Bulgarian Journal of Agricultural Science, 24 (Supplement 1) 2018, 52-58

KARYOLOGICAL STUDY OF GENUS *ATRIPLEX* L. IN BULGARIA

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Abstract

GROZEVA, N. H., 2018. Karyological study of genus *Atriplex* L. in Bulgaria. *Bulg. J. Agric. Sci.*, 24 (Suppl. 1): 52–58

The study is a part of a multi-annual research on family Chenopodiaceae within the Bulgarian flora and aims to investigate the karyotype morphology of taxa from *Atriplex*. The karyotype morphology of eight species – *Atriplex hastata* L., *A. heterosperma* Bunge, *A. hortensis* L., *A. nitens* Schkuhr, *A. oblongifolia* Walds. & Kit., *A. patula* L., *A. rosea* L., *A. tatarica* L. from Bulgarian populations was reported for the first time. Two chromosome numbers: $2n = 2x = 18$ and $2n = 4x = 36$ have been found in the genus *Atriplex*. The karyotypes of the investigated populations consist of metacentric and submetacentric chromosomes and in one of the studied populations of *A. nitens* were established chromosomes with satellites. The idiograms of all studied populations were illustrated.

Key words: *Atriplex*; chromosome number; karyotype; idiograms; Bulgaria

Introduction

The genus *Atriplex* L. (Chenopodiaceae) includes about 260 species that are mostly spread in the arid and semiarid regions of Europe, Asia, America and Australia (Al-Turki et al., 2000; Sukhorukov and Danin, 2009). Most of the species are halophytes of coastal or inland saline habitats; a few are widespread ruderals of disturbed ground (Bassett et al., 1983). For the Bulgarian flora so far eight species have been reported – *A. hastata* L., *A. heterosperma* Bunge (syn. *A. micrantha* Lebed.), *A. hortensis* L., *A. nitens* Schkuhr, *A. oblongifolia* Walds. & Kit., *A. patula* L., *A. rosea* L. and *A. tatarica* L. (Markova, 1966; Assyov and Petrova, 2012).

A. hastata forms populations along coastal and river bank saline locations. It also inhabits inland ruderalized territories, along roads and fields up to 800 m a.s.l.

A. heterosperma inhabits saline sands and clays along the Black Sea coast.

A. hortensis is cultivated in gardens and yards as food and fodder plant. It seldom occurs on ruderalized terrains in some parts of the country.

A. nitens forms populations on ruderalized and weedy locations up to 1000 m a.s.l. all over the country. It often grows along roads. It occurs as a weed in cultivated crops as well.

A. oblongifolia forms populations in plains and lowlands all over the country on ruderalized terrains up to 500 m a.s.l.

A. patula inhabits ruderalized terrains in the lowland parts of the country up to 600 m a.s.l. It also occurs as a weed in cultivated crops.

A. rosea prefers saline habitats, forms populations on ruderalized terrains up to 800 m a.s.l. all over the country.

A. tatarica occurs on saline sands and clays along the Black Sea coast and the inland up to 800 m a.s.l. It rarely forms populations on ruderalized terrains.

The study is a part of a multi-annual research on family Chenopodiaceae within the Bulgarian flora. In previous studies (Grozeva, 2010a, 2010b, 2013, 2015) the chromosome number of the species of genus *Atriplex* L. spread in Bulgaria has been established.

The objective of the present study was to investigate the karyotype morphology of taxa from *Atriplex* in Bulgaria and thus to help in taking taxonomic decisions and elucidate evolutionary problems.

Materials and methods

Karyological analyses were carried out on 14 natural Bulgarian populations of the genus *Atriplex*, referred to

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eight species: *A. hastata*; *A. heterosperma*; *A. hortensis*; *A. nitens*; *A. oblongifolia*; *A. patula*; *A. rosea*; *A. tatarica* (Table 1). Chromosome numbers and karyotypes have been 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 by the centromere index I = s/s+l, according to the classification proposed by Grif and Agapova (1986). Three metaphase plates were measured from each population. The idiograms were obtained with the help of the Adobe Photoshop CS6.0 program.

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 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).

Table 1
Studied populations from genus *Atriplex* L.

