

Palynological study of some Iranian *Amaranthus* taxa

Seyed Mehdi Talebi*, Mitra Noori, Zeinab Nasiri

Department of Biology, Faculty of Science, Arak University, Arak, Iran

*Corresponding author, E-mail: seyedmehdi_talebi@yahoo.com

Abstract

There has been much discussion about infrageneric taxonomy of the genus *Amaranthus*. In the present study, in order to solve taxonomical problems and to aid identification of Iranian *Amaranthus* taxa, the palynological characteristics of seven taxa from two subgenera (*Amaranthus* and *Albersia*) were examined. Pollen grains were investigated with light microscopy and scanning electron microscopy using the prolonged acetolysis procedure. Twelve quantitative and qualitative characteristics were studied. Results showed that all of the studied taxa had poly pantoporate pollen and surface sculpturing was seen as scabrate, while its aggregation differed between samples. Statistical analysis showed that some quantitative morphological features were more valuable in identification of the studied taxa. The studied taxa were separated from each other using multivariate analysis. Our data together with the results of previous investigations confirmed that the present infraspecific classification of *A. blitum* requires change. It is advisable to return to the previous taxonomical status of the genus.

Key words: *Amaranthus*, pollen characteristics, taxonomy.

Abbreviations: PCA, principal coordinate analysis; PCO, principal coordinate ordination; UPMGA, unweighted paired group using average.

Introduction

Approximately 60 *Amaranthus* L. species are native to the Americas, and an additional 25 species are present in the temperate and tropical regions of Africa, Asia, Australia, and Europe (Sauer 1967). Often collectively called “pigweeds,” at least 10 *Amaranthus* species are considered troublesome weeds in the North America (Horak et al. 1994; Wax 1995). The cultivated taxa of the genus are utilized as food grains, leafy vegetables, and forage crops in different geographic areas of America, Asia and Europe (Stallknecht, Schulz-Schaeffer 1993).

The genus *Amaranthus* naturally grows in Iran and is found in several regions of the country (Azadi 2013). There has been much discussion about its species number: Mobayen (1979) reported six species, Ghahreman (1980–2002) more than seven species and Rechinger (1998) 11 species in Iran. These differences were due to hybridization and domestication of some of the species.

Identification of species of *Amaranthus* is necessary for an effective weed management (Mayo et al. 1998). Because of the large genetic and morphological diversity within the genus *Amaranthus*, species identification is difficult. The presence of flowers is required to identify most species. In addition, interspecific hybridization between *Amaranthus* species causes further difficulties in identification, as these hybrids can exhibit characteristics of both parents (Horak et al. 1994).

In modern systematics pollen morphology has been

extremely useful in clarifying systematic relationships within plant taxa. Moreover, palynology of the species and subspecies can have taxonomic value as supporting evidence to morphological and phylogenetic traits. Also, studies on pollen grains morphology is considered as the basic necessity for palynology due to its fundamental value in the recognition and identification of grains found in various conditions (Arora, Modi 2008). Iwanami et al. (1988) considered that the morphology of the pollen grain is generally a conserved characteristic, which is an excellent means for identification of most species.

Previous studies (Eliasson 1988; Costea 1998a; Costea 1998b; Costea et al. 2004) have confirmed that in *Amaranthus* taxa, flowers lack nectar glands and pollen grains are small (diameter 18 to 28 μm), usually with 30 to 45 pores uniformly distributed on their surface. In addition, they are tectate with granules and spinules. The relationship between monoecious and dioecious *Amaranthus* as well as different ploidy level and interspecific hybrids can be determined using pollen grain features. Pollen grains of dioecious species have a larger number of apertures on the visible surface. The arrangement of pores creates high air turbulence, which minimizes the friction between the pollen grain and the air, therefore increasing the distance at which the pollen grain can be dispersed (Franssen et al. 2001). Roulston and Buchmann (2000) believed that *Amaranthus* pollens contain starch (up to 7.5%), which protects them against desiccation.

Studies showed that the genus *Amaranthus* is a

problematic genus from a taxonomic point of view (Brennan 1961; Robertson 1981). As a result, infrageneric classification of *Amaranthus* species in sections or subgenera is always controversial or in correction. Yet, many classification schemes have been proposed for the genus and its species have been classified in several subgenera (Sauer 1955; Mosyakin, Robertson 1996) or subgenera with sections (Carretero 1979; Hugin 1987; Klopper, Robel 1989). In addition, the distinctions between some sections are also not clear.

