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Morphological and karyological variability in Bulgarian populations of Amaranthus albus L. (Amaranthaceae)

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Abstract. *Amaranthus* L. is represented by 12 species in the flora of Bulgaria. Most of them are ruderal plants, widely distributed in the country. The representatives of amaranth in the Bulgarian flora are insufficiently studied. The aim of the present study is to study the morphological and karyological variability of one of the most widely distributed annual representatives of the genus in the country - *Amaranthus albus* L. A diploid chromosome number 2n = 2x = 32 was established. The total length of the haploid chromosome number in the studied populations varied from 14.57 μm to 27.39 μm. The pollen morphology and the seed morphology were examined using a scanning electron microscope (SEM).

Key words: *Amaranthus albus* L., morphology, karyology, Bulgaria.

Introduction

Genus *Amaranthus* is one of the most numerous genera in the family Amaranthaceae. Worldwide it is represented by over 70 species, most of which from America, and the rest from Eurasia, South Africa and Australia (Bayan, 2015). The representatives of this genus inhabit mainly tropical, subtropical and temperate climate zones (Iamonico, 2020). The names of most of the plants are duplicated, which has led to a confusion in the nomenclature and incorrect application of the names (Mosyakin & Robertson, 1996; Costea et al., 2001; Iamonico, 2020).

The species in the genus are characterized by high ecological plasticity, similar morphological characteristics and spontaneously appearing hybridization between them (Brenner et al., 2010; Das, 2014). Genetically similar species in genus *Amaranthus* easily achieve hybridization (Wassom & Tranel, 2005), and the resulting hybrids are fertile and often with wider ecological plasticity than the parent species. The species of the genus create a huge number of seeds with delayed germination, and sometimes forms, resistant to some of the most widely used modern herbicides (De Prado et al., 1993). These characteristics of the species in the genus and their late life cycle make them especially dangerous and competitive to crops. The effective control over these species often begins with understanding their biological characteristics.

One of the most widely represented species from the genus *Amaranthus* in Bulgaria is *A. albus*. The species is invasive for Bulgarian flora. The horological data show its expansive distribution in the country (Assyov & Petrova, 2012). It inhabits mainly polluted places, tilled and vegetable crops and others (Rushing et al., 1985; Samanipour et al., 2018). The studies in the past decades show an increase in the density of the species in different crops like cotton, sunflowers and others (Bükün, 2005; Moskova et al., 2016; Pala et al., 2016). It is distributed from 0 to 1000 m a.s.l.

Until now the Bulgarian populations have not been the object of such a study. The karyological data for the species are scarce. Cheshmedziev (1994) reported a diploid chromosome number 2n = 2x = 32 for a population of the species in the Thracian plain. Data for the karyotype of the Bulgarian populations of *A. albus* are missing.

The present study includes karyological and morphological characteristics of the Bulgarian populations of *A. albus*. The presented data is new for the country and could ease the further identification of this species and guarantee its future identification.

Materials and Methods

Karyological and morphological analysis of 7 Bulgarian populations of the species, on the territory of 5 floristic regions were completed (Table 1). The chromosome numbers and chromosome morphology were established. The chromosome number was determined on permanent squash preparations of the metaphase plates from the root tips of seeds germinated in laboratory conditions, gathered from the natural habitats of the species. The root tips were treated according to the methodology of Grozeva (2007). The metaphase plates were observed on light microscope Olympus BX51 with microscope zoom X100-oil and captured with camera U-TVO.5XC-3 with camera 15X zoom. The data were treated with the help of Adobe Photoshop 2023 and Karyo Type Win 2018 software. The type of the chromosomes was determined through the centromeric index I = s / s + l, according to the methodology of Grif & Agapova (1986). The interchromosome asymmetry was calculated by means of index A2 (Zarco, 1986). To determine intrachromosome asymmetry the following indexes were used: general shape stated as percentage - TF% (Huziwara, 1962); percentage of karyotype asymmetry - Ask% (Arano, 1963); symmetry index - Syi (Greilhuber & Speta, 1976); intrachromosome asymmetry A1 (Zarco, 1986); asymmetry level A (Watanabe et al., 1999); the four categories of Stebbins (1971) - SKS: from A to D according to the arm ratio and the centromere location in the chromosome. Each of the four categories has three subtypes determined according to the largest/smallest chromosomes ratio (Table 2). The following coefficients were used also: coefficient of variation of centromeric index (Paszko, 2006) – CVCI, coefficient of variation of chromosome length (Paszko, 2006) – CVCL, mean centromeric asymmetry (Peruzzi and Eroglu, 2013) – MCA, index of chromosomal size resemblance (Greilhuber & Speta, 1976) – Rec%, dispersion index (Lavania & Srivastava, 1992) – DI, and asymmetry index (Paszko, 2006) – AI.

