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Karyological study of genus *Oxybasis* Kar. & Kir. in Bulgaria

Neli Grozeva[1], Stefka Atanassova[2], Svetoslava Terzieva[1]

[1]*Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, 6000 Stara Zagora Bulgaria*
[2]*Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, 6000 Stara Zagora Bulgaria*
[*]*Corresponding author:* grozeva@uni-sz.bg

Abstract

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A karyological study of *Oxybasis chenopodioides*, *O. glauca*, *O. rubra* and *O. urbica* was conducted and the karyotype morphology from their Bulgarian populations was reported for the first time. The studied species showed $2n = 2x = 18$ and differed significantly in total size of chromosomes, the size of the long and the short arms. The karyotype consists of metacentric and submetacentric chromosomes and their size varied from 0.8 to 3.12 μm. Clustering of the species based on karyotype features grouped them in 2 clusters: *O. chenopodioides* with *O. glauca* and *O. rubra* with *O. urbica* with greater karyotype similarity found between *O. rubra* and *O. urbica*. The idiograms of all studied populations have been illustrated.

Keywords: Oxybasis; karyotype; Bulgaria; idiograms

Introduction

Chenopodium L. is one of the big and most difficult taxonomically genera in the family Amaranthaceae. Molecular phylogenetic studies in recent years revealed that *Chenopodium* L. is polyphyletic, and its representatives have been placed in several genera – *Chenopodium*, *Blitum, Chenopodiastrum, Oxybasis, Lipandra, Dysphania, Teloxys* (Kadereit et al., 2003; 2010; Fuentes-Bazan et al., 2012a,b). Genus *Oxybasis* Kar. & Kir. comprises the species from sect. Pseudoblitum of genus *Chenopodium* s.l.

Genus *Oxybasis* is characterised in having flowers with a hyaline or greenish perianth of 2-4(5) free or connate segments, a reduced number of stamens (2-4, rarely 5) and usually red seeds with the outer layer (testa) impregnated with tannin-like substances and a diversely oriented seed embryo (Fuentes-Bazan et al., 2012b; Sukhorukov & Zhang, 2013; Sukhorukov et al., 2013).

For the Bulgarian flora up to this moment a total of 4 species of genus *Oxybasis* have been reported – *Oxybasis chenopodioides* (L.) S. Fuentes, Uotila & Borsch. (syn.: *Chenopodium chenopodioides* L.), *Oxybasis glauca* (L.) S. Fuentes, Uotila & Borsch L. (syn.: *C. glaucum* L.), *Oxybasis rubra* (L.) S. Fuentes, Uotila & Borsch (syn.: *C. rubrum* L.), *Oxybasis urbica* **(**L.) S. Fuentes, Uotila & Borsch (syn.: *Chenopodium urbicum* L.) (Yordanov et al., 1966; Andreev, 1992; Assyov & Petrova, 2012).

O. chenopodioides was reported for the first time for the Bulgarian flora by Andreev (1992) for the area of the Northern Black sea coast without any clear motives about that. Upon review of the materials in the Bulgarian scientific herbaria it has been found that prof. P. Uotila upon review of the herbarium samples deposited at the Faculty of Biology at Sofia University in 1993 referred two of them to *C. chenopodioides*: SO No82609 (sub *C. rubrum* L.) from the region of Durankulak lake and SO No18126 (sub *Atriplex hastata* L.) from Shabla lake. The species prefers saline coastal sunny ruderalized terrains (Uotila, 2001).

Oxybasis glauca occurs most frequently along the Black Sea coast and in the inland of the country it usually grows

in close proximity to water basins. It prefers saline soils and open sunny terrains. It is either a dominant or asectorial species in ruderal or anthropophytic communities from the sea level up to 800 m a.s.l.

Oxybasis rubra has been reported for the Black Sea coast: Danubian plane; North-East Bulgaria; Fore Balkan area; Stara planina Mts.; Sredna gora Mts.; Znepole region; West Frontier Mountains; Sofia region; Thracian Plane, Tundzha hilly plain. Asectorial species in ruderal or anthropophytic communities, it often occurs on ruderalized terrains in settlements. It forms populations of small number on moderately moist terrains from the seal level up to 850 m a.s.l.

