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CHROMOSOME AND POLLEN MORPHOLOGY OF *SALSOLA SODA* L. AND *SALSOLA TRAGUS* L. IN BULGARIA

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Abstract

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Chromosome and pollen morphology of *Salsola soda* L. and *Salsola tragus* L. in Bulgarian populations were studied. A diploid chromosome number and karyotypes of $2n = 18$ m, $2n = 14$ m + 4 sm were established for *Salsola soda* populations. The metaphase chromosome length was ranging from 1.21 to 2.64 μm and the total haploid chromosome length was from 14.76 to 15.97 μm. A tetraploid chromosome number and karyotypes of $2n = 34$ m + 2 sm, $2n = 32$ m + 4 sm were established for *Salsola tragus* populations. The metaphase chromosome length was ranging from 1.03 to 3.06 μm and the total haploid chromosome length was from 30.44 to 34.53 μm. The scanning electron microscope investigations revealed pantoporate, spheroidal, with spinulose tectum pollens in the two studied species. The pollen diameter of *S. soda* varied from 18.3 to 27.12 μm and of *S. tragus* – from 28.3 to 33.2 μm. Between the two species were found differences also in the number, diameter and area of pores.

Key words: Salsola soda; *Salsola tragus*; chromosome number; karyology; pollen morphology; idiograms; Bulgaria

Introduction

Genus *Salsola* L. comprises of about 130 species, with wide distribution in the Mediterranean area, Europe, Asia, South Africa, Australia and North America (Wilson, 1984; Pyankov et al., 2001; Mosyakin, 2003). Most of its representatives are ruderal and weed species. The others are halophytes with habitats in coastal and inland saline territories.

In Bulgarian flora the genus is represented by two species – *Salsola tragus* L. (= *S. ruthenica*) and *Salsola soda* L. (Markova, 1966; Assyov and Petrova 2012). The first of the species is wider spread in the country – along the coastal sands, in ruderalized places, along roads, as a weed in the fileds. *S. soda* has more limited spread on saline soils along the Black Sea coast and around the Straldzha marsh, Yambol region.

Until now the Bulgarian populations of both species have not been an object of a specific study. There were some horological data about *S. soda* and *S. tragus* in some publications about halophyte plants in Bulgaria and the flora and vegetation in the Burgas wetlands (Ganchev et al., 1971; Filipova-Marinova et al., 2002; Ivanov et al., 2002; Grozeva et al., 2004; Grozeva, 2005; Tzonev et al., 2005). According to data by Grozeva (2012), *S. tragus* was among the accompanying species in the communities of the new invasive species for Bulgarian flora – *Chenopodium pratericola* Aellen. Markova (1966) mentioned *S. tragus* as a weed in cultivated crops, but data about its spread in the country as a weed and which crops it most commonly weeds have not been published. In our previous studies data have been published about the chromosome number of both species – diploid $2n = 18$ for a population of *S. tragus* on the Southern Black Sea coast, town of Ahtopol (Grozeva, 2013) and tetraploid $2n = 36$ for a population of *S. soda* on Southern Black Sea Coast, town of Ahtopol (Grozeva, 2015). The objective of the present study was to investigate the karyotype morphology and analyse the

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pollen morphology of *S. tragus* and *S. soda* in their Bulgarian populations.

Materials and Methods

A total of 3 populations of *S. soda* and 5 populations of *S. tragus* from the Black Sea coast were studied (Table 1).

Chromosome numbers and karyotypes were reported on lasting preparations of metaphase root apex plates of seeds germinated in laboratory conditions, collected in the natural habitats of the species. The root tips were treated and squashed according to the accepted methods (Grozeva, 2007). The chromosomal type was determined after the centromere index I = s/s + l, according to the classification proposed by Grif and Agapova (1986). The karyograms and idiograms were obtained with the help of the Adobe Photoshop CS6.0 program. Three metaphase plates were measured from each population. The voucher specimens are kept in the herbarium of the Bulgarian Academy of Sciences (SOM).