In order to solve the above mentioned taxonomical problems and to facilitate better identification of Iranian *Amaranthus* taxa, in the present study, palynological characteristics of seven taxa from two subgenera [*Amaranthus* and *Albersia* (Kunth) Gren. & Godr.] were investigated in Iran.

Materials and methods

Plant collection, determination and sample preparation

Plant samples of seven taxa from two subgenera were collected: *Amaranthus*, including *A. coadatus* L. and *A. retroflexus* L.; and *Albersia* (Kunth) Gren. & Godr., including *A. albus* L., *A. blitoides* S. Wats., *A. blitum* L. subsp. *blitum* and *A. blitum* subsp. *emarginatus*. The samples were collected from natural populations in different regions of Tehran Province, Iran during spring and summer of 2013. In each locality three to four individuals were collected randomly. Details of localities are given in Table 1. Flowers of all collected plant species were fixed in Carnoy's solution (a fixative composed of glacial acetic acid and ethanol, 1:3).

Table 1. Localities of samples of the studied taxa of the genus *Amaranthus*

Taxon	Locality	Altitude (m)
<i>A. albus</i>	Tehran province, Firuozkooh.	1930
<i>A. blitoides</i>	Tehran province, Shahr-e Rey	1062
<i>A. blitum</i> subsp. <i>blitum</i>	Tehran province, Robat Karim	1024
<i>A. blitum</i> subsp. <i>emarginatus</i>	Tehran province, Qaleh Morqi	1062
<i>A. coadatus</i>	Tehran province, Shariati Park	1062
<i>A. retroflexus</i>	Tehran province, Jajroud	1133
<i>A. tricolor</i>	Tehran province, Firuozkooh	1900

Light microscopy

Pollen grains were obtained from flowers of inflorescences. For each taxon, three specimens were used, and from each specimen at least three anthers were examined. Pollen grains were prepared for light microscopy and scanning electron microscopy using the prolonged acetolysis procedure (Erdtman 1952). For light microscopy, the pollen grains were mounted in glycerine jelly and sealed with paraffin. Some palynological characteristics, such as equatorial diameter (*E*), polar axis length (*P*), *P/E*, log *P/E*, exine ornamentation and pore dimensions, were described for each sample (Table 2).

Table 2. Some important palynological characteristics of the studied *Amaranthus* taxa (all measured values are in μm)

Taxon	Polar length (P)	Equatorial length (E)	P/E	Pore diameter	Polar length/pore diameter ratio	Equatorial length/pore diameter ratio	Polar shape	Equatorial shape
<i>A. albus</i>	24.5 \pm 1.3	26.0 \pm 1.5	0.94	1.14 \pm 0.75	21.5	22.8	circular	elliptic-truncate
<i>A. blitoides</i>	24.4 \pm 2.0	25.1 \pm 1.7	0.97	1.63 \pm 0.86	15.0	15.4	circular	elliptic-truncate
<i>A. blitum</i> subsp. <i>blitum</i>	20.5 \pm 0.6	21.0 \pm 0.5	0.97	2.45 \pm 1.15	8.4	8.6	circular	elliptic-truncate
<i>A. blitum</i> subsp. <i>emarginatus</i>	23.9 \pm 1.0	25.1 \pm 0.9	1.05	0.98 \pm 1.23	24.4	25.6	circular	elliptic-truncate
<i>A. coadatus</i>	24.5 \pm 1.0	25.3 \pm 0.8	0.95	1.31 \pm 0.98	18.7	19.3	circular	elliptic-truncate
<i>A. retroflexus</i>	24.0 \pm 1.8	25.0 \pm 1.1	0.96	0.81 \pm 0.83	29.6	30.9	circular	elliptic-truncate
<i>A. tricolor</i>	25.4 \pm 0.8	25.9 \pm 0.8	0.98	1.47 \pm 0.73	17.3	17.6	circular	elliptic-acuminate