Species	Population location	Population №	2*n*
***A. hastata* L.**	Danube plain, Svishtov town, ruderal places, at 50 m, 43°37.11'N, 25°20.43'E	300	18*
	Thracian Lowland, Chirpan town, ruderal terrains, at 493 m, 42°12.21'N, 25°22.03'E	305	18*
***A. heterosperma* Bunge**	Southern Black Sea Coast, Nessebar, ruderal sandy terrains, at 0 m, 42°39.21'N, 27°44.08'E	707	36****
***A. hortensis* L.**	Danubian plain, Svishtov town, ruderal terrains, at 50 m, 43°37.31'N, 25°20.05'E	262	18**
	Thracian Lowland, Bogomilovo village, cultural community, at 192 m, 42°23.32'N, 25°33.15'E	280	18**
***A. nitens* Schkuhr**	Eastern Rhodope Mts, Kurdzhali town, ruderal places, at 275 m, 41°39.11'N, 25°38.08'E	265	18**
	Eastern Sredna Gora Mt., Gorno Novo Selo village, ruderal places, at 597 m, 42°27.31'N, 25°14.17'E	249	18**
***A. oblongifolia* Walds. & Kit.**	Southern Black Sea coast, Nessebar town, on the sands in the eastern end of Old Nessebar town, at 0 m н.в. 42°39.13'N, 27°44.04'E	702	36***
	Thracian Lowland, Rakitnitsa village, ruderal places, at 182 m, 42º 20.13'N', 25º 31.07'E	278	36**
	Tundza Hilly Country, the village Golyam Manastir, ruderal places, at 213 m, 42°12.32'N, 26°21.14'E	247	36**
***A. patula* L.**	Eastern Rhodope Mts, Kurdzhali town, ruderal places, at 275 m, 41º 39' N, 25º 38' E	277	36*
***A. rosea* L.**	Southern Black Sea coast, Burgas town, ruderal places, at 30 m, 42°30.06'N, 27°28.12'E	298	18**
	Thracian Lowland, Stara Zagora town, ruderal places, at 198 m, 42°25.17'N, 25°38.07'E	295	18**
***A. tatarica* L.**	Northern Black Sea coast, Varna town, ruderal sandy places near nVarna lake, at 79 m, 43°11.15'N, 27°50.24'E	701	18***

* - data published by Grozeva (2010a);** - data published by Gozeva (2010b);
*** - data published by Gozeva (2013);**** - data published by Gozeva (2015)

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

The four categories of A to D according to the proportion of acrocentric and/or telocentric chromosomes in a karyotype and subtypes 1 to 3 according to the ratio between the largest/smallest chromosomes

Table 3
Karyomorphometric data for the representatives of the genus *Atriplex* in Bulgaria

Population №	Karyotype formula	S	L	hcl	Inter index	Intrachromosomal index					
					A_2	SKS	TF %	Ask%	Syi	A_1	A
A. hastata L.											
300	2*n*=18m	1.40	2.97	17.99	0.02	1A	46.86	55.98	83.71	0.15	0.01
305	2*n*=16m+2sm	1.55	2.60	19.84	0.06	2A	43.75	56.20	77.85	0.20	0.01
***A. heterosperma* Bunge**											
707	2*n*=18m+18sm	0.81	2.48	28.57	0.07	1A	41.23	58.73	70.20	0.30	0.01
***A. hortensis* L.**											
262	2*n*=12m+6sm	1.42	2.88	18.07	0.06	2A	40.29	59.66	67.53	0.28	0.02
280	2*n*=12m+6sm	1.92	2.65	20.06	0.05	1A	42.67	57.43	74.31	0.25	0.02
A. nitens Schkuhr											
265	2*n*=8m+10sm	1.58	3.48	20.42	0.06	2A	40.01	60.09	66.59	0.32	0.02
249	2*n*=12m+4sm+2sm-SAT	1.41	3.59	21.03	0.05	2A	42.18	57.77	73.00	0.24	0.02
***A. oblongifolia* Walds. & Kit.**											
702	2*n*=24m+12sm	1.16	2.62	31.82	0,05	1A	42.74	57.26	74.64	0.25	0.01
278	2*n*=28m+8sm	1.02	2.68	27.76	0.07	2A	45.61	54.39	83.84	0.15	0.00
247	2*n*=28m+8sm	0.90	2.77	28.56	0.06	2A	42.82	57.18	74.89	0.22	0.01
***A. patula* L.**											
277	2*n*=36m	1.07	2.48	28.20	0,08	2A	45.85	54.15	84.68	0.15	0.00
***A. rosea* L.**											
298	2*n*=6m+12sm	1.04	2.36	15.22	0.09	2A	39.07	59.32	66.08	0.34	0.02
295	2*n*=14m+4sm	1.20	2.97	16.1	0.07	2A	41.37	59.12	69.74	0.29	0.02
***A. tatarica* L.**											
701	2*n*=10m+8sm	1.65	3.61	20.97	0.06	2A	41.06	59.85	68.61	0.29	0.02