Oxybasis urbica occurs sparsely all over the country. It is either a dominant or asectorial species in anthropophytic or ruderal communities. As a weed it has been registered mainly in row crops. It forms populations of small number on moderately moist soils from the seal level up to 1000 m a.s.l.

Up to this moment there have been no data found in reference sources concerning the karyotype of the Bulgarian populations of the species from genus *Oxybasis,* and the objective of the present study is to trace the karyological variability of the species in Bulgaria.

Materials and Methods

A total of 9 populations of *O. chenopodioides***,** *O. glauca*, *O. rubra* and *O. urbica* have been researched from 5 floristic regions (Table 1).

The chromosome number has been determined from durable squash preparations on metaphase plates according to the methods by Grozeva (2007b). During the autumn-winter period roots were collected between 11 and 12 noon, and during the spring-summer one between 9 and 10 a.m., when the most active division has been observed. The metaphase plates were studied on a light microscope Olympus BX51. Idiograms were made using the mean values of chromosome length. Idiograms have been obtained by the Adobe Photoshop CS6 software. From each populations at least five metaphase plates have been measured. The herbarium samples of the karyologically studied plants have been deposited at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences.

The type of chromosomes was determined by the method of Grif & Agapova (1986). The coefficient of variation of chromosome length ($CV_{CL} = A_2.100$) according to Paszko (2006) and the mean centromeric asymmetry ($M_{CA} = A.100$) proposed by Peruzzi & Eroğlu (2013) were used to assess karyotype symmetry. Interchromosomal asymmetry index A_2 has been calculated by the method of Romero Zarko (1986) and interchromosomal index A - by Watanabe et al. (1999).

The results obtained for the parameters related to the karyotype characteristics were statistically processed with Statistica 10 for Windows (for Cluster analysis) and Pirouette 4.5 (for Principal Component Analysis) software. Cluster analysis (CA) was applied to group studied populations by similarity of karyotypic parameters. Principal component analysis (PCA) was performed to evaluate the contribution of each karyotypic parameter to the ordination of species.

Results and Discussion

For all studied populations of the species from genus *Oxybasis* diploid chromosome number $2n = 2x = 18$ has been registered (Table 1). According to Runemark (1996) and

Table 1. Studied populations of genus *Oxybasis.*

№	Species, Population`s location	2*n*	GPS coordinates, altitude
	Oxybasis chenopodioides (L.) S. Fuentes, Uotila & Borsch		
1.	Black Sea Coast (Northern), town of Obzor, not far from the seashore in the northern part of the town – chen1.	18**	42°49′43″N, 27°52′55″E, 1 m
2.	Black Sea Coast (Southern), town of Burgas, ruderalized terrains – chen2.	18	42°30.14′ N, 27° 28.17′ E, 30 m
3.	Rhodope Mts (Easthern), town of Kardzhali, ruderalized terrains – chen3.	18	41°39.12′ N, 25° 38.18′ E, 275 m
	Oxybasis glauca (L.) S. Fuentes, Uotila & Borsch		
4.	Black Sea Coast (Northern), town of Balchik, ruderalized terrains – gla1.	18*	43°25′ N, 28°10′ E, 190 m
5.	Danube plain, town of Svishtov, ruderalized terrains – gla2.	18*	43°37′ N, 25º20′ E, 50 m
	Oxybasis rubra (L.) S. Fuentes, Uotila & Borsch		
6.	Northeastern Bulgaria, town of Tutrakan, ruderalized terrains – rub1.	18*	44º03′ N, 26°37′ E, 107 m
7.	Thracian Lowland, town of Stara Zagora, ruderal terrains – rub2.	18**	42°25′ N, 25°38′ E, 195 m
	Oxybasis urbica (L.) S. Fuentes, Uotila & Borsch		
8.	Northeastern Bulgaria, town of Targovishte, ruderalized terrains – urb1.	18	43°32′ N, 22°42′ E, 170 m
9.	Thracian Lowland, village of Tselina, ruderalized terrains – urb2.	18**	42°07′ N, 25°27′ E, 193 m