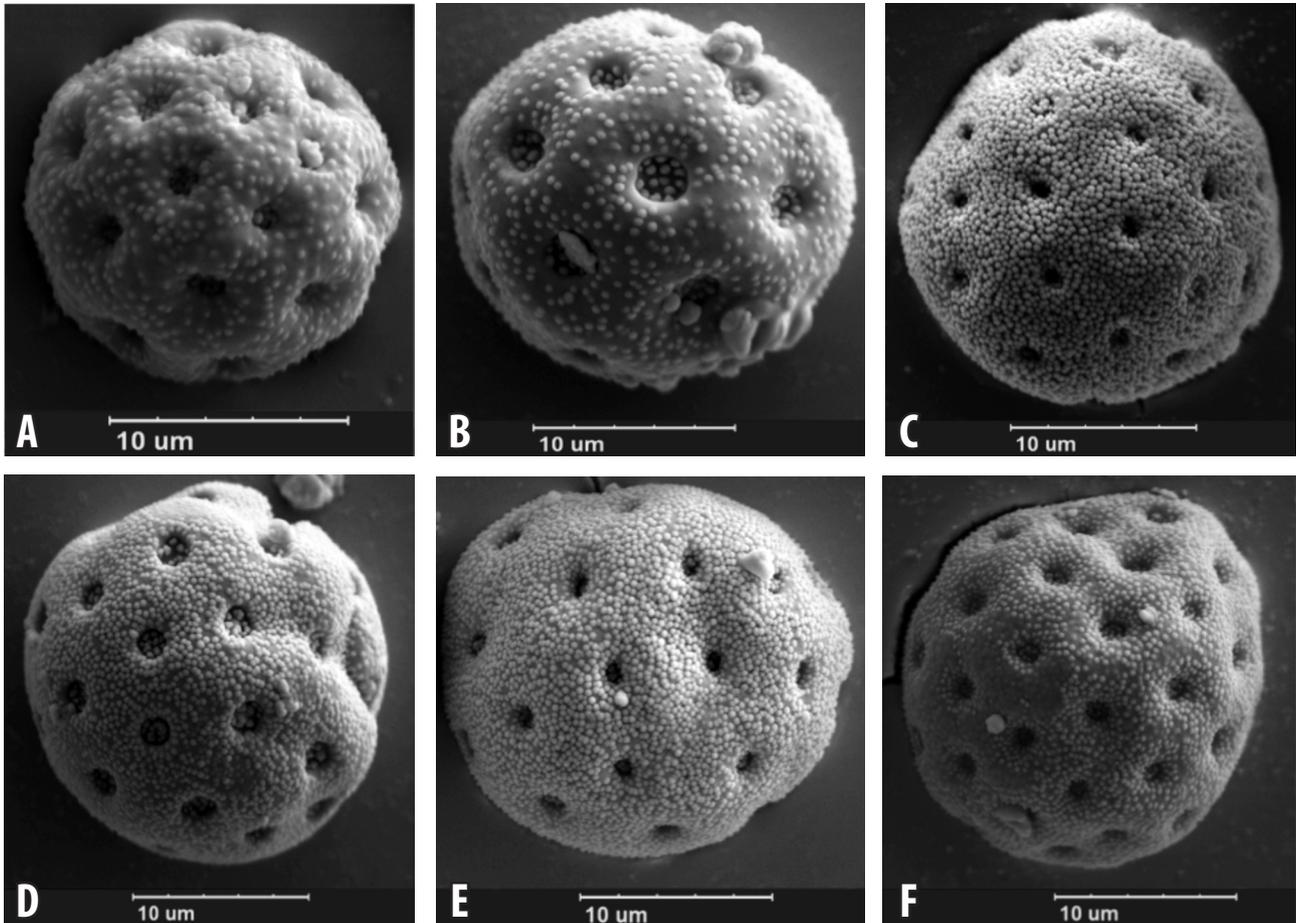


Fig. 1. Scanning electronic microscope images of pollen in studied *Amaranthus* taxa. A, *A. blitum* subsp. *emarginatus*; B, *A. blitum* subsp. *blitum*; C, *A. coadatus*; D, *A. blitoides*; E, *A. retroflexus*; F, *A. tricolor*.

Statistical analysis

For grouping the studied taxa, data were standardized (mean = 0, variance = 1) and used for the multivariate analyses with unweighted paired group average (UPGMA), principal coordinate ordination (PCO), and principal coordinate analysis (Podani 2000). MVSP (version 2) and SPSS Ver. 9 (1998) were used for the analysis.