The interchromosomal asymmetry was calculated using index A_2 (Zarco, 1986). The following indices were used to determine the intrachromosomal asymmetry: the total form percentage – TF% (Huziwara, 1962); the karyotype asymmetry index percentage – Ask% (Arano, 1963); the symmetric index – Syi (Greilhuber and Speta, 1976); the intrachromosomal asymmetry index A_1 (Zarco, 1986); asymmetry index

Table 1
Studied populations from genus *Salsola* L.

Population location	Population №	2*n*
***Salsola soda* L.**		
Southern Black Sea Coast, Pomorie town, on the sands of the beach, at 1 m, 42°35.036'N, 27°37.92'E	597	18
Southern Black Sea Coast, Burgas town, on the sands of the beach, at 5 m, 42°29.409'N, 27°28.87'E	598	18
Southern Black Sea Coast, Chernomorets town, on the sands of the beach, at 5 m, 42°26.891'N, 27°38.40'E	599	18
***Salsola tragus* L.**		
Southern Black Sea Coast, Pomorie town, on the sands of the beach, at 2 m, 42°35.224'N, 27°37.93'E	582	36
Southern Black Sea Coast, Burgas town, on the sands of the beach, at 6 m, 42°29.406'N, 27°28.89'E	583	36
Southern Black Sea Coast, Chernomorets town, on the sands of the beach, at 5 m, 42°26.917'N, 27°38.45'E	584	36
Northern Black Sea Coast, Shkorpilovtsi village, on the sands of the beach, at 3 m, 42°58.43'N, 27°53.38'E	585	36
Northern Black Sea Coast, Irakli area, on the sands of the beach, at 5 m, 42°44.46'N, 27°53.28'E	586	36

Table 2
Intrachromosomal asymmetry indexes (Stebbins, 1971)

Ratio: largest/smallest chromosomes	Proportion of chromosomes with arm ratio > 2:1			
	0.0	0.01 – 0.5	0.51 – 0.99	1.0
< 2 : 1	1 A	1 B	1 C	1 D
2:1 – 4:1	2 A	2 B	2 C	2 D
> 4:1	3 A	3 B	3 C	3 D

A (Watanabe et al., 1999); the four categories of Stebbins (1971) – SKS: from A to D according to the proportion of acrocentric and/or telocentric chromosomes in a karyotype. The four categories have subtypes 1 to 3 according to the ratio between the largest/smallest chromosomes (Table 2).

Specifics of pollen morphology were investigated using the Scanning Electron Microscope (SEM) method. The research was performed on herbarized plant parts in the laboratory of the Faculty of Chemistry and Pharmacy at Sofia University „St. Kliment Ohridski" – Sofia. At least ten pollen grains from each population were studied. Dried plant parts were mounted on a metallic stub, coated with gold in ion sputtering chamber and examined under JEOL scanning electron microscope (JSM5510). Pollen terminology used is in accordance with Erdthman (1952), Kremp (1965), Walker and Doyle (1975). The following pollen characteristics became object of study: 1) Pollen diameter D_1 (maximum diameter, μm) – D_1; 2) Polar axis D_2 (diameter perpendicular to D_1, μm) – D_2; 3) Chord distance between three neighboring pores, forming a triangle with sides as equal as possible near the highest focus of the grain (μm) – C; 4) C/D_1 ratio; 5) Total number of pores – TNP; 6) Pore diameter (μm) – PD; 7. Pore area (μm^2) – PA; 8) Number of spinules of 100 μm^2 – NS/100 μm^2; 9) Number of spinules in pore – NSP. In determining the above indicators and defining the number of pores the method proposed by Toderich (2008) was used.

Statistical analysis was performed with Statistical 10 for Windows, StatSoft.