* – data published by Grozeva (2007a), ** – data published by Grozeva (2013)

Table 2. Karyomorphometric data for the studied *Oxybasis* populations, chromosome size variation (µm) – short (S) and long (L), total haploid chromosome length (hcl, µm), interchromosomal asymmetry indices (A_2); coefficient variation of chromosome length (CV_{cl}), degree of asymmetry of karyotype (A), mean centromeric asymmetry (M_{ca})

Species/population	Karyotype formula	S	L	Hcl	A2	A	CVcl	Mca
1. *O. chenopodoides*, Obzor	2m + 16sm	1.23	2.93	17.87	0.08	0.03	8.41	3.28
2. *O. chenopodoides*, Burgas	2m + 16sm	1.20	2.90	17.88	0.07	0.03	7.44	3.43
3. *O. chenopodoides*, Kardzhali,	10m + 8sm	1.03	2.31	15.10	0.06	0.02	6.32	2.44
4. *O. glauca*, Balchik	10m + 8sm	1.20	3.12	16.30	0.06	0.02	6.23	2.37
5. *O. glauca*, Svishtov	10m + 8sm	1.20	3.05	16.34	0.07	0.02	7.34	2.24
6. *O. rubra*, Tutrakan	16m + 2sm	0.84	2.17	13.03	0.09	0.01	9.41	1.41
7. *O. rubra*, Stara Zagora	16m + 2sm	0.85	2.10	13.01	0.10	0.01	10.17	1.23
8. *O. urbica*, Targovishte	16m + 2sm	0.80	1.84	11.85	0.09	0.01	9.08	1.33
9. *O. urbica*, Tselina	16m + 2sm	0.85	1.87	11.98	0.10	0.01	10.42	1.28

Goldblatt & Johnson (2000) the basic chromosome number in the Chenopodiaceae family is x = 9 and the most frequent chromosome number is $2n = 18$. Karyomorphometric data about the studied populations is presented in Table 2 and idiograms are presented in Fig. 1 and Fig. 2. In all studied populations of the species from genus *Oxybasis* two types of chromosomes have been registered: metacentric and submetacentric. The dimesions of the longest chromosome vary from 1.84 µm in the population of *O. urbica* from the town of Targovishte to 3.12 µm in that of *O. glauca* from the town of Balchik while those with the shortest chromosome from 0.8 µm in the population of *O. urbica* from the town of Targovishte to 1.23 µm for that of *O. chenopodoides* from the town of Obzor, respectively.

The established diploid chromosome number for the three studied populations of *O. chenopodioides* is in accordance with the one known from literature data about populations of the species from various parts of its area (Winge, 1917; Kjellmark, 1934; Cooper, 1935; L*ö*ve, 1954; Kawatani & Ohno, 1956; Gadella & Kliphuis, 1963; Schwarzowa, 1978; Granado et al., 1988; Lomonosova, 2006). Our data correlated with the diploid chromosome number stated by Grozeva (2007a) about a population of the species from the Northern Black Sea coat in the region of Shabla lake. The mean length of chromosomes in the three studied populations was 1.99 µm for the populations from the Black Sea coat – town of Burgas and town of Obzor, 1.68 µm for the population from the town of Kardzhali, respectively. The longest chromosome was 2.93 µm long, while the shortest one was 1.03 µm. The ratio between the longest and the shortest chromosome in the population from the town of Kardzhali was 2.24:1, with a difference in their size of 1.28 µm. The ratio between the longest and the shortest chromosome in the population from the town of Obzor was 2.44:1, with a difference in the size of 1.73 µm. The ratio between the longest and the shortest chromosome in the population from the town of Burgas was 2.42:1, with a difference in the size of 1.7 µm. Karyotype of 5 pairs of metacentric and 4 pairs of submetacentric chromosomes has been established (Fig. 1C) for the population from the town of Kardzhali, while in the two populations from the Black Sea coast: town of Burgas and town of Obzor 8 of the chromosome pairs were submetacentric (Fig. 1A,B).

