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Morphological studies on three *Amaranthus* species

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Abstract

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The study examined indumentum, stomata and pollen morphology of *Amaranthus deflexus* L., *A. hybridus* L. and *A. retroflexus* L. from their Bulgarian populations. Mature pollen and herbarized plant parts have been observed and photographed with a scanning electron microscope (SEM). The indumentum of the three species is of multicellular, un-branched, uniseriate, glandular trichomes. Three types of stomata have been registered – anomocytic, paracytic and anisocytic with the latter type being the dominant one. Paracytic type were observed only in *A. deflexus.* Pollen grains were spheroidal, pantoporate, scrabrate, with diameter from 15.4 to 24.2 μm. Among the studied three species differences in pollen morphology have been found in *A. deflexus.*

Keywords: A. deflexus; *A. hybridus*; *A. retroflexus*; indumentum; stomata; pollen

Introduction

The genus *Amaranthus* L. (Amaranthaceae Juss.) consists of about 65-80 species, which are mostly prevalent in tropical, subtropical, and warm-temperate zones, some taxa are at present almost worldwide as introduced and naturalized weeds (Mosyakin & Robertson, 1996; Bojian et al., 2003; Mosyakin & Robertson, 2003; Mujica & Jacobsen, 2003; Iamonico, 2014). The representatives of the species are mostly edible and nutritious plants and a considerable part of them are problematic agricultural weeds worldwide (Mosyakin & Robertson, 2003).

In Bulgarian flora *Amaranthus* is represented by 13 species (Kovachev, 1966; Assyov & Petrova, 2012; Petrova, 2018). Its representatives are usually found in ruderalized habitats and very often they are among the weeds, especially in row crops. The subjects of the present study were 3 species from genus *Amaranthus – A. deflexus, A. hybridus, A. retroflexus.*

A. deflexus originates from South America and is relatively widely distributed in the country from sea level up to 600 m a.s.l. It forms populations along roads, gardens, registered as a weed in row crops, as well (Kovachev, 1966).

A. hybridus is native to tropical and subtropical America. Widespread weed in crop-fields, gardens, orchards and vegetable crops, often in irrigated areas. It is considered one of the most dangerous weeds in the world. Recorded in all floristic regions, up to about 1000 m a.s.l. (Petrova et al., 2013).

A. retroflexus is native to tropical and subtropical America. It is recorded in all floristic regions, up to about 1000 m a.s.l. The species was found in man-made habitats – abandoned, ruderal places, as a weed in spring field and vegetable crops, especially row crops, orchards and vineyards, stubble fields, along roads and railways, waste places (Petrova et al., 2013).

A. hybridus and *A. retroflexus* were introduced in Europe in the 19th century, naturalized and invasive species in many countries, including Bulgaria (Petrova et al., 2013).

Up to this moment there is no data in scientific literature about *A. deflexus, A. hybridus, A. retroflexus* from their Bulgarian populations. The objective of the present research is to study pollen morphology, stomata and indumentum of the three species.

Materials and Methods

Terrain studies were conducted during the vegetation period of 2018. A total of 3 populations from *A. deflexus, A. hybridus, A. retroflexus* were researched (Table 1).

To study the morphology of stomata, pollen and details of the indumentum Scanning Electron Microscope (SEM) has been used. At least 15 pollens, and herbarized branches with leaves and flowers have been examined from each population. The study has been performed in the laboratory of the Faculty of Chemistry and Pharmacy at Sofia University "St. Kliment Ohridski". The samples were coated with gold, viewed, and photographed. The fastening of the contact holder was made with a silver paste.

To trace pollen morphology the technique for observation of air-dried pollen has been used. Anthers were isolated from flowers, put into petri dishes and kept at room temperature for 48 h. The pollen was sprinkled on microscope slides and dried at room temperature. The following traits have been established: pollen diameter; number of apertures on the visible surface area of the pollen; aperture density; diameter of apertures. Pollen diameter was determined as the mean arithmetic value of the x-axis and y-axis. Aperture density was determined as a function of visible surface area and aperture number. The visible surface area was taken as one-half the surface area of the sphere. Aperture density was calculated as the visible aperture number was divided by the visible surface area (μm^{-2}).

The data from the Scanning Electron Microscope have been presented for each species due to lack of significant variability in and among their populations.

The voucher specimens are kept in the herbarium of the Agricultural University, Plovdiv (SOA).