Table 1. Data about the studied *A. albus* L. populations in Bulgaria.

Location	Floristic regions	Coordinates	Altitude (m)	Area, m²	Numbers
Plovdiv	Thracian plane	N42º08.017″ E024º48.049″	155	2000	120
Plodovitovo	Thracian plane	N42º10.359″ E025º12.405″	161	320	250
Elin Pelin	Sofia region	N42º62.844'' E023º55.779''	554	600	80
Byala	North-Eastern Bulgaria	N43º46.860'' E025º66.948''	16	1000	50
Pavlikeni	Danubian plain	N43º24.443'' E025º30.841''	87	200	80
Petrich	Struma valley	N41º40.827'' E023º20.888''	93	3000	250
Sandanski	Struma valley	N41º54.215'' E023º25.155''	95	2000	80

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 - 099**	**1.0**
< 2:1	1 A	1 B	1 C	1 D
2:1 - 4:1	2 A	2 B	2C	2 D
> 4:1	3 A	3 B	3 C	3 D

Scanning electron-microscopic method (SEM) was used for a more detailed study of the morphology of the pollen and seeds. The study was conducted in the laboratory of the Faculty of Chemistry and Pharmacy of the University of Sofia "St. Kliment Ohridski". Herbarized plant parts were used for the work. The herbarized materials were mounted on a metal pedestal, covered with gold particles in an ionization chamber and observed with a scanning electron microscope (JEOL 5510). The used pollen terminology is in accordance with Erdthman (1952), Kremp (1965), Walker & Doyle (1975). Determined were the following morphological characteristics: 1) Pollen diameter D1 (maximum diameter, μm) – D1; 2) Polar axis D2 (diameter, perpendicular to D1, μm) – D2; 3) Distance among three adjacent pores forming a triangle with sides as close as possible to the highest grain focus (μm) – C; 4) C/D1 ratio; 5) Pollen radius – R (μm); 6) Pollen area – A (μm^2); 7) Total number of pores - TNP; 8) Pore density; 9) Pore diameter (μm) – d; 10) Pore area (μm^2); 11) Number of spinules in the pores – NSP; 12) Number of spinules over 100 μm^2 – NS / 100 μm^2; 13) Polar shape; 14) Equatorial shape. The methodology suggested by Toderich (2008) was used. For the morphological characterization of the seeds was used the terminology adopted by Costea et al. (2001). The following terms were used: seed length, seed width, sculpture covering, seed edge. The qualitative characteristics describing the form and color of the seed were noted.

Results and Discussion

Karyology

As a result of the karyological study of *A. albus* in Bulgaria for each of the studied populations was found diploid chromosome number 2n = 2x = 32. The karyomorphological data are presented in Table 3 and 4. These results are in accordance with the results of Dmitrieva et al. (1986), Queiros (1989), Song et al. (2002) and Probatova et al. (2021). In contrast, Sharma & Banik (1965) reported 2n = 34 from India. Cheshmedziev (1994) determined for the population of *A. albus* in the Thracian plane a diploid chromosome number 2n = 2x = 32, but did not describe the karyotype. For the studied populations were registered 4 types of chromosomes - metacentric, submetacentric, telocentric and subtelocentric, while dominant in all are the metacentric chromosome pairs. The ideograms are shown in Figure 1.

Table 3. Karyomorphometric data for the representatives of *A. albus* L. in Bulgaria.
Legend: Chromosome size variation (μm) – short (S) and long (L); total sum of the haploid chromosome length (μm) - hcl.