Results and discussion

Karyology

As a result of the karyological study, for the three studied populations of *S. soda* diploid chromosome number 2*n* = 2x = 18 was established, while for the five studied populations of *S. tragus* the chromosome number was tetraploid 2*n* = 4x = 36 (Table 1). Karyomorphometric data about the studied populations is shown in Table 3. Idiograms of the studied populations are presented in Figures 1 and 2. Two types of chromosomes: metacentric and submetacentric have been established in the karyotypes. According to Runemark (1996), Snogerup (1995), Goldblatt and Johnson (2000) the basic chromosome number in Chenopodiaceae family is x = 9 and most species have chromosome number 2*n* = 18. The chromosome number 2*n* = 18 found for the Bulgarian populations of *S. soda* is in accordance with most reports (Wulff, 1937; Polya, 1948; Zosimovič, 1965; Labadie, 1976; Scrugli and Bocchieri, 1977; Tarnavschi and Lungeanu, 1982; Zakharyeva, 1985; Lomonosova, 2013; Goldblatt and Johnson, 2014; Michalková and Letz, 2014). Additionally, Queiros (1975) reported for the same species 2*n* = 36. Among the three studied populations of *S. soda* some differences in the morphology and in the size of chromosomes have been registered (Figure 1, Table 3). For the one from Pomorie a karyotype of 7 pairs of metacentric and 2 pairs of submetacentric chromosomes was found (Figure 1, A), while in the other two studied population from Burgas and Chernomorets a karyotype of 9 pairs

Table 3
Karyomorphometric data for the representatives of the genus *Salsola* L. in Bulgaria

Population №	Karyotype formula	S	L	hcl	Inter index	Intrachromosomal index					
					A_2	SKS	TF %	Ask%	Syi	A_1	A
S. soda											
597	2n = 14m + 4sm	1.21	2.14	14.76	0.07	1A	44.51	55.49	44.51	0.19	0.01
598	2n = 18m	1.30	2.54	15.78	0.06	1A	47.34	52.66	47.34	0.10	0.01
599	2n = 18m	1.29	2.34	15.97	0.06	1A	44.96	55.04	44.96	0.19	0.01
S. tragus											
582	2n = 34m + 2sm	1.21	3.18	34.53	0.05	1A	45.47	54.53	45.47	0.17	0.01
583	2n = 32m + 4sm	1.03	2.93	30.44	0.06	1A	45.73	54.27	45.73	0.16	0.00
584	2n = 34m + 2sm	1.08	2.95	31.72	0.05	1A	45.68	54.32	45.68	0.15	0.00
585	2n = 34m + 2sm	1.17	3.06	32.45	0.05	1A	44.59	55.41	44.59	0.17	0.01
586	2n = 34m + 2sm	1.32	2.95	34.12	0.05	1A	45.55	54.45	45.55	0.14	0.00

Chromosome size variation (μm) – short (S) and long (L); total sum of the haploid chromosome lenght (hcl, μm)

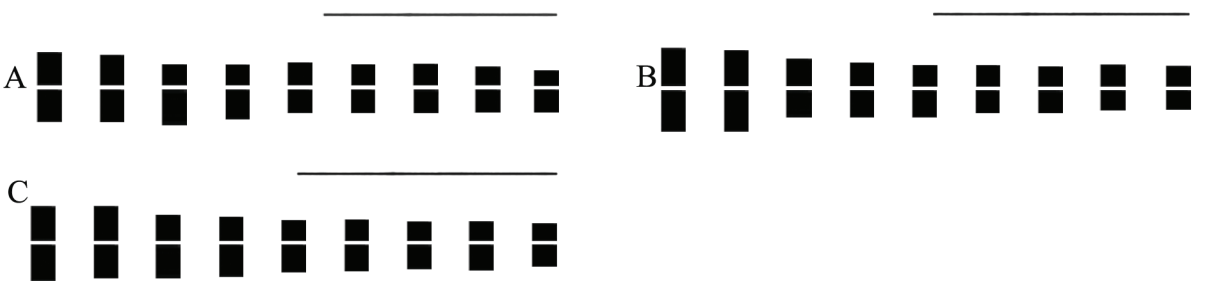

Fig. 1. Idiograms of *Salsola soda* L. populations, 2*n* = 18:
A) № 597 from Pomorie town;
B) № 598 from Burgas town; C) № 599 from Chernomorets town, scale bar 10 μm.