Results and discussion

Indumentum

Stems, leaves and branches of the inflorescences of *A. deflexus* were coated with multicellular, un-branched, uniseriate, glandular trichomes (Fig. 1, A-B). On the stem and the branches of the inflorescences the hairs are scattered rarely and were usually of 7-9 cells and with a total length of 0.04 to 0.54 mm. The leaves had hairs of 4-6 cells and a length of 0.04 to 0.2 mm, also rarely spaced and on the leaf petiole the hairy coating is along the midrib only.

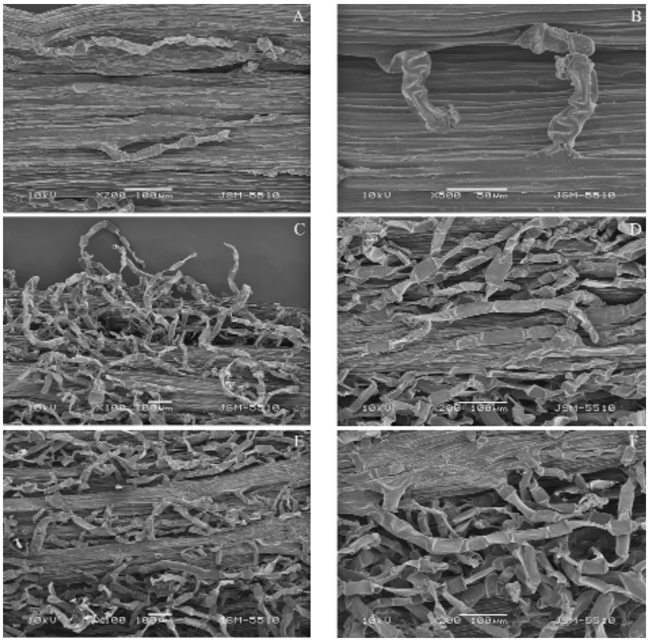

Fig. 1. Scanning electron micrographs showing indumentum of studied *Amaranthus* species: A-B stem and leaf petiole surface of *A. deflexus*; C-D stem and leaf petiole surface of *A. hybridus*; E-F stem and leaf petiole surface of *A. retroflexus*

Table 1. Studied *Amaranthus* populations

Species	Population	Locality	Floristic region
A. deflexus L.	Stara Zagora	N42°25.145″, E025°37.220″, 205 m a.s.l.	Thracian Plane
	Shabla	N43°32.532″, E028°32.607″, 0 m a.s.l.	Black Sea coast (North)
	Strelets	N42°17.542″, E025°44.804″, 172 m a.s.l.	Thracian Plane
A. hybridus L.	Zvanichevo	N42°11.380″, E024°15.000″, 221 m a.s.l.	Thracian Plane
	Varnensko lake	N43°12.183″, E027°47.999″, 7 m a.s.l.	Black Sea coast (North)
	Nova Zagora	N42°29.910″, E026°00.337″, 124 m a.s.l.	Tundzha Hilly Plain
A. retroflexus L.	Shipka	N42°42.832″, E025°19.844″, 538 m a.s.l.	Tundzha Hilly Plain
	Balchik	N43°24.587″, E028°09.914″, 65 m a.s.l.	North-Eastern Bulgaria
	Velingrad	N42°01.519″, E023°59.659″, 715 m a.s.l.	West Rhodope Mts

Stems, leaf petioles and branches of the inflorescences of *A. hybridus* were densely coated with multicellular, un-branched, uniseriate, glandular trichomes of 5-9 cells and length of 0.4 to 0.6 mm (Figure 1, C-D). On the leaf lamina and flowers hairs were rarely spaced and usually had 5-8 cells and length of 0.1-0.3 mm, while the stem and leaf petioles had denser indumentum.

Stems, leaves and branches of the inflorescences of *A. retroflexus* were densely coated with multicellular, un-branched, uniseriate, glandular trichomes 0.11 to 0.6 mm long with the densest hairy coating on the stem and the branches of the inflorescence (Fig. 1, E-F). Most often hairs consisted of 7-10 cells.

Trichomes may have a characteristic form in a species and possess taxonomic value (Esau, 1977). The types of trichomes vary in Amaranthaceae (Metcalfe & Chalk, 1950). According to El-Ghamery et al. (2017) in *A. deflexus* and *A. hybridus* the hairy coating is of uniseriate, glandular, multicellular, unbranched trichomes, while in *A. retroflexus* it is of uniseriate and biseriate, multicellular, glandular, unbranched trichomes. Our data about the first two species correlate fully with the ones by El-Ghamery et al. (2017), but in the studied samples from the Bulgarian populations of the third species – *A. retroflexus* – biseriate trichomes were not registered. Of course, this does not exclude their existence.