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

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

Results and discussions

As a result of the karyological study, 2 chromosome numbers: $2n = 2x = 18$ and $2n = 4x = 36$ have been found in the genus *Atriplex* (Table 1). The basic chromosome number in the Chenopodiaceae family is x = 9 (Runemark, 1996; Snogerup, 1995; Goldblatt and Johnson, 2000) and most species have a chromosome number $2n = 18$. Karyomorphometric data about the studied populations is presented in Table 3. Two types of chromosomes: metacentric and sub-metacentric have been established in the karyotypes. Idiograms of the studied populations are presented in Figures 1 and 2.

Diploid chromosome number $2n = 18$ has been found in 5 of the studied 8 species – *A. hastata*, *A. hortensis*, *A nitens*, *A. rosea* and *A. tatarica*. The average size of their chromosomes varied from 1.7 μm in the population of *A. rosea* from the town of Burgas to 2.3 μm in the studied populations of *A. nitens* and *A. tatarica* (Table 3). The total sum of haploid chromosome length (hcl) ranged from 15.22 to 21.03 μm.

In the two populations of *A. hastata* some differences have been found in the morphology and size of chromosomes (Figure 1, A-B). For the one from the town of Svishtov karyotype of 9 pairs of metacentric chromosomes has been established, while for the population from the town of Chirpan 1 pair of submetacentric chromosomes has also been registered (Table 3). The average chromosome length in the first studied population was 1.92 μm, the shortest one being 1.4 μm and the longest - 2.97 μm. In the second population the average chromosome length was 2.2 μm and their size ranged from 1.55 to 2.6 μm. Our data was somewhat similar to that of Juan and Pastor (1990), who also reported for two of the populations of *A. hastata* studied by them from Southern Spain karyotype $2n = 18$m and $2n = 16$m + 2m-SAT and to that of Ruas et al. (2001), who reported a North American population of the species had $2n = 18$m. Greater differences have been observed in chromosome length. Ruas et al. (2001) reported about the North Ameri-