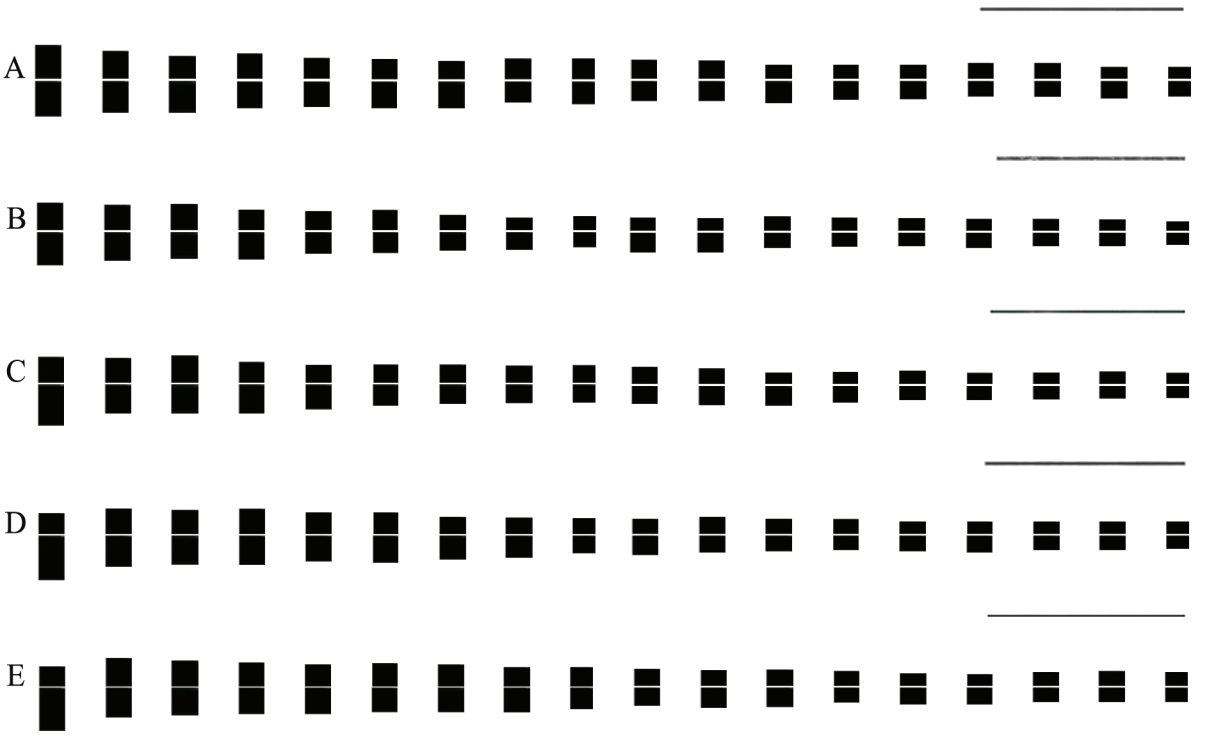

Fig. 2. Idiograms of *Salsola tragus* L. populations, 2*n* = 36:
A) № 582 from Pomorie town; B) № 583 from Burgas town;
C) № 584 from Chernomorets town; D) № 585 from Shkorpilovtsi village;
E) № 586 from Irakli area; scale bar 10 μm.

of metacentric chromosomes has been registered (Figure 1, B-C). The size of chromosomes varied from 1.21 μm for the population from Pomorie to 2.64 μm for that from Burgas (Table 3). Their average length was from 1.64 μm for the population in Pomorie to 1.77 μm for that from Chernomorets.