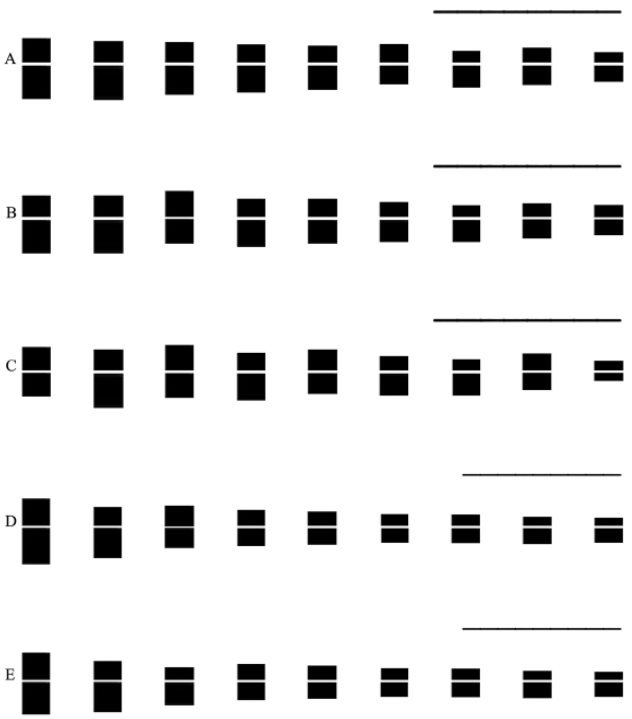

Fig. 1. Idiograms of *Oxybasis* populations, $2n = 18$: A) *O. chenopodioides*, Obzor; B) *O. chenopodioides*, Burgas; C) *O. chenopodioides,* Kardzhali; D) *O. glauca*, Balchik,; E) *O. glauca*, of Svishtov, scale bar 10 µm

The registered diploid chromosome number for the populations of *O. glauca* from the town of Balchik and the town of Svishtov confirmed to the ones stated by many researchers about the species from different parts of the world (Cooper, 1935; Wullf, 1937; Polja, 1948; Löve, 1954; Kawatani & Ohno, 1956; Scalinska et al., 1961; Gadella & Kliphuis, 1963; Keener, 1970; Löve & Löve, 1974; Murin & Schwarzova, 1976; Bouchard et al. 1978; Gervais, 1979; Dvořák et al., 1980; Murin et al., 1980; Pogan, 1980; Schwarzova, 1980; Arohonka, 1982; Dvorák, 1989; Měsíček, 1992; Lomonosova & Krasnikov, 1994; Al-Turki et al., 1999; 2000; L**ö**vkvist & Hultg**å**rd, 1999; Lomonosova et al., 2003; Rahiminejad & Gornall, 2004; Rahiminejad, 2006; Lomonosova, 2006). The mean length of chromosomes in the two studied populations was 1.81-1.82 μm. The longest chromosome was 3.12 μm long, while the shortest one was 1.2 μm. The ratio between the longest and the shortest chromosome in the population from the town of Balchik was 2.6:1, with a difference in their size of 1.92 μm. The ratio between the longest and the shortest chromosome in the population from the town of Svishtov was 2.54:1, with a difference in the size of 1.85 μm. The karyotype of 5 pairs of metacentric and 4 pairs of submetacentric chromosomes has been established (Fig. 1D,E). The results from the present study were similar to the ones by Tanaka & Tanaka (1980) about the populations of *O. glauca* from Japan for which diploid chromosome number of $2n = 18$ and presence of 2 types of chromosomes (metacentric and submetacentric ones) have been reported. The longest chromosome pair both in the Bulgarian and in the Japanese populations was always metacentric while the shortest one was submetacentric. Differences have been noticed in the size of chromosomes in the chromosome pairs which was from 2 to 3 μm in the Japanese populations. In the Japanese populations satellites on one of the pairs of metacentric chromosomes have been registered, while in the Bulgarian ones there were no satellites.

