1.23	1.36	1.28	1.12	58.14
	VC	5.12	10.72	9.46	10.21	5.93	3.01	8.26	
19	X	1.14	1.11	1.24	1.13	1.14	1.21	1.09	55.62
	VC	4.36	6.41	5.34	5.33	7.01	5.79	7.16	
20	X	0.45	0.46	0.43	0.42	0.41	0.46	0.42	57.43
	VC	11.13	10.53	12.07	11.54	8.36	10.58	10.16	

no variability has been registered for the trait lower stem leaf length/width ratio (Table 3).

The interpopulation variability is more pronounced in the studied populations. This is mainly due to differences in the environmental conditions among populations, and is probably influenced by the registered differences in karyotype. The intrapopulation variability is generally less pronounced, which is in line with the smaller number and area of the population and the homogeneous environmental conditions within it.

When comparing the *S. salsa* populations along the entire morphological set of traits, as can be seen from the dendrogram, a certain grouping can be established (Figure 3). Six of the studied populations, for which a karyotype of 14 pairs of metacentric and 4 pairs of submetacentric chromosomes has been established, are grouped and form two clusters. The population No. 513 from town of Nessebar, which has 1 submetacentric chromosome pair less, has larger differences compared to the populations from the two clusters.

The first cluster includes the two populations from the town of Pomorie: No. 504 from the Salt Museum and No. 506 from the Pomorie Saltworks. The registered similarity is in accordance with the geographical proximity and the similar environmental conditions.

The second cluster comprises 4 populations: the two populations from Burgas Saltowrks (No. 507 and No. 514), the population from Atanasovsko Lake along the road Burgas-Pomorie (No. 505), and the population from the saltworks near town of Aheloy (No. 508). The registered greater similarity between the populations from Burgas Saltworks-south (No. 514) and Burgas Saltworks-north (No. 507) compared to the other two populations in the cluster is in accordance

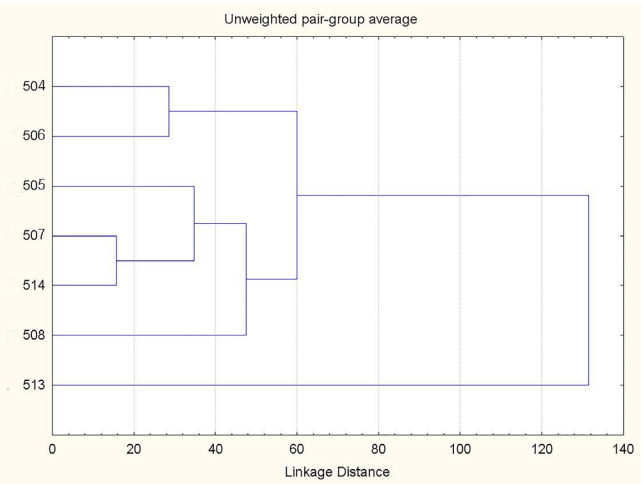

Fig. 3. Dendrogram of the cluster analysis of the studied populations of *Suaeda salsa*

with their geographical proximity and similar environmental conditions on their territory.

The observed differences between population No. 513 from the town of Nessebar and the other six populations are probably influenced by the registered differences in the karyotype of that population. For the population from Atanasovsko Lake a karyotype of 15 pairs of metacentric and 3 pairs of submetacentric chromosomes has been found, $2n$ = 30m +6sm, while in the other populations the submetacentric pairs of chromosomes are one more – $2n$ = 28m +8sm. The specimens from the population from town of Nessebar, compared to the specimens from other populations, have narrower lower and upper stem leaves, and longer perianth

Table 4. Values of the distances of Mahalanobis of the studied populations of *Suaeda salsa*

Population	No. 504	No. 505	No. 506	No. 507	No. 508	No. 513
No. 504						
No. 505	65.8466					
No. 506	28.5968	52.8435				
No. 507	78.5203	44.8622	38.1629			
No. 508	96.0092	46.9722	62.6492	53.9178		
No. 513	63.5822	147.628	106.562	166.889	161.651	
No. 514	57.768	24.5749	28.052	15.7626	41.6816	142.459

