

CHROMOSOME AND POLLEN MORPHOLOGY OF *AMARANTHUS HYBRIDUS* L. AND *AMARANTHUS RETROFLEXUS* L. IN BULGARIA

SVETOSLAVA R. TERZIEVA*, NELI H. GROZEVA

Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, 6000, Stara Zagora, BULGARIA

E-mail corresponding author: sv.terzieva@uni-sz.bg

INTRODUCTION

Genus *Amaranthus* L. (Amaranthaceae Juss.) comprises about 65-80 species, spread mainly in tropical, subtropical and warm-moderate areas. Worldwide, some of the species are spread as introduced and naturalized weeds (Mosyakin & Robertson, 1996, 2003; Bojian et al., 2003; Mujica & Jacobsen, 2003; Iamónico, 2014). The representatives of the genus are predominantly edible food plants and a significant part thereof are categorized as agricultural weeds or pseudocereals all over the world (Costea and Halmajan, 1996; Mosyakin & Robertson, 2003). Some species are grown due to their ornamental qualities (Brenner et al., 2000; Jäger & al., 2008; Shukla et al., 2004, 2006, 2010).

In the Bulgarian flora the genus is represented by 13 species (Kovachev, 1966; Assyov & Petrova, 2012; Petrova, 2018). The present study includes two species - *Amaranthus hybridus* L. and *Amaranthus retroflexus* L. Up to that moment the Bulgarian populations of both species have not been an object of a detailed study. Karyological data are scarce and incomplete, partially due to difficult taxonomic differentiation of species (Pal, 1982). The latest report about the chromosome number of the genus is from the end of the XXth century.

MATERIALS AND METHODS

The present study includes three populations of each species. The data comprise three floristic regions in the country (Table 1). To establish the species chromosome number and karyotype durable preparation were prepared from the metaphase plates of root tips. All root tips were prepared from seeds collected natural habitats of *A. hybridus* and *A. retroflexus* and germinated under laboratory conditions. The root tips were treated and squashed following the methodology by Grozeva (2007). Karyograms and ideograms were been processed by the Adobe Photoshop 2020 and Karyo Type Win 2018 software (Table 3). The morphological characterization of pollen was done by scanning electron microscope JEOL 5510 (Table 5).

Table 5. Some important palynological characteristics of the studied *Amaranthus* taxa (all measured values are in μm)

Populations	D ₁	D ₂	C	C/D ₁	TNT	PD	PA	NS/100 μm^2	NSP	Polar shape	Equatorial shape
<i>Amaranthus hybridus</i> L.											
Elin Pelin	21,78	20,25	3,37	0,152	34,33	1,74	2,38	336	8,45	circular	elliptic-truncate
Pavel banya	21,57	19,89	3,64	0,167	33,20	1,84	2,66	414	10,00	circular	elliptic-truncate
Plovdiv	20,93	20,30	3,97	0,176	40,00	1,62	2,06	486	9,50	circular	elliptic-truncate
<i>Amaranthus retroflexus</i> L.											
Asenovgrad	18,57	14,93	3,57	0,190	32,00	1,65	2,14	279	7,25	circular	elliptic-truncate
Zvanichevo	22,43	22,00	3,37	0,160	42,00	1,84	2,65	331	6,00	circular	elliptic-truncate
Plovdiv	21,43	19,00	3,81	0,200	34,66	1,69	2,24	369	8,00	circular	elliptic-truncate

