Taeckholmia 30: 17-27 (2010)

Taxonomical Studies on Some Taxa of the Genus *Chenopodium* L. (*Chenopodiaceae*) Using Pollen Grain Characters and Elctrophoretic Patterns of Seed Protein

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El-Kholy, M. A, Kasem, W. T, Farghal, I. I, Gaafar. A. S., 2010. Taxonomical Studies on Some Taxa of the Genus *Chenopodium* L. (*Chenopodiaceae*) Using Pollen grain Characters and Elctrophoretic patterns of Seed protein. Taeckholmia **30**: 17-27.

Taxonomical studies using palynological and seed protein electrophoretic profile have been made on 15 taxa belonging to 9 species of the genus *Chenopodium* namely *C. album*, *C. ambrosioides*, *C. amranticolor*, *C. bonus-henricus*, *C. ficifolium*, *C. glaucum*, *C. murale*, *C. quinoa*, and *C. strictum*. Pollen grain characters were described using light microscopy (LM) and Scanning Electron Microscopy (SEM). Numerical analysis of pollen grain characters alone provided no useful criteria for reclassification of this genus. Electrophoretic patterns of seed protein profiles using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) isolated *C. amranticolor, C. bonus-henricus, C. ficifolium* and *C. quinoa at* different levels and classified the remainder taxa into three groups.

Key Wards: *Chenopodium*, Pollen grains, Seed protein, (SDS-PAGE), Taxonomy, UPGMA.

Introduction

Received 15 December 2009, Revision accepted 8 February 2010

Chenopodium L. is a cosmopolitan genus of perennial or annual herbaceous plants it belongs to family *Chenopodiacae* known as the goosefoots. This genus includes 150 species of world wide distribution; most of them annual; principally adapted to temperate region. The genus is divided into 16 sections according to Aellen (1960). Several species of this genus are currently identified on the basis of seed scanning electron microscopy (Malekloo *et al*, 2008), but difficulties are still encountered in the separation of some taxa. Macro and micro morphological characters of pollen grains and electrophoretic pattern of seed protein have been utilized as a tool for separation of *Chenopodium* species (Erdtman, 1943, 1969, McAndrews & Swanson, 1967, Tsukada, 1967, Uotila, 1974, Münevver & Özden, 1999; Hamdi *et al.*, (2009). These criteria have been also used for solving taxonomic problems and explaining the origin and evolution of a numbers of cultivated plants (Badr 1995, Jha & Ohri 1996, Nath *et al.*, 1997, Gaafar 2006, Kasem & Mansour 2007, Kasem *et al.*, 2008). On the other hand, phylogenetic relationships between cultivated plants and their related wild taxa have been studied using isozyme polymorphism (Wilson 1976, Walters 1987). The present study aims to characterize the studied taxa of the genus *Chenopodium* using comparative analysis of pollen grain morphology, electrophoretic of seed protein patterns, as produced by SDS-PAGE technique.

Material and Methods

Seeds of *Chenopodium* L. accessions were obtained from the Royal Botanic Gardens at Kew, London, UK, Iowa State University (North Central Regional Plant Introduction Station), and from different localities in Egypt. A list of the species used in this study and their accessions numbers, origins, and their sub generic and sectional delimitation is given in Table 1.

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I- Pollen grains techniques:

Seeds of all plants were cultivated in the botanic garden of Faculty of Science, Al-Azhar University to obtain pollen grains of buds maturity upon flowering. Flower buds were collected and stored in the refrigerator until used. Pollen samples of accessions were acetolyzed according to Erdtman's technique (Erdtman, 1952). The acetolyzed and non acetolyzed samples were used for both light (LM) and scanning electron microscope (SEM). For

microscope with a pre-calibrated eye-piece micrometer was calculated to determine the shape class of pollen grains. For SEM, dried pollen grains were mounted onto clean stubs using double-slide adhesive, the samples were coated with a 30 nm layer of gold using fine coat ion sputter JEOL-JFC-1100E ion-sputtering device. The coated pollen grains were examined in SEM were operated at JEOL-JFC-5500V scanning electron microscope, which operated at accelerated voltage of 15 Kv at the scanning electron microscope unit, the Regional Center for Mycology and Biotechnology, Al-Azhar University.

II- Electrophoretic Techniques:

Extraction and analysis of seed proteins

Characterization of seed protein fractions was carried out by using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Preparation and running of the gel were carried out according to Stegemann *et al.*, (1981). The gel was stained with coomassie brilliant blue stain R-250. Bands of protein were determined, scanned by using Hoefer Scanning Densitometer GS300.