Location	Karyotype formula	L	S	L/S	hcl
Plovdiv	2n = 2x = 11m + 1m^{sat} + 4sm	1.02	0.69	10.79	27.39
Plodovitovo	2n = 2x = 12m + 1m^{sat} + 2sm + 1sm^{sat}	0.73	0.54	3.97	21.13
Elin Pelin	2n = 2x = 12m+ 1m^{sat} + 1sm^{sat} + 1st^{sat} + 1t	0.64	0.41	6.32	18.80
Byala	2n = 2x = 14m + 2sm	0.50	0.41	3.84	14.57
Pavlikeni	2n = 2x = 15m + 1sm	0.79	0.63	2.89	22.70
Petrich	2n = 2x = 13m + 1m^{sat} + 1sm + 1st	0.72	0.51	6.07	19.75
Sandanski	2n = 2x = 14m + 2sm	0.70	0.51	3.74	19.39

Table 4. Karyomorphometric data for the representatives of *A. albus* L. in Bulgaria.

Location	CVCI	CVCL	MCA	A2	SKS	TF%	Ask%	Syi%	A1	A	Rec%	DI	AI
Plovdiv	13.4	65.73	16.54	0.66	2C	40.38	59.62	67.72	0.27	0.17	26.49	31.3	8.81
Plodovitovo	13.46	31.89	15.84	0.32	2B	41.48	58.52	70.88	0.26	0.16	48.43	17.21	4.29
Elin Pelin	19.67	40.79	22.65	0.41	2C	37.07	62.93	58.91	0.35	0.23	43.4	21.08	8.02
Byala	11.17	31.00	9.84	0.31	2B	44.92	55.08	81.57	0.17	0.10	58.36	15.20	3.46
Pavlikeni	7.84	28.88	11.62	0.29	2B	44.28	55.72	79.48	0.20	0.12	63.98	13.25	2.27
Petrich	12.4	43.41	15.57	0.43	2C	41.51	58.49	70.97	0.26	0.16	49.23	17.57	5.38
Sandanski	11.47	26.57	15.17	0.27	2B	42.23	57.77	73.09	0.25	0.15	57.60	12.11	3.05