RESULTS AND DISCUSSIONS

As a result of the karyological study of the species *A. hybridus* and *A. retroflexus* in Bulgaria diploid chromosome number $2n = 34$ was established (Table 1). Two types of chromosomes were reported: meta- and submetacentric. Predominant in both species are the metacentric chromosomes (Table 3, Figure 1). The chromosome size varies from 0,74 μm for the representatives from Elin Pelin to 1,68 μm for the ones from Zvanichevo. From all studied karyological data the shortest arm is 0,11 μm long and the longest one - 1,38 μm . The total sum of the haploid chromosome length is within small limits - from 11,44 μm to 13,50 μm for the *A. hybridus* populations and from 13,01 μm to 23,32 μm for *A. retroflexus*. The base chromosome number for the three studied *A. hybridus* populations is $n = 17$. The karyotype formulas of the following populations in the country have been established: Elin Pelin - $2n = 34m$, Pavel banya - $2n = 32m + 2sm$ and Plovdiv - $2n = 27m + 7sm$. They do not confirm the diploid chromosome number of $2n = 32$ established so far, but conform to the taxonomic analyses worldwide. The base chromosome number for the three studied *A. retroflexus* populations is $n = 17$. The karyotype formulas of the following populations in the country have been established: Asenovgrad - $2n = 31m + 3sm$, Zvanichevo - $2n = 33m + 1sm$ and Plovdiv - $2n = 31m + 3sm$. The results are new for Bulgaria.

Palynological analysis was made on the basis of ten qualitative and quantitative traits (Table 5, Figure 2). The smallest pore diameter was measured in the population of *A. hybridus* from Plovdiv. The highest value of the diameter - 1,84 μm was measured in both species. Pollen area varies from 2,06 μm to 2,66 μm . The smallest total number of spinules per 100 μm^2 - 279 and number of spinules in the pores - 7,25 were reported in the population from Asenovgrad. The currently existing morphological characteristics of pollen give grounds to classify it to *Amaranthus* type with pores type II typical of that type of pollen (BORSCH, 1998). Our data largely confirm the already published ones. Pollen in all six populations has typical spherical shape covered by numerous perforations. It is characterized by small size and a big number of pores. Difference between the species is found in the pollen size, which is bigger in *A. retroflexus* (18,57 - 22,43 μm). The C index is high (3,81 μm , 3,97 μm) in both species. The values have been reported for the same region. That could be due to the same conditions of the environment and could be used as a chorological trait in current studies of the species (Arora, Modi 2008).

Table 1. Data about the studied *A. hybridus* L. and *A. retroflexus* L. populations in Bulgaria.

Species	Population locality	Coordinates	Altitude (m)	Floristic regions	2n
<i>A. hybridus</i> L.	Elin Pelin	N42°6'04.70" E023°59'01.7"	544	Sofia Region	34
	Pavel banya	N42°35.344" E025°12.515"	406	East Sredna Gora	34
	Plovdiv	N42°08.086" E024°47.862"	157	Thracian Plane	34
<i>A. retroflexus</i> L.	Asenovgrad	N42°00.745" E024°52.317"	238	Thracian Plane	34
	Zvanichevo	N42°11.380" E024°15.000"	221	Thracian Plane	34
	Plovdiv	N42°08.017" E024°48.049"	155	Thracian Plane	34

Table 3. Karyomorphometric data for the representatives of *A. hybridus* and *A. retroflexus* in Bulgaria

Population	Karyotype formula	S	L	hcl	Inter index	Intrachromosomal index					
						A ₂	SKS	TF%	Ask%	Sy _i	A ₁
<i>A. hybridus</i> L.											
Elin Pelin	$2n = 34m$	0,17	0,57	11,44	0,21	1B	44,78	55,22	81,11	0,19	0,11
Pavel banya	$2n = 32m + 2sm$	0,17	0,84	13,00	0,44	1C	45,27	54,73	82,72	0,18	0,10
Plovdiv	$2n = 27m + 7sm$	0,13	1,00	13,50	0,37	2C	42,10	57,90	72,71	0,26	0,15
<i>A. retroflexus</i> L.											
Asenovgrad	$2n = 31m + 3sm$	0,11	1,38	13,01	0,74	1C	44,34	55,66	79,66	0,19	0,11
Zvanichevo	$2n = 33m + 1sm$	0,39	1,29	23,32	0,31	1B	44,88	55,12	81,43	0,17	0,10
Plovdiv	$2n = 31m + 3sm$	0,16	1,33	13,58	0,47	2C	42,53	57,47	74,01	0,24	0,14