Results

In this study palynological traits of seven *Amaranthus* taxa, including *A. albus*, *A. blitoides*, *A. blitum* subsp. *blitum*, *A. blitum* subsp. *emarginatus*, *A. coadatus*, *A. retroflexus* and *A. tricolor* were studied. Twelve quantitative and qualitative characteristics were investigated (Table 2). Results showed that all of the studied taxa had poly pentaporate pollen, with many pores distributed on the surface of pollen. Pollen polar shapes were stable in the studied taxa and were circular. In addition, equatorial views in the all of taxa were elliptic-truncate with the exception of *A. tricolor* that was elliptic-acuminate (Fig. 1).

In all examined taxa, surface sculpturing were seen as scabrate, while its aggregation differed between samples.

Dense aggregations were recorded in *A. retroflexus*, while sparse aggregation occurred in *A. blitum* subsp. *blitum*, *A. albus* and also *A. blitum* subsp. *emarginatus* (Fig. 2). Furthermore, quantitative traits varied between taxa. For example, polar axis length (20.5 µm) as well as equatorial diameter (21.0 µm) was shortest in *A. blitum* subsp. *blitum*, and largest polar (25.4 µm) and equatorial (26.0 µm) length in *A. tricolor* and *A. albus*. Pore diameter varied between the studied taxa. the largest pores were found in *A. blitum* subsp. *blitum* (2.45 µm), and smallest in *A. retroflexus* (0.81 µm).

Significant negative/positive correlation were found between the studied quantitative palynological traits. For example, a significant positive correlation ($r = 0.97, P < 0.01$) was recorded between polar axis length with equatorial diameter. Equatorial diameter had a negative correlation ($r = -0.80, P < 0.05$) with pore diameter.

Box and whisker plots showed that some quantitative morphological features were more valuable in identification of the studied taxa. For example, *P/E* ratio was a distinct characteristic in identification of *A. blitum* subsp. *emarginatus* from the other taxa. Pore diameter, equatorial and also polar axis length were the main characteristics for