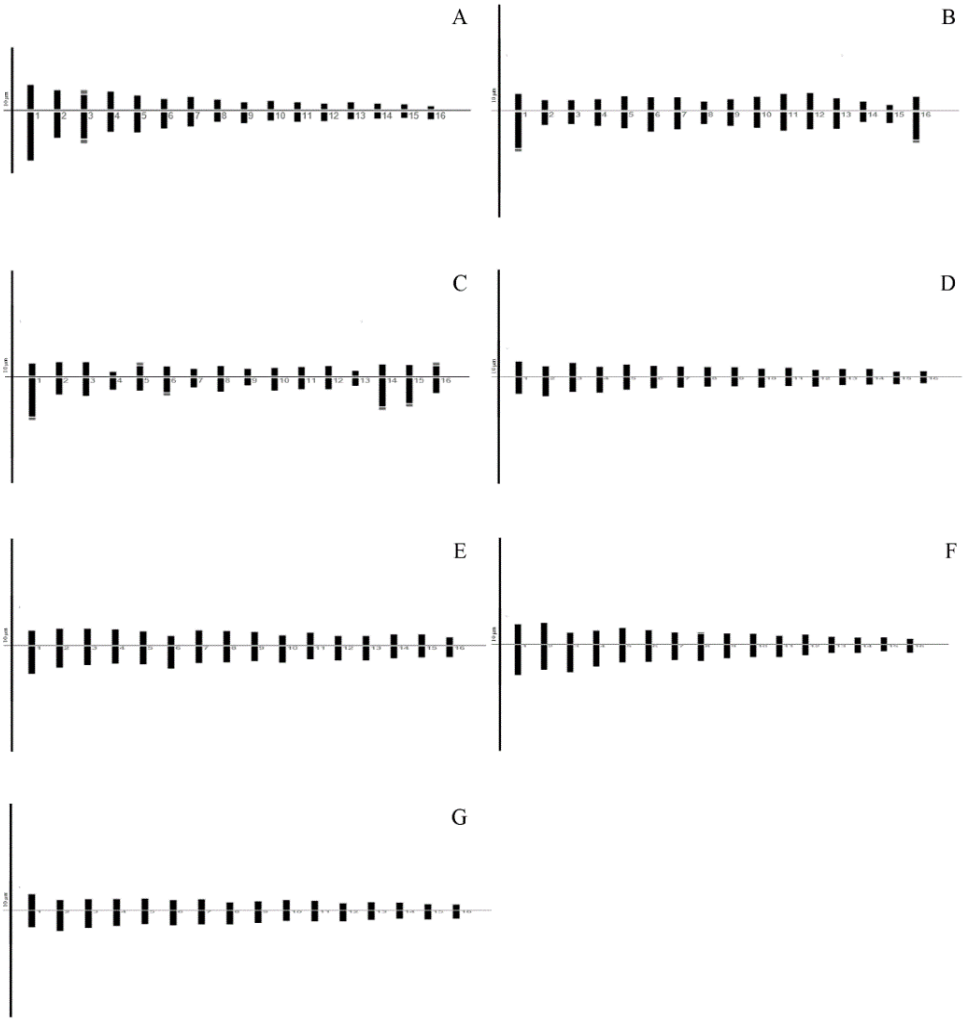

Fig. 1. Ideograms of the studied populations of *A. albus* L.: A) Plovdiv; B) Plodovitovo; C) Elin Pelin; D) Byala; E) Pavlikeni; F) Petrich; G) Sandanski; rock – 10 μm.

The longest chromosome is 1.02 μm and it is registered for the population from Plovdiv, while the shortest – 0.41 μm for the populations of the towns Byala and Elin Pelin. The sum of the length of the haploid chromosome number in the studied populations ranged from 14.57 μm for the population from Byala to 27.39 μm for the population from Plovdiv.

According to the classification of Stebbins (1971) the established karyotypes for the populations of *A. albus* are type 2B, 2C. The most symmetric, in accordance with the data for the chromosome index

A1, is the karyotype from the population of Byala, and the most asymmetric is that from Plovdiv. The values of the rest of the chromosome indexes for asymmetry confirm these data. The results are new for Bulgaria.

Morphology

The results from the SEM studies showed that for all studied individuals from the 7 populations of *A. albus* the pollen was spherical-shaped, polypantoporate (Table 5, Fig. 2-4).

Table 5. Some important palynological characteristics of the studied populations of *A. albus* in Bulgaria.

Population	D1, µm	D2, µm	D, µm	C, µm	C/D	R, µm	A, µm	TNP	pore density	d pore, µm	pore area, µm²	NSP	NS/100 µm²	Polar shape	Equatorial shape
Plovdiv	17.25	17.04	17.14	3.39	0.198	8.57	932.64	26.67	0.005	1.85	2.80	5.53	540.67	circular	elliptic-trucate
Plodovitovo	17.81	17.52	17.67	2.87	0.162	8.83	980.20	29.33	0.006	1.83	2.66	6.55	543.00	circular	elliptic-trucate
Elin Pelin	17.81	16.33	17.07	3.06	0.179	8.54	916.30	31.33	0.007	1.53	1.84	5.84	506.67	circular	elliptic-trucate
Byala	17.76	16.33	17.05	3.27	0.192	8.52	912.70	28.67	0.006	1.71	2.32	5.62	885.67	circular	elliptic-trucate
Pavlikeni	17.67	17.05	17.36	2.99	0.172	8.68	946.54	28.67	0.006	1.74	2.42	4.81	686.00	circular	elliptic-trucate
Petrich	17.79	16.86	17.32	2.52	0.145	8.66	943.55	39.00	0.008	1.19	1.11	4.08	808.50	circular	elliptic-trucate
Sandanski	17.71	17.00	17.36	3.14	0.181	8.68	948.70	34.00	0.007	1.50	1.77	6.43	563.50	circular	elliptic-trucate