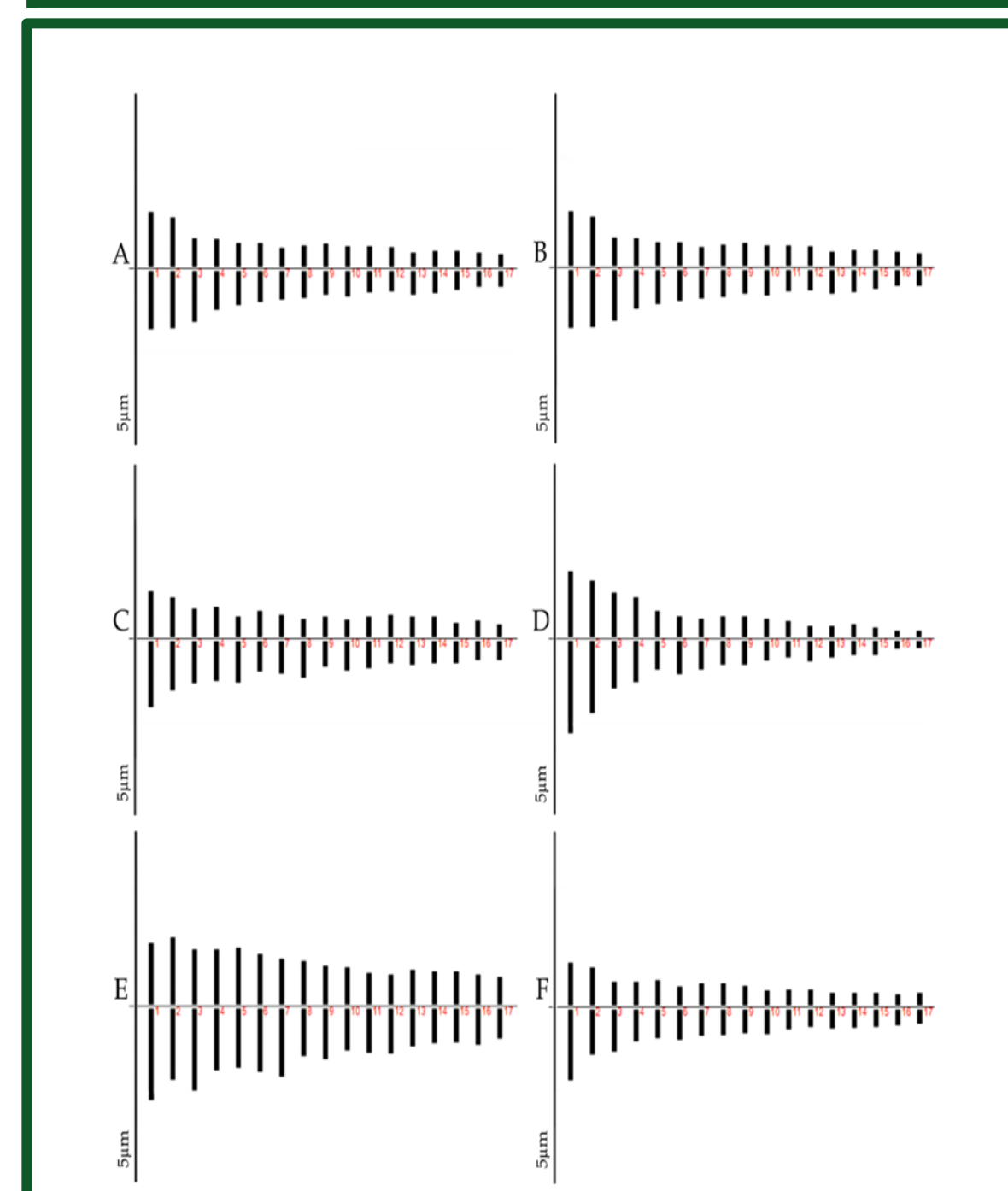


Fig. 1. Idiograms of *Amaranthus hybridus* and *Amaranthus retroflexus*, $2n = 34$.
A. *hybridus*: A) Elin Pelin, B) Pavel banya, C) Plovdiv;
A. *retroflexus*: D) Asenovgrad, E) Zvanichevo, F) Plovdiv; scale bar 5 μm .