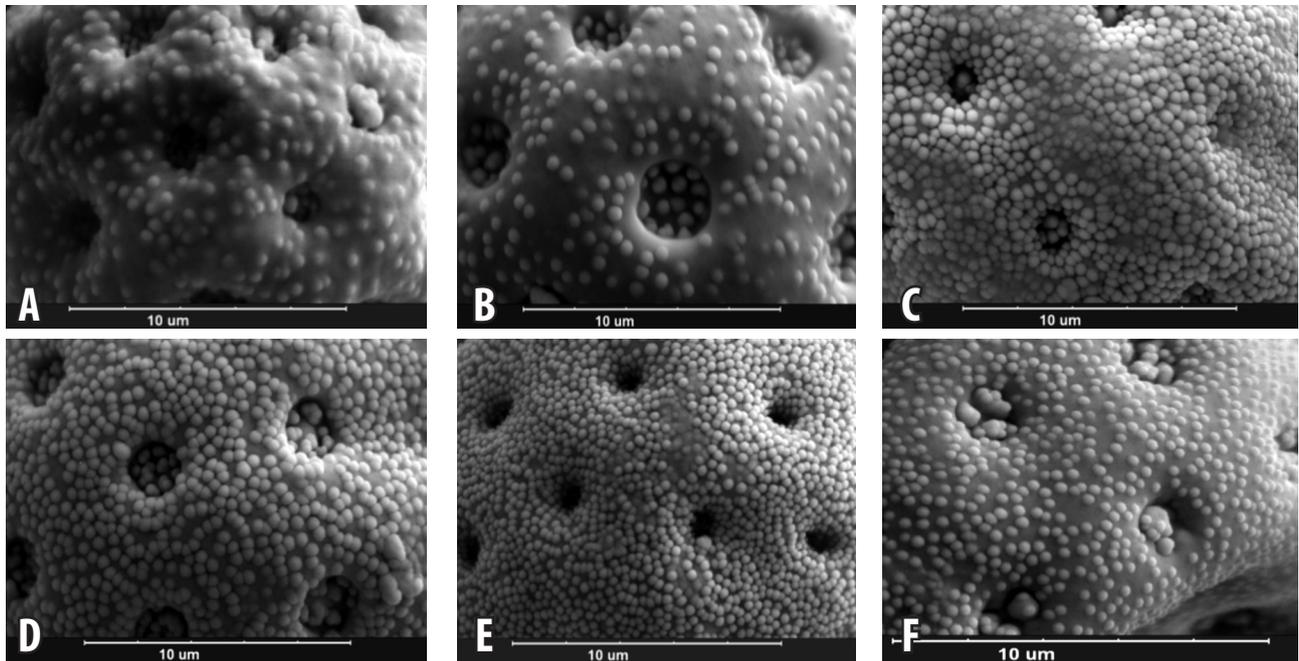


Fig. 2. Surface sculpturing shapes of pollen in the studied *Amaranthus* taxa. A, *A. blitum* subsp. *emarginatus*; B, *A. blitum* subsp. *blitum*; C, *A. coadatus*; D, *A. blitoides*; E, *A. retroflexus*; F, *A. tricolor*.

recognition of *A. blitum* subsp. *blitum* from the other taxa (Fig. 3). PCA analysis showed two factors which together explained 78% of total variance. The first factor (54% of variance) had significant positive correlation with polar and equatorial axes length, and a negative significant correlation with pore diameters (for details see Tables 3 and 4). The first few PCA axes represent the majority of variation in the data set and thus represent significant differences among taxa (Fig. 5).

The studied taxa were separated from each other in a UPGMA tree (Fig. 4). Furthermore PCA and PCO plots (Fig. 5 and 6) showed similar results. In the UPGMA analysis two main clusters were seen. One contained *A. blitum* subsp. *blitum* and the other taxa were clustered in other branch, which had two sub-clusters: one of them

included *A. retroflexus* and the other had two distinct groups. In small group *A. albus* and *A. blitum* subsp. *emarginatus* showed similarity and *A. blitoides*, *A. coadatus* and *A. tricolor* clustered closely in the larger group.

Discussion

There has been much discussion about infrageneric taxonomy of the genus *Amaranthus* (Carretero 1979; Hugin 1987; Klopper, Robel 1989), and in spite of the fact that the genus has been the subject of many taxonomic studies, it is still poorly understood and is widely considered to be a difficult genus. On the other hand, its infrageneric classification is still unresolved. For these reasons, in this study palynological features were used for identification of infraspecific and infrageneric taxa in the genus. Some studies (for example, Pandey, Misra 2009; Zhigila et al. 2014a) showed that pollen morphological characteristics play a major role in solving taxonomic problems. Palynological characteristics have been able to reposition several disputed genera and interpret problems related to the origin and evolution of many taxa (Nair 1980) and to derive a classification of angiosperms (Cronquist 1981). The obtained results showed that pollen sizes were small and also thin-walled in the studied taxa. Since these taxa are anemophilous, these conditions facilitate their pollination. Sosnoskie et al. (2009) reported that some pollen physical traits in the genus *Amaranthus*, such as density, specifically size as well as settling velocity, can influence flight of pollen and also pollination. This was also true for the other taxa in the genus *Amaranthus*. For example, Zhigila

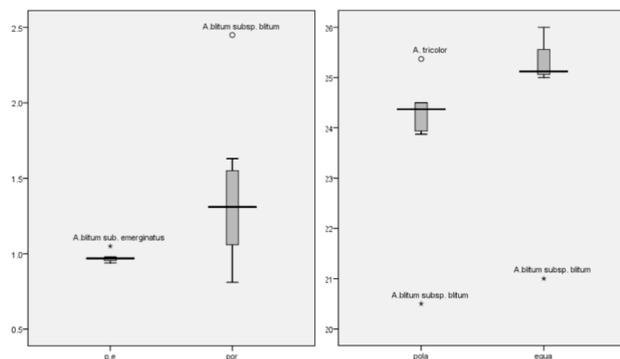


Fig. 3. Box and Whisker plots of palynological traits of the studied *Amaranthus* taxa. p.e, *P/E* ratio; por, pore diameter; pola, polar axis length; equa. equatorial axis length

Table 3. Total variance explained for principal component axes analysis for pollen characteristics in the studied *Amaranthus* taxa