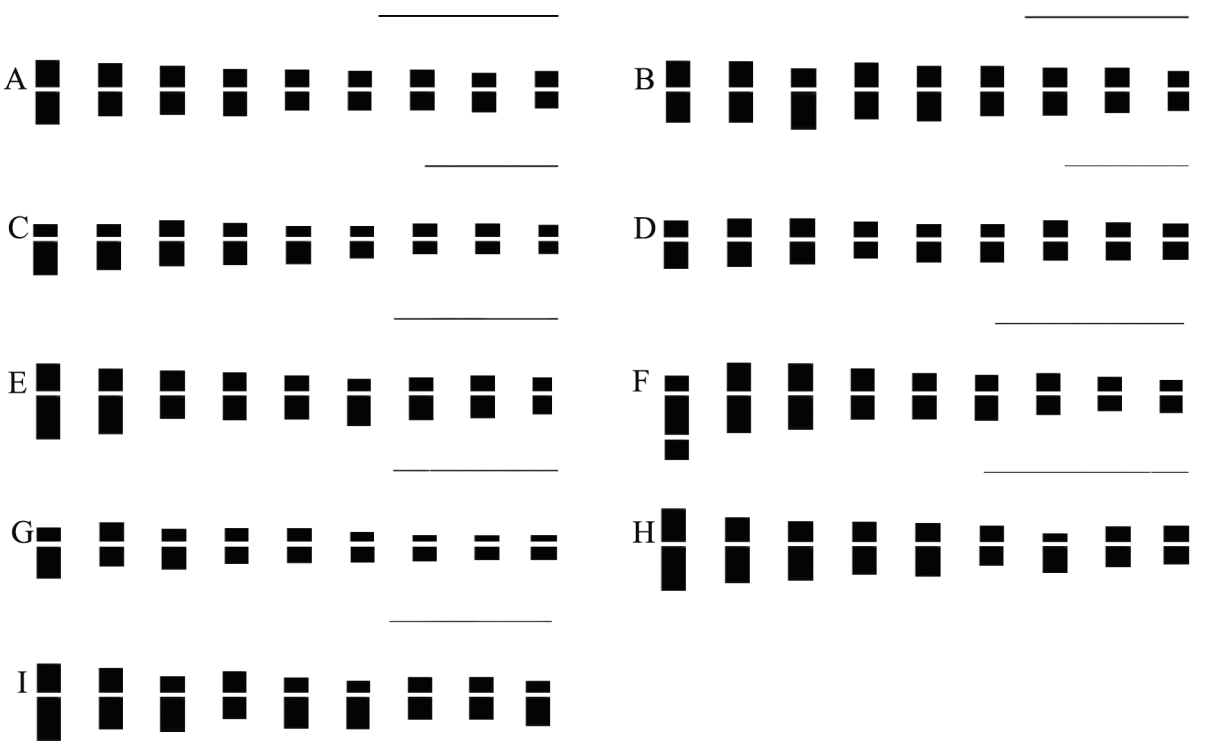

Fig. 1. Idiograms of *Atriplex* populations, $2n = 18$:
A) *A. hastata* from Svishtov; B) *A. hastata* from Chirpan; C) *A. hortensis* from Svishtov;
D) *A. hortensis* from Bogomilovo; E) *A. nitens* from Kardzhali; F) *A. nitens* from Gorno Novo Selo;
G) *A. rosea* from Burgas; H) *A. rosea* from Stara Zagora; I) *A. tatarica* from Varna; scale bar 10 μm

can *A. hastata* populations' chromosome size from 1.3 to 1.6 μm and Juan and Pastor (1990) about the Spanish sizes from 2.26 to 3.6 μm.

In the two studied populations of *A. hortensis* a karyotype of 6 pairs of metacentric and 3 pairs of submetacentric chromosomes has been established (Figure 1, C-D). The average chromosome length in the population from the town of Svishtov was 2 μm, the shortest one being 1.42 μm, the longest - 2.88 μm (Table 3). In the population from the village of Bogomilovo the average chromosome length was 2.2 μm and their size ranged from 1.92 to 2.65 μm (Table 3). Our data differs from the one by Ruas et al. (2001), who reported for a *A. hortensis* population from North America karyotype of 18 metacentric chromosomes with chromosome size from 1.6 to 2.1 μm and average length of 1.8 μm.

Some differences in the morphology and size of chromosomes have been registered in both studied populations of *A. nitens* (Table 3; Figure 1, E-F). For the one from the town of Kardzhali a karyotype of 4 pairs of metacentric and 5 pairs of submetacentric chromosomes has been established, and for the population from the village of Gorno Novo Selo a karyotype of 6 pairs of metacentric and 3 pairs of submetacentric chromosomes, one of the pairs of submetacentric chromosomes being registered satellites. The average length of chromosomes in both populations was 2.3 μm and in the population from the town of Kardzhali the size of chromosomes ranged from 1.58 to 3.48 μm, and in the population from the village of Gorno Novo Selo - from 1.41 to 3.59 μm. Our data about the morphology of chromosomes in both studied populations differs from that by Ruas et al. (2001) who reported in a population of *A. nitens* from North America karyotype of 18 metacentric chromosomes with chromosomes size from 1.6 to 2.0 μm and average length of 1.79 μm.

Differences in the morphology and size of chromosomes have also been found in the two studied populations of *A. rosea* (Table 3; Figure 1, G-H). For the one from the town of Burgas a karyotype of 3 pairs of metacentric and 6 pairs of submetacentric chromosomes has been established and for the population from Stara Zagora - karyotype of 7 pairs of metacentric and 2 pairs of submetacentric chromosomes. The average length of chromosomes in the two populations was 1.7 - 1.8 μm and in the population from the town of Kardzhali their size ranged from 1.04 to 2.36 μm and in the one from the town of Stara Zagora - from 1.2 to 2.97 μm. Juan and Pastor (1990) reported in a population of *A. rosea* from Southern Spain karyotype of 8 pairs of metacentric and 1 pair of submetacentric chromosomes.

A karyotype of 5 pairs of metacentric and 4 pairs of submetacentric chromosomes has been established for the studied population of *A. tatarica* from the town of Varna (Table 3; Figure 1, I). The average chromosome length was 2.3 μm and their size ranged from 1.65 to 3.61 μm.

Tetraploid chromosome number $2n = 36$ has been registered for 3 species – *A. heterosperma, A. oblongifolia* and *A. patula* (Table 1). The average size of their chromosomes ranged from 1.5 μm in the population of *A. oblongifolia* from the village of Rakitnitsa to 1.8 μm in the population of *A. oblongifolia* from the town of Nessebar (Table 3). The total sum of the haploid chromosome length (hcl) ranged from 27.76 to 31.82 μm.