The registered diploid chromosome number for the two populations of *O. rubra* from Sinite Kamani Nature Park and from the town of Tutrakan correlated to the results by great part of the researchers of the species for populations from various parts of the world (Kjellmark, 1934; Kawatani & Ohno, 1962; Zosimovič, 1965; Keener, 1970; Kliphuis & Wieffering, 1972; Uotila, 1973; Dvorak et al., 1980; Bassett & Crompton, 1982; Dvorak & Dadakova, 1984). For O. rubra tetraploid chromosome number 2n = 36 was reported by Dvorak et al. (1980), Löve & Löve (1982), Dvorák (1989), Lomonosova & Krasnikov (1994), Lövkvist & Hultgård (1999). The mean length of chromosomes in the Bulgarian populations of the species was 1.44-1.45 μm. The longest chromosome was 2.17 μm long, while the shortest one was 0.84 μm. The ratio between the longest and the shortest chromosome in the population from Sinite Kamani Nature Park was 2.47:1, with a difference in their size of 1.25 μm. The ratio between the longest and the shortest chromosome in the population from the town of Tutrakan was 2.54:1, with a difference in size of 1.33 μm. The karyotype of 8 pairs of metacentric and 1 pair of submetacentric chromosomes has been established (Fig. 2A, B). Dvorak & D**a**dakova (1984) specify about a population of *O. rubra* from the village of *Sedlec* in the South Moravian Region of the *Czech* Republic a karyotype of 5 pairs of metacentric and 4 pairs of submetacentric chromosomes, with the mean size of chromosomes in the Czech population being similar to the one established for the Bulgarian populations.

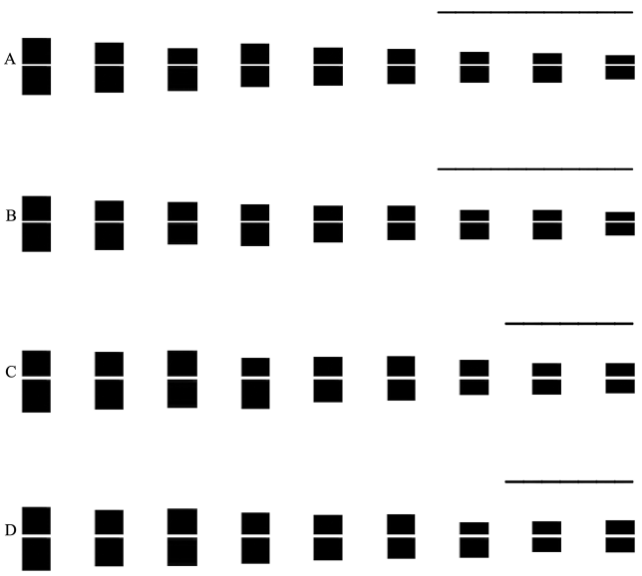

Fig. 2. Idiograms of *Oxybasis* populations, $2n = 18$: A) *O. rubra*, Tutrakan; B) *O. rubra*, Stara Zagora; C) *O. urbica*, Targovishte; D) *O. urbica*, Tselina, scale bar 10 μm

The registered diploid chromosome number in the two studied populations of *Oxybasis urbica* from the town of Targovishte and the village of Tselina correlated to the data by a number of reasearchers from all over the world (Kawatani & Ohno, 1956; Májovský et al., 1970; Uotila, 1973; Queiros, 1975; Dvorák, 1989; Lomonosova & Krasnikov, 1994; Dobea & Hahn, 1997; Lomonosova et al., 2003a,b; Probatova et al., 2009). For O. urbica tetraploid chromosome number 2n = 36 has also been reported by Cole (1962). The mean length of chromosomes for the Bulgarian populations of the species was 1.32-1.33 μm

with the longest chromosome being 1.87 µm long and the shortest one being 0.82 µm. The ratio between the longest and the shortest chromosome in the population from the town of Targovishte was 2.30:1, with a difference in their size of 1.04 µm. The correlation between the longest and the shortest chromosome in the population from the village of Tselina was 2.20:1, with a difference in their size of 1.02 µm. The karyotype of 8 pairs of metacentric and 1 pair of submetacentric chromosomes has been registered (Fig. 2C, D).