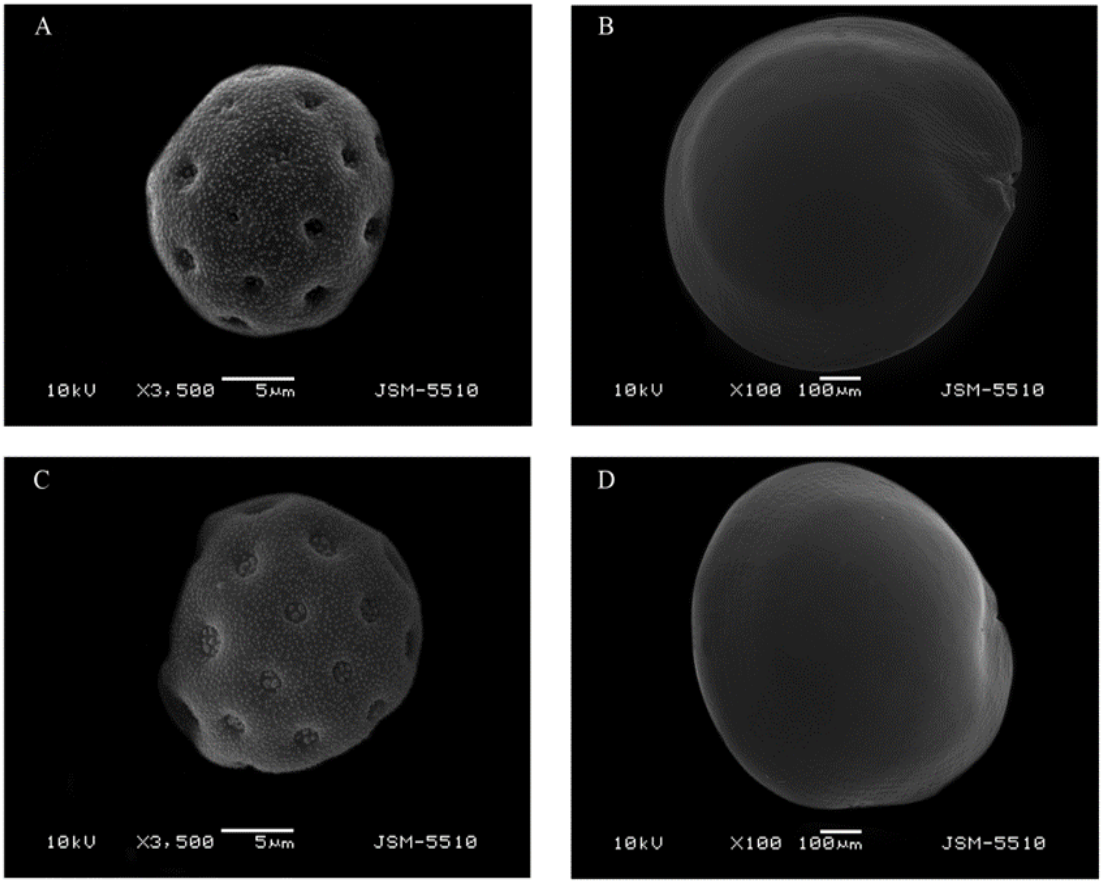

Fig. 2. Scanning electron-microscopic images of pollen and seed of *A. albus* L.: A) and B) Plovdiv; C) and D) Plodovitovo.