The chromosome number 2*n* = 36 registered for the Bulgarian populations of *S. tragus* conformed to the data by Váchová and Májovský (1978), Hindáková and Schwarzová (1980), Tomšovic (1990), Michalková and Letz (2014). Some differences in the morphology and size of chromosomes have been registered in the studied five populations of *S. tragus* as well (Figure 2, Table 3). In four of these – from Pomorie, Chernomorets, Shkorpilovtsi and Irakli a karyotype of 17 pairs of metacentric and 1 pair of submetacentric chro-

mosomes has been found (Figure 2, A-D). In the population from Burgas two of the chromosome pairs were submetacentric, while the other 16 were metacentric (Figure 2, E). The size of chromosomes varied from 1.03 μm for the population from Burgas to 3.18 μm for that from Pomorie. Their average length was from 1.7 μm for the population from Burgas to 1.9 μm for the ones from Pomorie and Irakli.

The variations in the values of inter- and intrachromosomal indices in the studied species are presented in Table 3. The overall ratio of largest to the smallest chromosome in studied populations of *S. soda* and *S. tragus* was less than 4:1 and the proportion of chromosomes was less than 2:1. According to classification of Stebbins (1971) karyotypes of the studied two species were classified into index 1A, which is considered as mainly primitive index in this system. The most symmetrical karyotype, regarding to interchromosomal index A_2, was found in four of the studied five populations of *S. tragus* and the most asymmetrical was found in *S. soda* from Pomorie (Table 3). The intrachromosomal index A_1 varied between 0.1 and 0.19. The TF% index varied from 44.51 to 47.34. Population of *S. soda* from Pomorie had the smallest TF% and the biggest A_1 indexes and that of *S. soda* from Burgas had the biggest TF% index and the smallest A_1. The index Ask% varied between 52.6 and 55.04. The Syi index varied between 80.2 and 89.8. The calculated values for the intrachromosomal asymmetry indices Ask% and Syi provided the same results as the other two intrachromosomal indexes TF% and A_1: the most symmetrical karyotype was found in *S. soda* from Burgas and the most asymmetrical one was found in that of *S. soda* from Pomorie. The sizes of chromosomes in the karyotypes of all studied species were relatively constant and this was confirmed by the zero and very close to zero values of interchromosomal index A_2.

Although TF% had a statistically significant negative correlation with the two indexes Ask% and A_1, it had a high positive correlation with the Syi index (Table. 4). Intrachromosomal index Ask% had a high positive correlation with A_1. The Syi index had statistical significant negative correlation with A_1.

Pollen morphology

The palynological data showed that all investigated specimens from the three studied populations of *S. soda* had pantoporate, spheroidal pollen with spinulose tectum (Figure 3, A-B). Data about the examined specimens is presented in Table 5. Dominant in the overall variability was the intrapopulation one. Statistically significant differences among the studied populations have been found for three of the studied features – pollen diameter, number of pores and pore area. For the studied specimens in the population from Chernomorets greater diameter of pollen, bigger numbers of pores and bigger pore area have been established. The summarized data from the pollen analysis of all three populations was as follows: diameter of pollen 18.3 – 27.12 μm; polar axis 17.78 – 23.67 μm; inter pore distance 1.94 – 3.05 μm; number of pores 44 – 54; C/D ratio 0.075 – 0.132; pore area 4.36 – 8.61 μm^2; diameter of pores 1.5 – 1.85 μm; number of spinules per 100 μm^2: 129 – 150; number of spinules in pore 7 – 21.

The palynological data showed that all investigated specimen from the five studied populations of *S. tragus* had pantoporate, spheroidal pollen with spinulose tectum, too (Figure 3, C-D). Data for the examined specimen is presented in Table 6. Statistically significant differences among the studied populations have been found for three of the studied features – polar axis, number of spinules per 100 μm^2; number of spinules in pore. The summarized data from the pollen analysis of the five populations was as follows: diameter of pollen 28.3 – 33.2 μm; polar axis 24.6 – 27.8 μm; inter pore distance 2.29 – 3.86 μm; number of pores 34 – 46; C/D ratio 0.069 – 0.132; pore area 7.09 – 12.15 μm^2; diameter of pores

Table 4
Corelations for asymmetry indexes of the studied *Salsola* species from Bulgaria