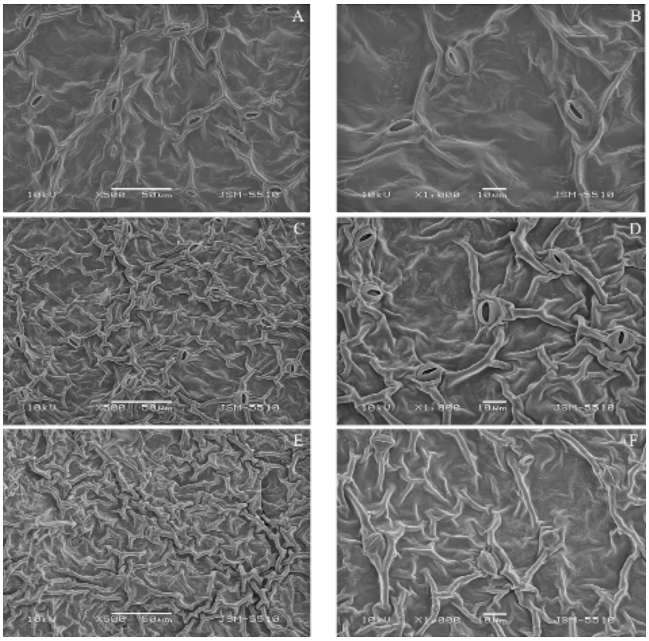

Fig. 2. Scanning electron micrographs showing stomata of studied *Amaranthus* species: A-B abaxial and adaxial leaf surface of *A. deflexus*; C-D abaxial and adaxial leaf surface of *A. hybridus*; E-F abaxial and adaxial leaf surface of *A. retroflexus*

Stomata

Surface view of both sides of the leaf petiole of the studied specimens from the studied populations of *A. deflexus* showed anisocytic, anomocytic and paracytic stomata with the first type – anisocytic – being the dominant one (Fig. 2, A-B). This type was also found along the stem of the studied plants.

In the second studied species *A. hybridus* two types of stomata – anomocytic, anisocytic – were registered along the stem and on both sides of the leaf petiole in all studied specimens from the three populations with the second type being prevalent (Fig. 2, C-D).

In the populations from the thirst species *A. retroflexus* two types of stomata were also registered – anomocytic and anisocytic, with the anisocytic stomata being prevalent again (Fig. 2, E-F).

According to Aege & Daudu (2014) anisocytic and paracytic stomata types were the most common types of stomata on both the adaxial and abaxial surfaces of the genus *Amaranthus*, while anomocytic type was found occasionally at low frequency among the studied species, this suggested that anomocytic is a recent stomata type that can be used for the delimitation of the genus *Amaranthus*. Our data from the Bulgarian populations of the three species complemented the information known from other parts of their area and confirmed the dominating presence of anomocytic type stomata. Aege & Daudu (2014) specified for *A. hybridus* three types of stomata:

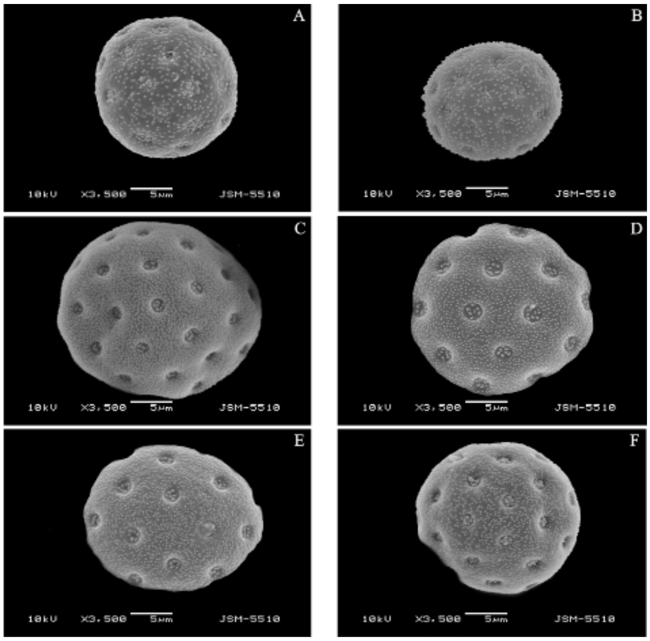

Fig. 3. Scanning electron micrographs of pollen in studied *Amaranthus* species: A-B *A. deflexus*; C-D *A. hybridus*; E-F *A. retroflexus*

Table 2. Comparison of pollen characteristics of *Amaranthus deflexus* L., *A. hybridus* L. and *A. retroflexus* L.