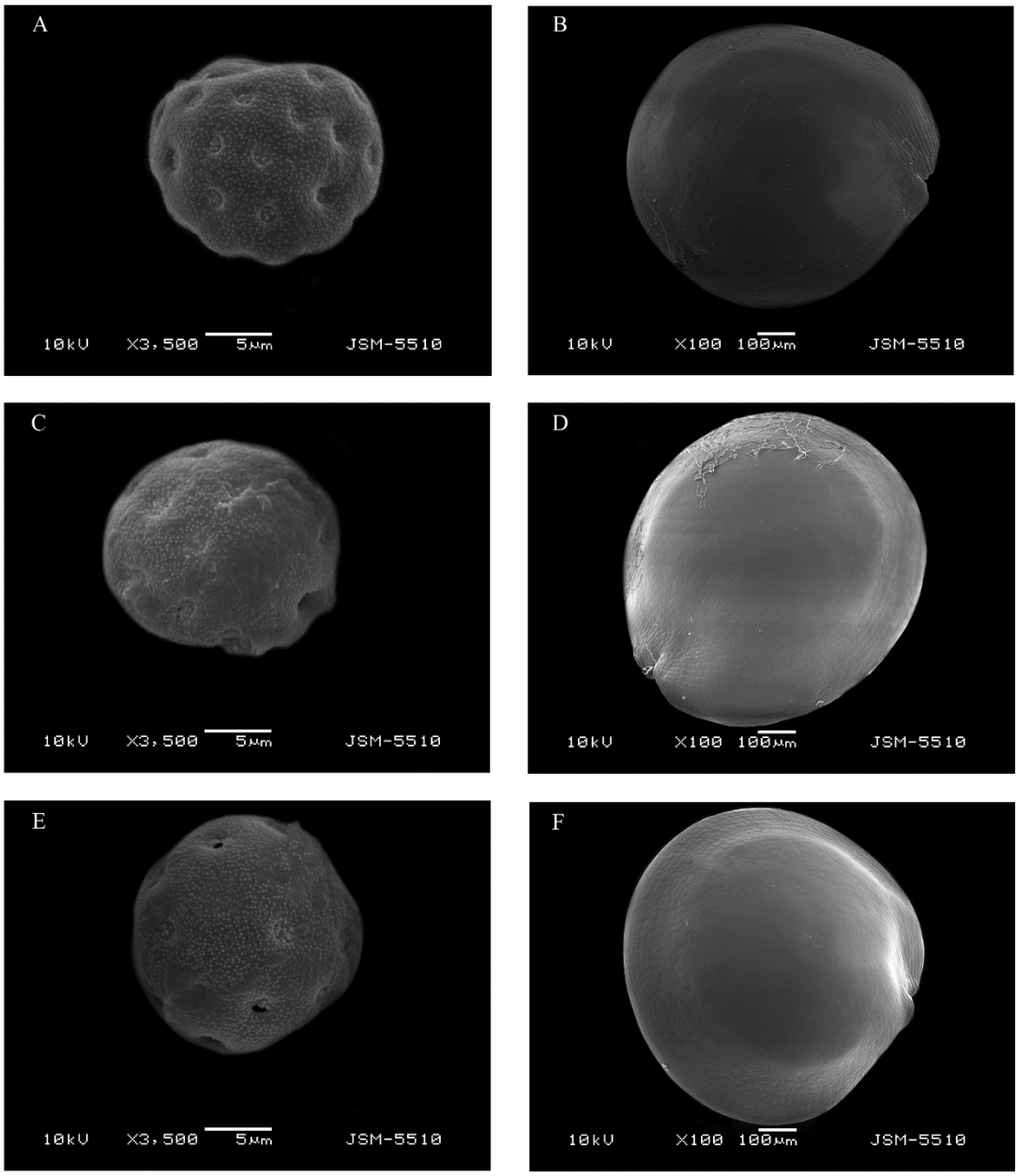

Fig. 3. Scanning electron-microscopic images of pollen and seed of *A. albus* L.: A) and B) Elin Pelin; C) and D) Byala; E) and F) Pavlikeni.

The diameters ranged from 16.33 µm to 17.81 µm, with a mean of 17.28 µm. The distance between three adjacent pores ranged from 2.52 µm for the population from Petrich to 3.39 µm for the one from Plovdiv, for which was established the highest values of pore diameter – 1.85 µm. In this population was also registered the pore area. The biggest area is registered for Plodovitovo village, and the biggest density and number of pores was established for the town of Petrich. The data for the morphology of the pollen allows it to be established as type *Amaranthus*.

The data from the SEM studies of the seeds show that they were smooth, shiny and slightly flattened with a lens-shaped form. Its size varied between 0.8-1.1 mm of length and 0.7-1 mm of width (Fig. 2-4). The seed edge is clearly established, blunt. The seed surface is with the characteristic sculpture in the form of polygonal cells, more pronounced in the periphery, close to the seed edge.