Indexes	A_2	TF %	Ask %	Syi	A_1	A
A_2	1.000					
TF %	-0.074	1.000				
Ask %	0.074	**-1.000***	1.000			
Syi	-0.061	**1.000***	**-1.000***	1,000		
A_1	0.236	**-0.916***	**0.916***	**-0.918***	1,000	
A	0.325	-0.162	0.162	-0.143	0,246	1,000

*Correlation is significant at P<0.05

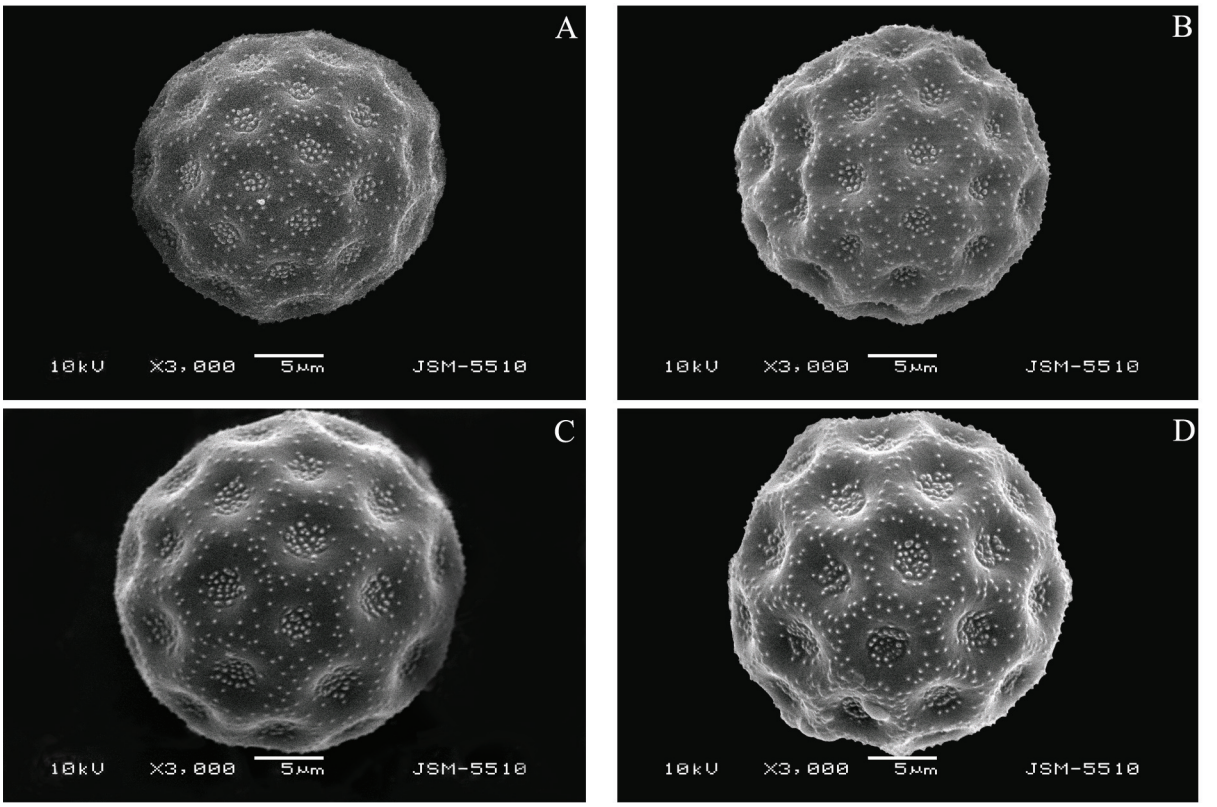

Fig. 3. Pollen microphotography of *Salsola* populations:
A) *S. soda* – №597 from Pomorie town; B) *S. soda* – №599 from Chernomorets town;
C) *S. tragus* – №585 from Shkorpilovtsi village; D) *S. tragus* – №586 from Irakli area.