Component	Initial Eigen values			Extracted sums of squared loadings			Rotation sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	2.691	53.823	53.823	2.691	53.823	53.823	2.655	53.094	53.094
2	1.223	24.459	78.282	1.223	24.459	78.282	1.259	25.188	78.282
3	0.788	15.761	94.043						
4	0.248	4.964	99.007						
5	0.050	0.993	100.000						

Table 4. Correlation of pollen characteristics of the studied *Amaranthus* taxa with two components of PCA

Characteristic	Component	
	1	2
Pore diameter	-0.905	
Equatorial diameter	0.891	
Polar axis length	0.868	
<i>P/E</i>	0.535	-0.518
Log <i>P/E</i>		0.896

et al. (2014b) provided evidence that *Amaranthus* species are eurypalynous (multipalynous) and that the genus is anemophilous. The pollen grains are generally small, rounded, smooth, thin-walled and dry but vary among species within this genus.

Quantitative palynological features varied between the studied taxa and were useful in identification of taxa. For example, based on equatorial shape, *A. tricolor* can be distinguished from the other taxa. Degree of sculpturing aggregates was useful in identification of *A. retroflexus* from the other taxa. The analyses (PCA and Box and Whisker plots) confirmed that most of the quantitative traits were useful in classification of *Amaranthus* species and had taxonomic value. For example polar and equatorial axes lengths were useful features in separating *A. blitum*

subsp. *blitum* from other taxa. In addition, some pollen qualitative characteristics differed between taxa. For example, equatorial shape is a good feature in identification of *A. tricolor* from the rest. The marginal shape of pores also differed between the studied taxa. Various palynological investigations on different species of the genus *Amaranthus* confirmed importance of pollen traits for distinguishing taxa. For example, Zhigila et al. (2014b) studied palynological characteristics in five species (*A. spinosus*, *A. viridis*, *A. hybridus*, *A. australis* and *A. tricolor*) of this genus. Their results indicated that pollen characteristics may be used to delimit the species in *Amaranthus*.

Our results did not confirm species classifications in to the traditional subgenera of the members of subgenera (*Amaranthus* and *Albersia*) present in Iran, *A. coadatus* and *A. retroflexus* (two studied samples of subgenus *Amaranthus*) clustered separately and were placed far from the others. This was also true for members of the *Albersia* subgenus; and also, two subspecies of *A. blitum* (subsp. *blitum* and subsp. *emarginatus*) were separated far from each other. There was high palynological variation between these subspecies. Noori et al. (2015) compared flavonoids components of these taxa. In their study, the mentioned taxa separated from each other and results of phytochemical clustering did not confirm species classifications in subgenera. Particularly members of

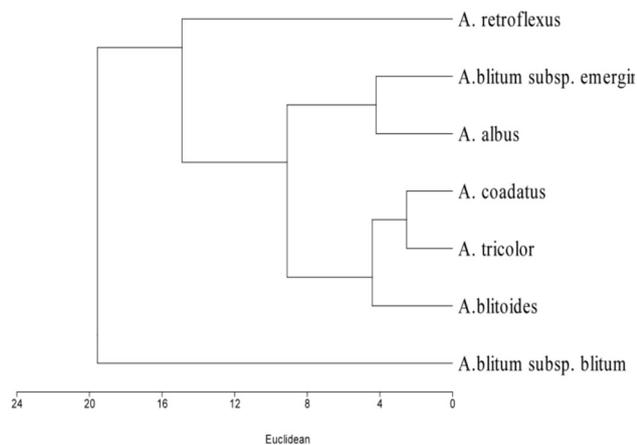


Fig. 4. UPGMA tree of the studied *Amaranthus* taxa based on palynological characteristics.

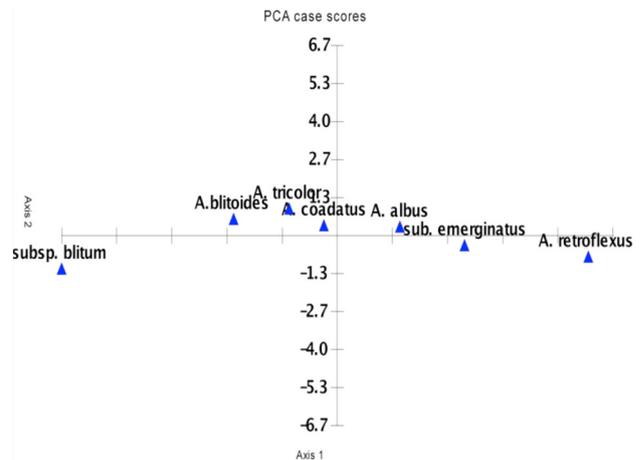


Fig. 5. PCA plot of the studied *Amaranthus* taxa based on pollen traits.