For the studied population of *A. heterosperma* from the town of Nessebar a karyotype of 9 pairs of metacentric and 9 pairs of submetacentric chromosomes has been established (Figure 2, A). The average chromosome length was 1.6 μm and their size ranged from 0.81 to 2.48 μm (Table 3).

Three populations of *A. oblongifolia* have been studied (Table 1). For the one from the town of Nessebar a karyotype of 12 pairs of metacentric and 6 pairs of submetacentric chromosomes has been established, the average chromosome length was 1.8 μm and their dimensions ranged from 1.16 to 2.62 μm (Table 3, Figure 2, B). For the other two populations from the village of Rakitnitsa and the village of Golyam Manastir a karyotype of 14 pairs of metacentric and 4 pairs of submetacentric chromosomes has been registered and an average length of 1.5 μm for the first and 1.6 μm for the second population (Figure 2, C-D). In the population from the village of Rakitnitsa the size of chromosomes ranged from 1.02 to 2.68 μm and in the one from the village of Golyam Manastir - from 0.9 to 2.77 μm.

For the population of *A. patula* from the town of Kardzhali a karyotype of 18 pairs of metacentric chromosomes has been established (Table 3). The average chromosome length was 1.6 μm, and their size ranged from 1.07 to 2.48 μm (Figure 2, E).

The overall ratio of the largest to the smallest chromosome in all studied *Atriplex* species was less than 4:1 and the proportion of chromosomes was less than 2:1. Karyotypes of all studied populations were classified into two indexes, according to classification of Stebbins (1971): 1A and 2A. The populations of *A. heterosperma* and *A. oblongifolia* from the town of Nessebar, the population of *A. hastata* from the town of Svishtov and this of *A. hortensis* from the village of Bogomillovo were included in 1A and the other 10 populations (see Table 3) – in 2A. The sizes of chromosomes in the karyotypes of all studied species were relatively constant and that was confirmed by the small and quite close to zero values of the interchromosomal index A_2. Regarding interchromosomal index A_2, the most symmetrical karyotype was found in population of *A. hastata* from the town of Svishtov and the most asymmetrical one was found in that of *A. rosea* from the town of Burgas. The TF% index varied between 39.07 and 46.86. The intrachromosomal index A_1 varied between