According to asymmetry indices, the highest were the values of CV_{CL} about the population of *O. urbica* from the village of Tselina and the lowest were the ones about that of *O. glauca* from the town of Balchik. Concerning M_{CA} the highest values have been registered for the population of *O. chenopodoides* from the town of Obzor and the lowest ones for that of *O. rubra* from the town of Stara Zagora. The LSD tests showed significant difference ($p < 0.05$) for CV_{CL} and M_{CA} among the studied populations and species.

The data from the cluster analysis based on Euclidean distances between population centroids revealed that by degree of similarity of the karyotype the studied populations form two big clusters (Fig. 3). The populations of *O. rubra* and *O. urbica* with the two populations of *O. rubra* having the smallest metric distance formed the first cluster. The second cluster grouped the populations of the other two species *O. glauca* and *O. chenopodioides* with the smallest metric distance between the two populations of *O. chenopodioides* from the Black Sea coast forming a subcluster. Greater similarity has been observed between the two populations of *O. glauca,* also forming a subcluster. The population of *O. chenopodioides* from Kardzhali had greater metric distance in the populations from the two subclusters.

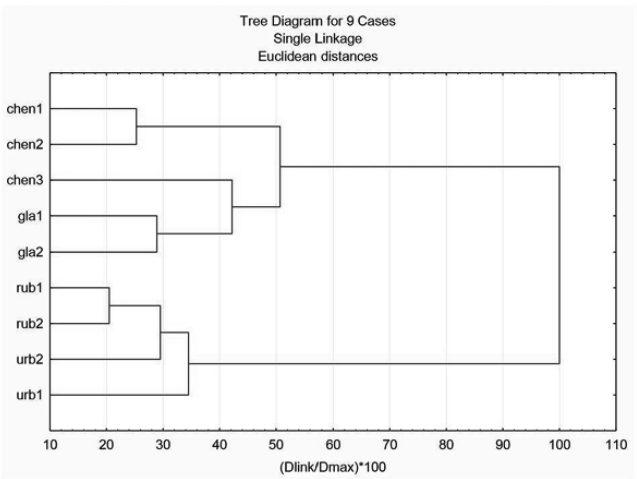

Fig. 3. Dendrogram of CA of *Oxybasis* populations based on karyotype data

*numbering of populations follows the indications in Table 1

The principal component analysis helps researchers to distinguish significant relationship between traits (Beheshtizadeh et al. 2013). The PCA of the mitotic data for the 4 species from genus *Oxybasis* revealed that the factor 1 was explained 98,3% of total variance and had the greatest positive correlation with the total sum of haploid chromosome length (Hcl), the coefficient of variation of chromosome length (CVcl), long arm (LA) and total length (TL) of chromosomes (Table 3). The factor 2 contributed 1.7% of the total variation and had the greatest positive correlation with the coefficient of variation of chromosome length (CVcl) and the lowest relations with the total sum of haploid chromosome length and long arm of chromosomes (LA).

Table 3. PCA based on karyotype parameters of studied *Oxybasis* populations

Parameter	Factor 1 (98.3%)	Factor 2 (1.7%)
S	0.058296	-0.037182
L	0.141450	-0.106876
L-S	0.083154	-0.069693
L/S	0.135884	0.062176
Mean	0.093814	-0.046315
Hcl	0.844324	-0.416833
A2	0.004451	0.008672
A	0.001039	-0.002356
CVcl	0.463086	0.863800
Mca	0.122720	-0.237373

Conclusion

The studied Bulgarian populations of *O. chenopodioides, O. glauca, O. rubra* and *O. urbica* had diploid chromosome number $2n = 2x = 18$ and karyotype of metacentric and submetacentric chromosomes and in the greater part of these the metacentric ones dominate. Submetacentric chromosomes dominated only in the populations of *O. chenopodioides* from the town of Obzor and from the town of Burgas. Satellites have not been registered. The size of the chromosomes varied from 0.8 to 3.12 ***µm***. The total sum of haploid chromosome length (hcl) ranged from 11.85 to 17.88 ***µm***. The data from CA revealed that the greatest was the similarity of karyotype between *O. rubra* and *O. urbica.* Certain similarity has been registered about these of *O. chenopodioides* and *O. glauca* as well. The karyotype morphology of *Oxybasis* species has been reported for the first time from Bulgarian populations.

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