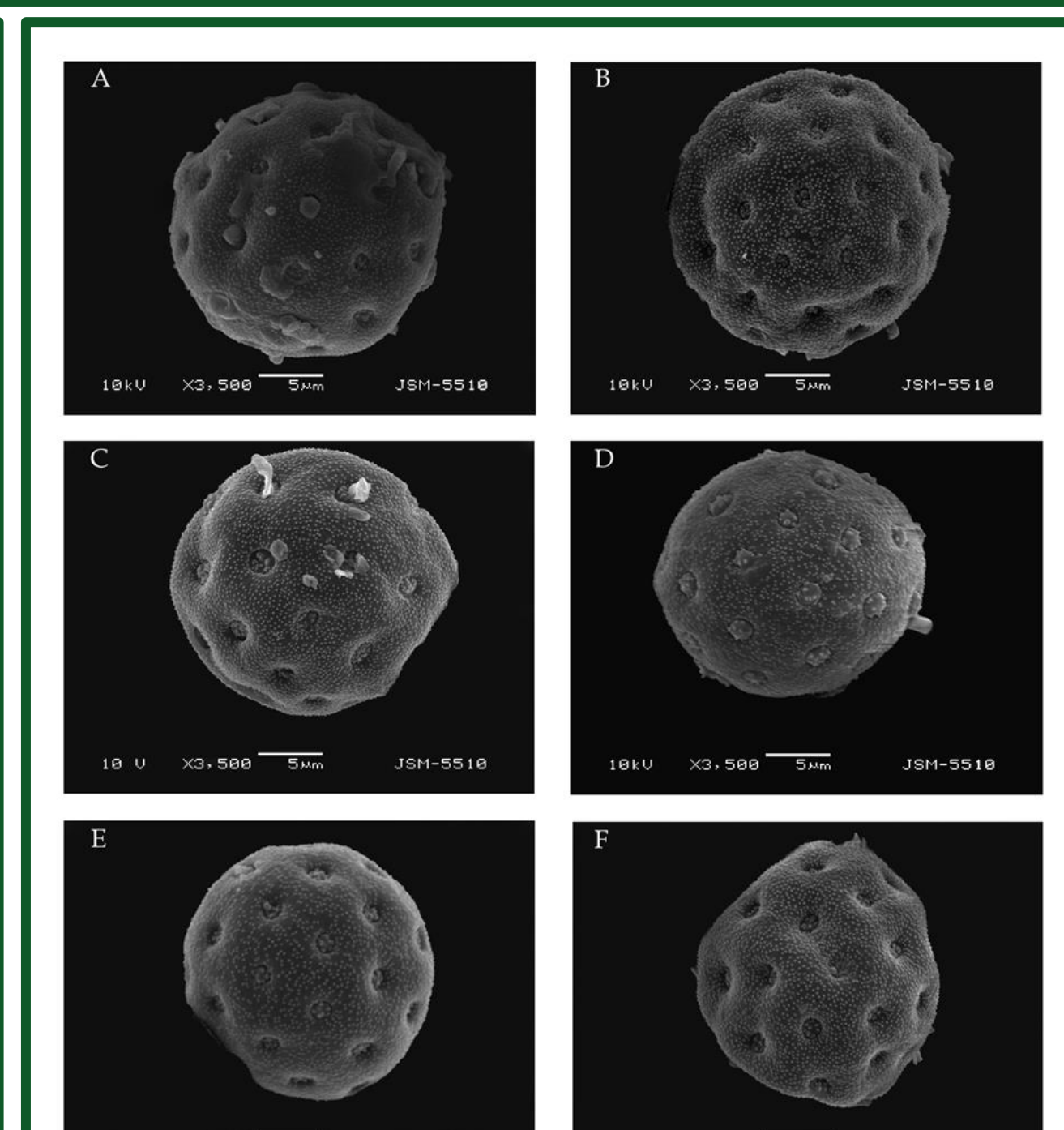


Fig. 2. Pollen microphotography.
Amaranthus hybridus: A) Elin Pelin, B) Pavel banya, C) Plovdiv;
Amaranthus retroflexus: D) Asenovgrad, E) Zvanichevo, F) Plovdiv.