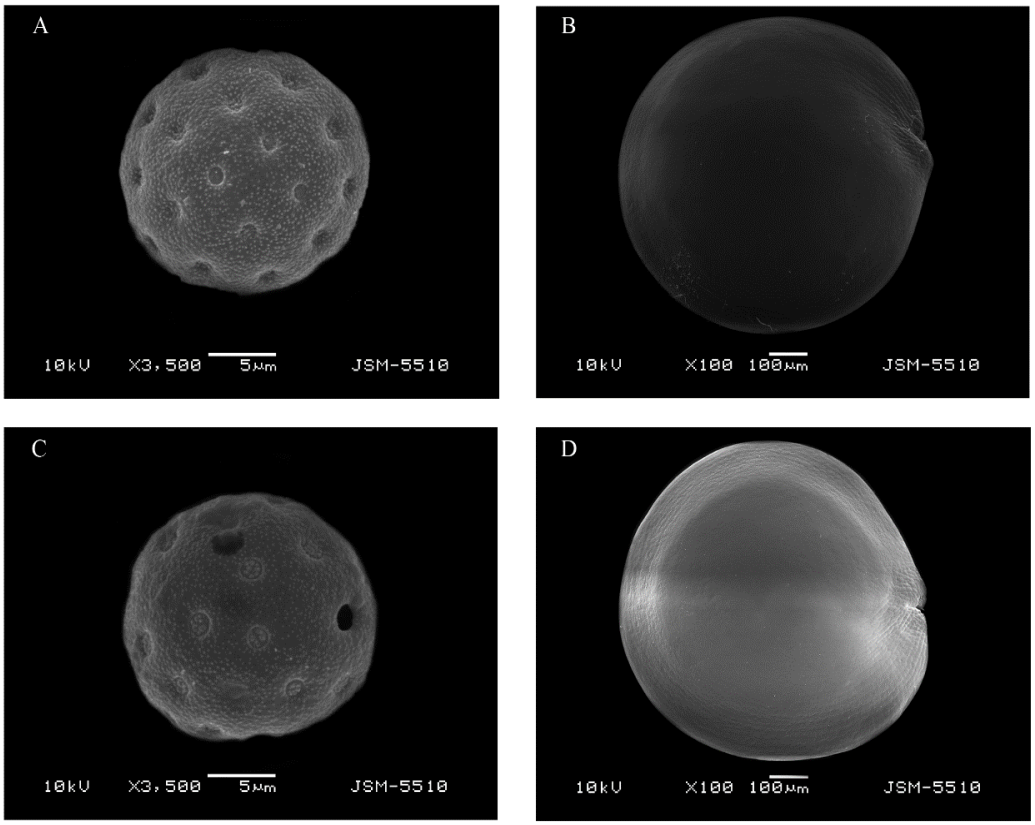

Fig. 4. Scanning electron-microscopic images of pollen and seed of *A. albus* L.: A) and B) Petrich; C) and D) Sandanski.

Conclusions

The species is an annual grass plant and it is a serious danger to agricultural crops. It is found in spring field and vegetable crops, mainly tilled, perennial plants, rare field perennial grasslands and uncultivated stubble fields. In modern agriculture the fight with the representatives from the genus *Amaranthus* is a relevant topic and in order for the fight to be successful it is necessary that the morphological and karyological characteristics of the plant be studied.

The presented results are new for the country. The present study describes for the first time the karyotype of *A. albus* from its Bulgarian populations and confirms the diploid chromosome number 2n = 2x = 32, reported by Cheshmedziev (1994). The studied morphology is useful. The pollen is spherical, type *Amaranthus*. Some important traits and characteristics describing the seed were reported. Its size varies between 0.8-11 mm of length and 0.7-1 mm of width.

Having in mind, on one hand, the invasive nature of the species, and on the other, the lack of a total study of its Bulgarian populations, it is recommended to continue the studies through inclusion of new populations and species. It is necessary to trace the morphological and karyological variability, as well as to search for possibilities and to plan adequate measures for limiting its distribution in natural habitats and agricultural lands.

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