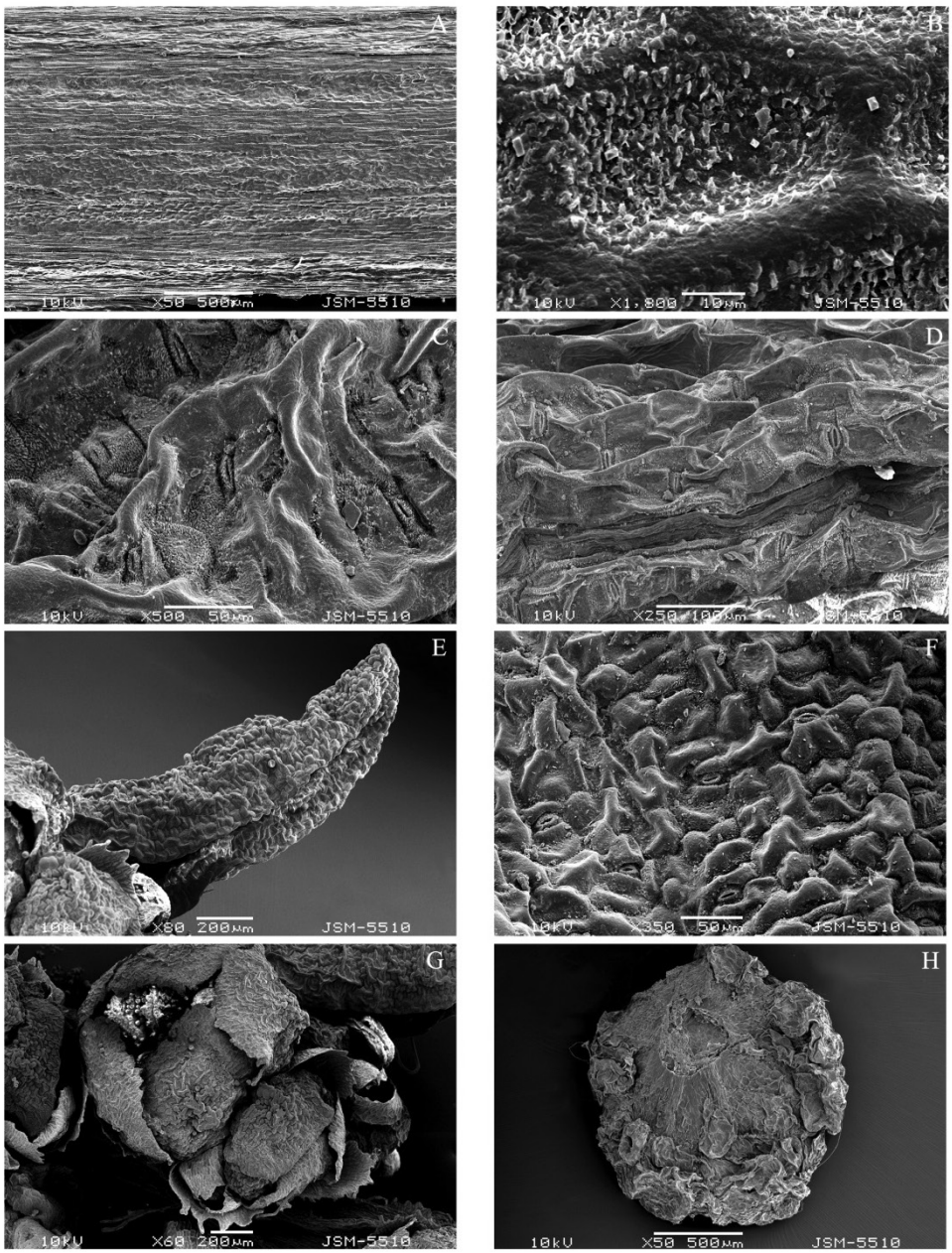

Fig. 4. Scanning electron micrographs of *Suaeda salsa*: A) stem surface; B) crystallized salts; C) adaxial (upper) leaf surface; D) abaxial (lower) leaf surface; E-F sepals surface of both sides; G) Flower cluster; H) Flower

leaflets (Table 3). The other traits of this population included in the morphological analysis are similar to traits from different populations. Squared Mahalanobis Distances given in Table 4 confirm the similarity between the populations of the species outlined in the Cluster analysis.

The data from the electron microscopic examinations showed that in all studied specimens from the seven *S. salsa* populations the stems, leaf lamina and sepals were not hairy (Figure 4A, C-F).