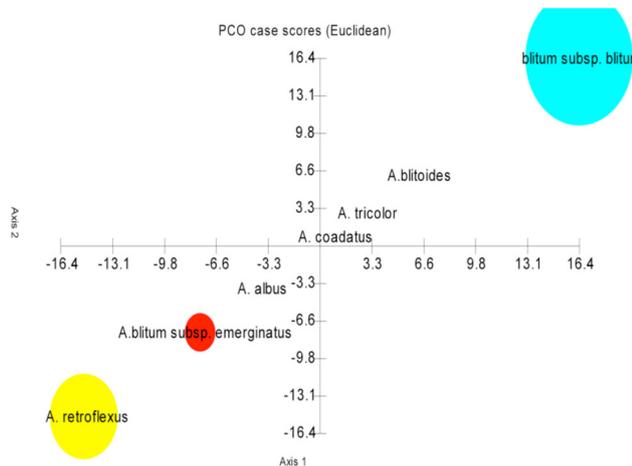


Fig. 6. PCO plot of the studied *Amaranthus* taxa based on pollen traits.

Albersia subgenus clustered separately from each other. In addition, two subspecies of *A. blitum* did not cluster closely.

Different patterns have been shown in infrageneric as well as infraspecific classifications of the genus: Sauer (1955) designated two subgenera: *Acnida* (L.) Allen ex K.R. Robertson, which includes dioecious species, and *Amaranthus*, which includes the monoecious species. Traditionally, the subgenus *Amaranthus* has been divided in two sections: *Amaranthus* and *Blitopsis* Dumert *sensu lato* (Carretero 1979, Hugin 1987). Carretero (1985, 1991) divided the section *Blitopsis sensu lato* in two sections *Blitopsis*, which includes those species having indehiscent fruits ($x = 17$); and *Pyxidium*, which includes those with dehiscent fruits ($x = 16$). Another section, *Puncticulatae*, was proposed by Kowal (1954), but was not validated by later studies (Klopper, Robel 1989). Recently the subgeneric rank (Subgenus *Albersia* (Kunth) Gren. & Godr.) has been given to the section *Blitopsis sensu lato*. Mosyakin and Robertson (1996) designated three subgenera in the genus; subgenus *Acnida* (L.) Aellen ex K.R. Robertson, *Amaranthus* and *Albersia* (Kunth) Gren. & Godr., on the basis of inflorescence and floral characteristics. *Acnida* comprises dioecious weeds. *Amaranthus* includes monoecious weeds and crop species, while *Albersia* contains many of the poorly characterized *Amaranthus*.

Wassom and Tranel (2005) believed that the classification of Mosyakin and Robertson (1996) was well substantiated by morphological features and also molecular data, but this pattern was challenged by the UPGMA tree of AFLP data. For example, all the dioecious taxa were grouped in the subgenus *Acnida*. This reveals the polyphyletic nature of *Acnida* and challenges the infrageneric classification of Mosyakin and Robertson.

Although, the infrageneric classification with three subgenera (*Acnida*, *Amaranthus* and *Albersia*) is based on classical characteristics, such as those of inflorescence and floral characteristics, the present results and also our

previous investigations did not confirm this.

In the infraspecific classifications in this genus different synonyms are used for some species. For example, Moquin-Tandon (1849) described *A. emerginatus* for the first time. Later Carretero (1979) made new contribution and altered as *A. blitum* subsp. *emarginatus*. In addition in some references (such as Ghahreman 1980–2002), it was described as a variety of *A. blitum*. Similarly *A. blitum* subsp. *blitum* has been named *A. lividus*. Our data with the results of previous investigations confirmed that the present infraspecific classification of *A. blitum* must change. Perhaps it is advisable to return to the previous taxonomical status of the genus.

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