Species	Pollen diameter	Surface area of the pollen	Apeture number	Aperture density	Diameter of apertures
	µm	µm	No. visible	No. μm^{-2}	µm
A. deflexus L.	17.24	937.50	17	0.004	1.79
A. hybridus L.	19.42	1315.50	15	0.002	1.72
A. retroflexus L.	20.04	1263.42	15	0.002	2.05

anisocytic (55%), paracytic (40%), and anomocytic (10%). El-Ghamery et al. (2017) found for *A. deflexus* and *A. retroflexus* anomocytic type of stomata and for *A. hybridus* found anomocytic and hemiparacytic type of stomata.

Pollen morphology

As seen from the SEM images of the *Amaranthus* species, pollen grains were spheroidal, pantoporate and surface sculpturing was scabrate.

The diameter of the pollen grains of *A. deflexus* ranged from 15.4 to 18.5 µm, with a mean of 17.2 µm (Table 2). The number of apertures on the visible surface area of the pollen was between 15-19 and their diameter varied from 1.4 to 1.9 µm.

For *A. hybridus* the diameter of the pollen grains was found to be from 17.4 to 24.2 µm, with a mean of 19.4 µm (Table 1). The number of apertures on the visible surface area of the pollen was between 17-24 and their diameter varied from 1.7 to 2.3 µm.

The diameter of the pollen grains registered for *A. retroflexus* was from 19.5 to 21.2 µm, with a mean of 20.4 µm (Table 1). The number of apertures on the visible surface area of the pollen was between 15-22 and their diameter varied from 1.7 to 2.2 µm.

According to Iwanami et al. (1988) morphology of the pollen grain was generally a conserved characteristic, which was an excellent means for identification of most species. Zhigila et al. (2014) studied palynological characteristics in five species of *Amaranthus,* including *A. hybridus* and the diameter of the pollen grain and pores registered by them was significantly greater than the one found by us for the Bulgarian populations of the species. Seied et al. (2016) studied the pollen of 7 species, including *A. retroflexus.* The characteristics published by them (Seied et al., 2016) about the pollen of *A. retroflexus* were similar to those found by us. Borsch (1998), stepping on profound studies of pollen in Amaranthaceae, distinguished 11 different types of pores and 17 pollen types. The pore characteristics established in the present study of *A. deflexus, A. hybridus, A. retroflexus* correlate to Type II (pores 1.0-2.0 µm in diameter, deeper seated and sharply set off against the tectum) as stated by Borsch (1998) and the pollen type described by the author as *Amaranthus-type* (spheroidal pollen, tectum punctate, with numerous evenly distributed microspines, pores of type II).

Conclusion

The indumentum of *A. deflexus, A. hybridus, A. retroflexus* was of multicellular, un-branched, uniseriate, glandular trichomes. With the densest hairy coating were the stems and branches of inflorescences of the latter 2 species. The hair length varied from 0.06 to 0.6 mm with shorter and more distantly located hairs in *A. deflexus*, and longer and more densely located ones in *A. hybridus* and *A. retroflexus*.

Stomata were evenly located on both sides of the leaf petiole and for *A. deflexus* 3 types have been registered: anomocytic, anisocytic and paracytic stomata, while for *A. hybridus* and *A. retroflexus* have been registered 2 types: anomocytic and anisocytic. In all studied samples prevailed anisocytic stomata type.

The pollen of the 3 species studied is *Amaranthus* type – spheroidal, pantoporate, scrabrate, with diameter from 15.4 to 24.2 µm, pore diameter from 1.4 to 2.3 µm and the number of apertures on the visible surface area of the pollen from 15 to 24. With the smallest dimensions of pollen and pores as well as weaker punctate surface is characterized *A. deflexus.* In *A. hybridus* and *A. retroflexus* the dimensions and morphological features of pollen were similar.

The present study reports for the first time data about indumentum, stomata type and pollen morphology for *A. deflexus, A. hybridus, A. retroflexus* from their Bulgarian populations. Having in mind, on the one hand, the invasive nature of the latter two species, and on the hand, the lack of complete research from their Bulgarian populations, it is advisable research to continue by including new populations. It‘s necessary to trace the morphological and karyological variability and to point out traits for distinguishing them, as well as to seek opportunities and to plan adequate measures to limit their spreading in natural habitats and agricultural areas.

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