The stomata are located on both sides of the leaf lamina and sepals and are of anomocytic and paracytic type, with the anomocytic type predominating (Figure 4C, D, F). Perveen et al. (2007) reported anomocytic stomata in the species of *Suaeda*. Farooqui et al. (2009) reported for the studied *Suaeda* species paracytic stomata type.

Crystallized salts are often observed on the surface of the stem, leaf lamina and sepals (Figure 4B, C).

The flowers are sessile, gathered of 5-7(9) per cluster, very rarely they are single (Figure 4G, H). Freitag & Lomonosova (2006) reported as typical for the species 5-9(11) flowers per cluster. The perianth segments are 5, fused at the base, with a blunt tip and a slightly scalloped membranous edge (Figure 4G, H; Figure 5A).

The pollen is of spherical pantoporate type, without perforations on the tectum, with its diameter ranging from 21.1 to 22.94 μm, and that of the pores – from 1.91 to 2.46 μm (Figure 5B). Pollen morphology of species from genus *Suaeda* has been studied by various researchers (Pinar & Inceoglu, 1997; Pinar, 1999; Akhani et al., 2003; Dehghani & Akhani, 2009) and the main characteristics used in the comparative analyses were pollen and pore diameter. Our data about pollen morphology are similar to those by Dehghani & Akhani (2009), who reported for *S. salsa* pollen diameter of 22.56 ± 0.82 μm and pore diameter of 3.90 ± 0.19.