Table 5
Numeric results from palynological measurements of the Bulgarian populations of *Salsola soda* L., N=30

Populations	1. D_1	2. D_2	3. C	4. C/D ratio	5. TNP	6. PD	7. PA	8. NS/ 100 μm^2	9. NSP
597	23.43a*	21.52a	2.28a	0.100a	46.00a	1.69a	5.45a	138.50a	13.70a
598	23.18a	19.90a	2.37a	0.103a	48.00ab	1.71a	6.26ab	142.70a	13.60a
599	26.99	21.20a	2.53a	0.094a	48.40b	1.69a	7.07b	140.80a	12.50a
All Grps	24.534	20.872	2.39	0.099	47.47	1.70	6.26	140.67	13.27
SD	3.42	1.87	0.30	0.015	2.67	0.08	1.20	6.11	2.98
CV	11.71	3.50	0.09	0.000	7.15	0.01	1.44	37.33	8.89
SE	0.62	0.34	0.06	0.003	0.49	0.01	0.22	1.12	0.54
Min	18.3	17.78	1.94	0.075	44	1.50	4.36	129	7
Max	27.12	23.67	3.05	0.132	54	1.85	8.61	150	21
SSv	73.26	85.55	88.79	93.00	84.06	98.32	68.69	91.83	96.56

*Different letters denote significant difference at P<0.05

2.26 – 3.71; number of spinules per 100 μm² 119 – 148; number of spinules in pore 12 – 33.

Summarized data on pollen morphology of both species is presented in Table 7. As it can be seen, statistically significant differences between them were found in 8 of the 9 studied traits. No statistically significant differences were found between *S. soda* and *S. tragus* for the C/D_1 ratio. The largest differences were found for diameter of pollen, polar axis, inter pore distance and pore area.

The classic taxonomy of Chenopodiaceae is very difficult (Balaei et al., 2004) and pollen morphological features play an important role for plant separation in this family. According to Pinar and Oybak (1997) and Toderich et al. (2010) the pollen size can be used in *Salsola* species' separation. Pol-

Table 6
Numeric results from palynological measurements of the Bulgarian populations of *Salsola tragus* L., N=50

Populations	1. D_1	2. D_2	3. C	4. C/D ratio	5. TNP	6. PD	7. PA	8. NS/ 100 μm²	9. NSP
582	31.08a*	26.29a	2.95a	0.095a	39.40a	3.02a	9.47a	131.70ab	20.40a
583	31.03a	26.22a	3.17a	0.102a	39.80a	2.98a	9.06a	136.20a	21.70ab
584	29.84a	25.41b	3.14a	0.105a	39.20a	2.85a	9.22a	131.60ab	24.00ab
585	30.12a	25.76ab	2.97a	0.099a	39.80a	2.82a	9.33a	132.20ab	24.70b
586	29.77a	25.71ab	2.89a	0.097a	38.40a	2.95a	9.79a	129.70b	20.60a
All Grps	30.37a	25.88	3.02	0.100	39.32	2.92	9.37	132.28	22.28
SD	1.58	0.86	0.41	0.014	3.82	0.30	1.07	7.06	4.41
CV	2.48	0.73	0.17	0.000	14.63	0.09	1.15	49.84	19.43
SE	0.22	0.12	0.06	0.002	0.54	0.04	0.15	1.00	0.62
Min	28.3	24.6	2.29	0.069	34	2.26	7.09	119	12
Max	33.2	27.8	3.86	0.132	46	3.71	12.15	148	33
SSv	86.46	84.78	92.67	93.09	98.15	92.95	94.58	90.65	83.71

*Different letters denote significant difference at P<0.05

Table 7
Numeric results from palynological measurements of genus *Salsola* L. in Bulgaria