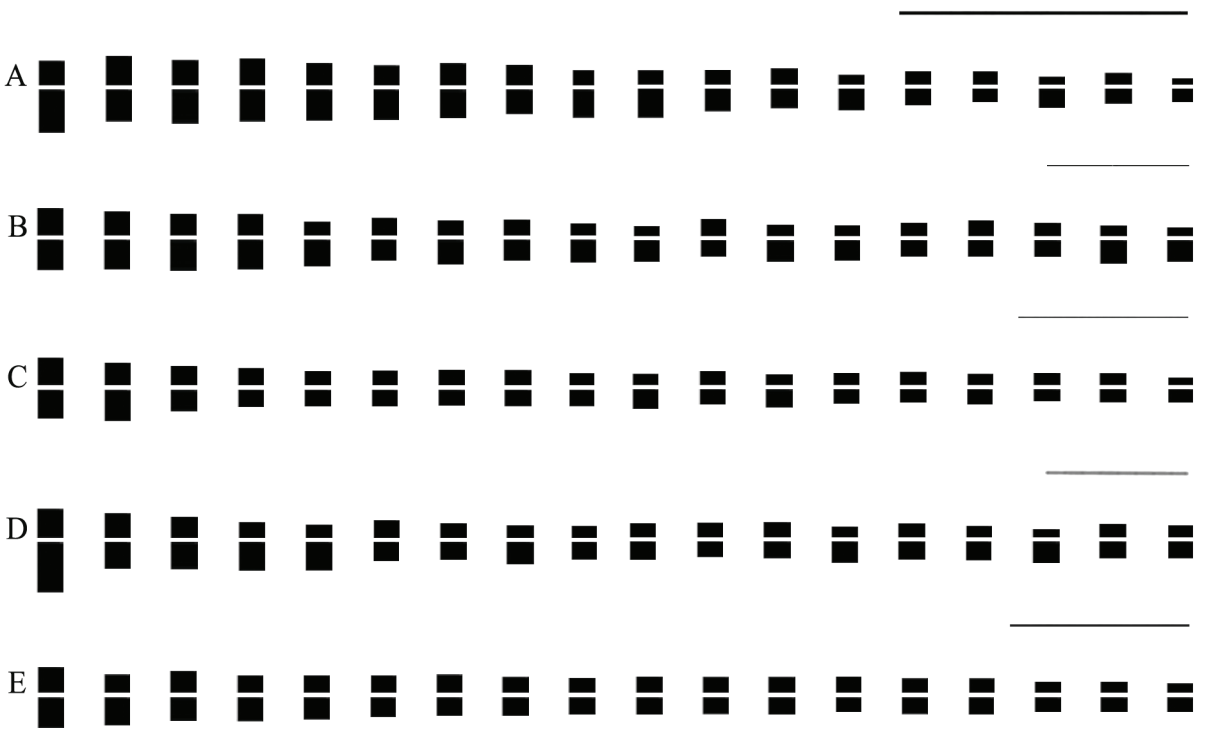

Fig. 2. Idiograms of *Atriplex* populations, 2*n* = 36:
A) *A. heterosperma* from Nessebar; B) *A. oblongifolia* from Nessebar; C) *A. oblongifolia* from Rakitnitsa; D) *A. oblongifolia* from Golyam Manastir; E) *A. patula* from Kardzhali; scale bar 10 µm

0.15 and 0.34. The population of *A. rosea* from the town of Burgas had the smallest TF% and biggest A_1 index and that of *A. hastata* from the town of Svishtov had the biggest TF% index and smallest A_1. The calculated values for the intrachromosomal asymmetry indices TF% and A_1 provided the same results as interchromosomal index A_2: the most symmetrical karyotype was found in a population of *A. hastata* from the town of Svishtov and the most asymmetrical one was found in that of *A. rosea* from the town in Burgas. The index Ask% varied between 54.15 and 60.09. The variations in the values of inter- and intrachromosomal indices in the studied species, as seen from Table 3, were not always one-directional and that is probably due to changes that had occurred in the size of chromosomes and/or the position of the centromeres.

Although TF% had a statistical significant negative correlation with the three indexes Ask%, A, A_1, it had a high positive correlation with the Syi index (Table. 4). Intrachromosomal index Ask% had a high positive correlation with A, A_1 and a high negative correlation with Syi and TF%. Between A and A_1 has been established a positive correlation. The Syi index had statistical significant negative correlation with A and A_1.

Conclusions

The data from the present study confirm that *Atriplex* species in Bulgarian flora had a basic chromosome number x = 9. Most of them – *A. hastata, A. hortensis, A. nitens, A. rosea, A. tatarica* were diploid and another three – *A. heterosperma, A. oblongifolia, A. patula* were tetraploid. The karyotypes of all populations consist of metacentric and submetacentric chromosomes with the prevalence of the metacentric. The submetacentric chromosomes predominate only in the karyotype of *A. heterosperma* from the town of Kurdzhali and in that of *A. rosea* from the town of Burgas. In one of the studied populations of *A. nitens* from the village of Gorno Novo Selo were established chromosomes with satellites. The size of the chromosomes in diploid *Atriplex* species varied from 1.7 to 2.3 µm and the total sum of haploid chromosome length ranged from 15.22 to 21.03 µm. The size of the chromosomes in tetraploid *Atriplex* species ranged from 1.5 to 1.8 µm and the total sum of the haploid chromosome length was from 27.76 to 31.82 µm. In terms of the Stebbins' system (Stebbins, 1971), the karyotypes of all populations were 1A and 2A, which are considered mainly primitive indexes in this

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

Indexes	A_2	TF %	Ask%	Syi	A_1	A
A2	1.000					
TF %	0.333	1.000				
Ask%	-0.233	**-0.913***	1.000			
Syi	0.319	**0.986***	**-0.965***	1.000		
A_1	-0.246	**-0.973***	**0.930***	**-0.978***	1.000	
A	-0.287	**-0.781***	**0.875***	**-0.839***	**0.786***	1.000

*correlation is significant at $p < 0,05$

system. The most symmetrical karyotype, regarding the interchromosomal index A_2 and the intrachromosomal asymmetry indices, was found in a population of *A. hastata* from the town of Svishtov and the most asymmetrical one – in *A. rosea* from the town of Burgas.

The karyotype morphology of eight species of genus *Atriplex* has been reported for the first time from Bulgarian populations.

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