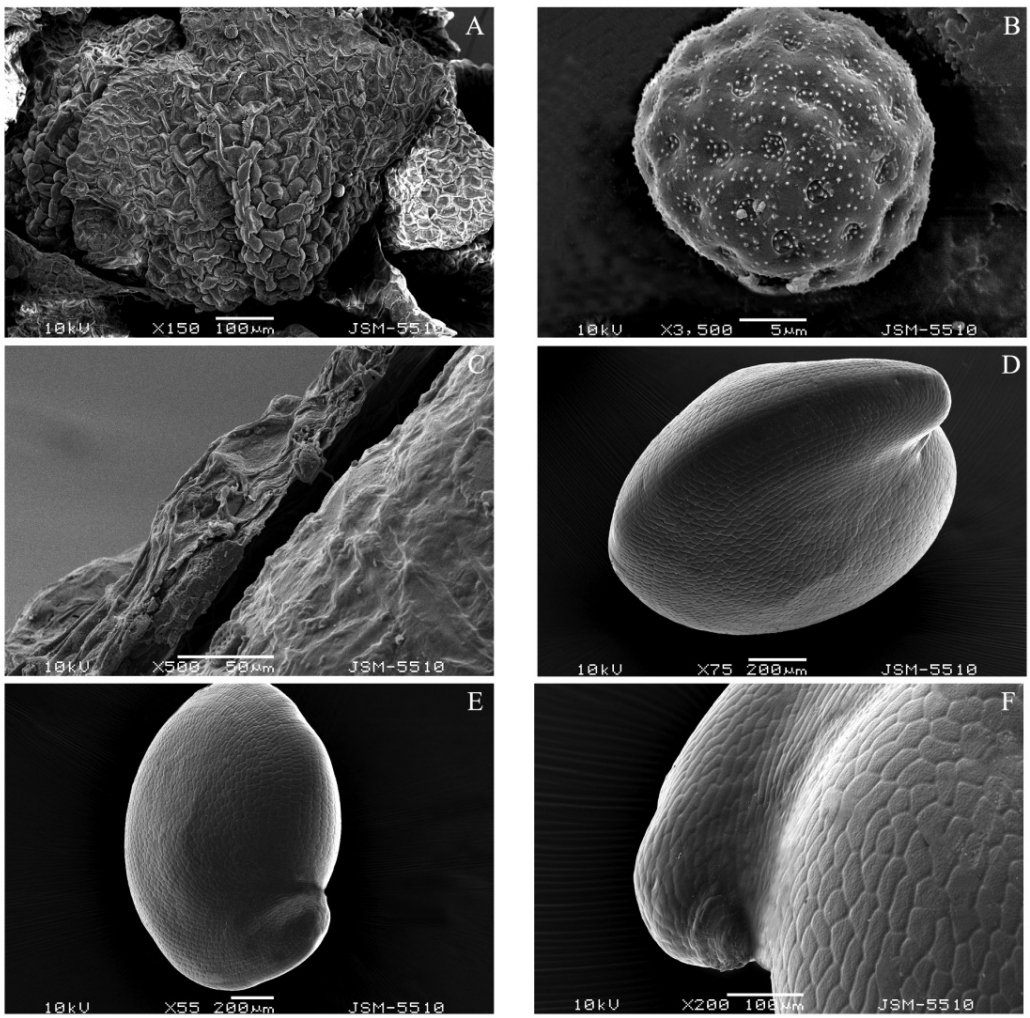

Fig. 5. Scanning electron micrographs of *Suaeda salsa*: A) perianth segments; B) pollen grain; C) pericarp; D-E seed surface from both sides; F) seed surface

The pericarp is translucent, colourless to light brown, layered and (6.67) 12 (16.67) µm thick (Figure 5C). The seeds are lenticular, with a blunt edge, a convex germinal root and a characteristic clear sculpture on the surface in the form of well-defined polygonal cells (Figure 5D-F). The characteristic quantitative and qualitative characteristics of the seeds are in full accordance with those indicated by Freitag & Lomonosova (2006) for *S. salsa*.

Morphological description

Annual herbaceous plant, not hairy. The stem (11.1)15.1 – 30(55.5) cm tall, erect, green at the beginning of vegetation, later usually light brown, branched at the top, slightly woody at base. Leaves are consecutive, sessile, fleshy, linear or narrowly elliptical, semi-cylindrical, entire, green, grey-green, yellow-green, often reddish.

The leaf lamina of the basal and middle stem leaves is (10)10.78 – 20.57(24) x (0.7)0.75 – 0.97(1.2) mm. The basal and middle stem leaf length/width ratio is within the range of (10)10.73 – 20.58(21) mm. The upper lead lamina is (6.5)8.24 – 19.37(20) x (0.5)0.61 – 0.77(1.1) mm. The upper leaf length/width ratio is within the range of (2.5)3.07 – 4.74(5). Along the abaxial and adaxial side of the leaf lamina two types of stomata have been registered anomocytic and paracytic, the first one being the dominant type. Very often crystallized salts are seen on the stem and the leaf lamina surface.

Flowers bisexual, sessile, 5-7(9) arranged in hemispherical sessile clusters, very rarely single, forming paniculate inflorescences at the tips of the stem and its branches. Flowers with a diameter of (0.9)1.1 – 1.29(1.40) mm. Perianth segments 5, with dimensions (0.77)0.81 – 1.31(1.35) x (0.4)0.49 – 0.58(0.70) mm, green to reddish, elliptical, membranous at the edge, tightly enclosing the fruit, with a blunt tip, entire edge and narrow dorsal edge as one part of the flowers of each perianth leaf or some of them have characteristic wing-like colourless growths running along the entire length of the perianth leaf as a dorsal keel, and on another part of them the perianth petals are without a wing-like dorsal keel. Anthers are 5. The pollen is spherical, pantoporate type, without perforations of the tectum, with a diameter from 21.1 to 22.94µm.

The pericarp is translucent, colourless to light brown, layered and (6.67) 12 (16.67) µm thick. The seeds are lenticular, black with dimensions (1.04) 1.24 – 1.28 (1.36) x (1.09) 1.16 – 1.19 (1.24) mm, with blunt edge, convex germinal root and with a characteristic sculpture on the surface in the form of well-defined polygonal cells, the interior of which has a very well-defined reticulated sculpture.

Conclusion

For the Bulgarian populations of *S. salsa*, a tetraploid chromosome number $2n = 4x = 36$ with the prevailance of metacentric has been established. The pollen is pantoporate, spherical, with a diameter from 21.1 to 22.94 µm. Two types of stomata, anomocytic and paracytic, have been registered. The interpopulation variability is more pronounced compared to the intrapopulation variation. A relationship has been established between the degree of morphological variability and the observed differences in environmental conditions and karyotype. The karyotype, pollen morphology and stomata type have been described for the first time in Bulgarian *S. salsa* populations.

The data from the present study can be used both for the correct identification of *S. salsa* and for getting familiar with the species characteristics.

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Received: January 4, 2022; *Accepted:* February 11, 2022; *Published:* April, 2022