Species	1. D_1	2. D_2	3. C	4. C/D ratio	5. TNP	6. PD	7. PA	8. NS/ 100 μm²	9. NSP
S. soda L	24.53***	20.87***	2.393***	0.099a	47.47***	1.698***	6.26***	140.67***	13.27***
S. tragus L	30.37***	25.88***	3.02***	0.100a	39.32***	2.923***	9.37***	132.28***	22.28***
All Grps	28.18	24.00	2.79	0.099	42.38	2.464	8.21	135.43	18.90
SD	3.73	2.77	0.48	0.014	5.24	0.643	1.88	7.83	5.88
CV	13.91	7.69	0.23	0.000	27.45	0.413	3.54	61.31	34.60
SE	0.42	0.31	0.05	0.002	0.59	0.072	0.21	0.88	0.66
Min	18.3	17.78	1.94	0.069	34	1.500	4.36	119.00	7
Max	33.2	27.8	3.86	0.132	54	3.710	12.15	150.00	33

***Statistical significant difference at P<0.001

len diameter and polar axis were among the most frequently used features for distinguishing the species in the genus. The results in the present study confirmed that these features can be used for differentiation of the two studied species *S. soda* and *S. tragus*. Our data showed that *S. tragus* had greater diameter and polar axis, bigger pore area and greater distance between them (Table 7). According to Zera and Keshararzi (2007) pollen size depends on a number of factors, incl. polyploidy. In the current studies it was found the species with tetraploid chromosome number *S. tragus* had bigger dimensions of pollen and pores, and one of the possible reasons for that is most probably the polyploidy found in it. The number of pores was also among the used in pollen analytical and taxonomic study of Chenopodiaceae taxa (Pinar and Ingeoglu, 1998). Between the two studied species *S. soda* and *S. tragus* differences in pollen have been found according to that feature as well. Pinar and Oybak (1997) studying the species from *Salsola* genus in Turkey registered correlation between pores diameter and pores number - when the pore diameter was small, the pore number increased. Our data about the Bulgarian populations of the species in the *Salsola* genus confirmed the dependence found by the researchers (Pinar and Oybak, 1997). According to Pinar (1999) the C/D ratio is one of the most diagnostic features in the family Chenopodiaceae used in pollen analytical and taxonomic investigations. Toderich et al. (2010) and Haidar (2012) noted that the C/D ratio has significant value in *Salsola* species. Between the two studied species *S. soda* and *S. tragus* no statistically significant differences have been found by that feature and it should not be used for their differentiation.

Conclusions

The chromosome number established from Bulgarian population of *S. soda* was diploid $2n = 18$, while for the populations of *S. tragus* it was tetraploid $2n = 36$. The karyotypes consist of metacentric and submetacentric chromosomes, the metacentric ones being prevalent. The total haploid chromosome length (hcl) for the studied populations of *S. soda* was from 14.76 to 15.97 µm and the chromosome length ranged from 1.21 to 2.64 µm. In the studied populations of *S. tragus* the total haploid chromosome length was from 30.44 to 34.53 µm, while the chromosome length ranged from 1.03 to 3.18 µm. Karyotypes of all studied populations were classified into index 1A, according to classification of Stebbins (1971), which is considered as mainly primitive index in this system. The most symmetrical karyotype, regarding to intrachromosomal indexes, was found in population of *S. soda* from Burgas and the most asymmetrical one was found in that of *S. soda* from Pomorie.

The pollen morphology of *S. soda* and *S. tragus* in general showed uniform type characteristics – pantoporate, spheroidal, with spinulose tectum. The basic differences between them were in the size of pollen, the number and the size of pores. The pollen of *S. soda* had diameter from 18.3 to 27.12 µm, with 44 to 54 pores with diameter of 1.5 and 1.85 µm, the area being from 4.36 to 8.61 µm^2. The pollen of *S. tragus* had diameter from 28.3 to 33.2 µm, with 34 to 46 pores with diameter from 2.26 to 3.71 µm and area from 7.09 to 12.15 µm^2. A possible reason for the established bigger pollen size of *S. tragus*, bigger diameter and bigger pore area is the polyploidy registered for the species.

The karyotype and pollen morphology of *S. soda* and *S. tragus* from Bulgarian populations was reported here for the first time.

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