This study is dependent upon the using of a total pollen grains characters as a binary one (0&1), on each of the 15 accessions of the genus *Chenopodium*. The used characters and their states have been subjected to numerical analysis using similarity and dissimilarity assessment percentage method (Rohlf, 1993). The method applied was based on clustering analysis UPGMA (Unweighted Pair-Group Method with Arithmetic means). The dendrogram illustrating the intraspecific relationships of the studied taxa was generated based on the percent of dissimilarity.

Table 1. The studied *Chenopodium* L. species and their accessions number, origin, subgenera and sections.

No.	Studied taxa	Acc. No.	Orig.	Subgenus	Section
1	*C. album* L.	-	Egypt		*Chenopodium*
2	*C. album* L.	5175	Portugal	*Chenopodium*	*Chenopodium*
3	*C. album* L.	84095	India		*Chenopodium*
4	*C. ambrosioides* L.	-	Egypt		*Ambrina*
5	*C. ambrosioides* L.	57738	Italy	*Ambrosia*	-
6	*C. amranticolor* L.	-	Italy	*Chenopodium*	*Chenopodium*
7	*C. bonus-henricus* L.	12050	Germany	*Blitum*	*Agathophyton*
8	*C. ficifolium* Smith.	-	Egypt	*Chenopodium*	*Chenopodium*
9	*C. glaucum* L.	30977	Denmark		*Agathophyton*
10	*C. glaucum* L.	612859	U.S,Iowa	*Blitum*	-
11	*C. murale* L.	-	Egypt		*Undata*
12	*C. murale* L.	37437	Portugal	*Chenopodium*	-
13	*C. quinoa* Wild	7534	Italy	-	*Leprophyllum*
14	*C. strictum* Roth.	23893	India		-
15	*C. strictum* Roth.	538	Germany	*Chenopodium*	-

Results and Discussion

I- Pollen grain morphology:

Pollen grain morphology of *Chenopodium* under LM showed diversity in the shape and size, in addition to the variations in their diameters and aperture types and pore numbers. Our obtained results fairly indicated some of the differentiation between the studied species, the main features of the investigated pollen grains are given in Table 2. Generally, the pollen grains of this genus were spherical with pantopolyporate shaped. The mean diameter values of pollen grains of the studied taxa ranged from 13.11µm to 28.85µm, the largest diameter was recorded in *Chenopodium album* accession (No. 3) from (India), whereas the lowest diameter (13.11µm) was found in *Chenopodium glaucum* accession (No. 9) from (Denmark). On the

other hand, the results of scanning electron microscope examination (Plate I; 1-15) showed that the pore diameter ranged from (0.65-1.7µm), were recorded in each of *Chenopodium glaucum* accession (No. 10) from (U.S. Iowa) and *Chenopodium amranticolor* accession (No. 6), from (Italy) respectively. The pore numbers of the studied taxa varied from (40-42) in *C. ficifolium* accession (No. 8) to (120-125) in *C. ambrosioides* accession (No. 4) respectively collected from Egypt.

Table 2. Summary of LM and SEM morphological characters of the pollen grains of the studied *Chenopodium* taxa.

No.	Studied taxa	Pollen diameter (µm)	Pore diameter (µm)	Pore No.
1-	*C. album*	21.36	1.1-1.3	100-110
2-	*C. album*	23.39	1.0-1.1	105-120
3-	*C. album*	28.85	1.15-1.2	103-115
4-	*C. ambrosioides*	18.18	1.46-1.5	120-125
5-	*C. ambrosioides*	18.95	1.3-1.4	119-122
6-	*C. amranticolor*	20.31	1.65-1.7	125-130
7-	*C. bonus-henricus*	25.31	0.95-0.98	45-48
8-	*C. ficifolium*	22.52	1.1-1.2	40-42
9-	*C. glaucum*	13.11	0.67-0.75	48-50
10-	*C. glaucum*	15.23	0.65-0.71	50-55
11-	*C. murale*	18.8	1.3-1.4	55-60
12-	*C. murale*	16.23	1.5-1.6	60-65
13-	*C. quinoa*	21.71	1.55-1.65	98-100
14-	*C. strictum*	17.72	0.98-1.0	61-63
15-	*C. strictum*	20.72	0.99-1.1	59-62

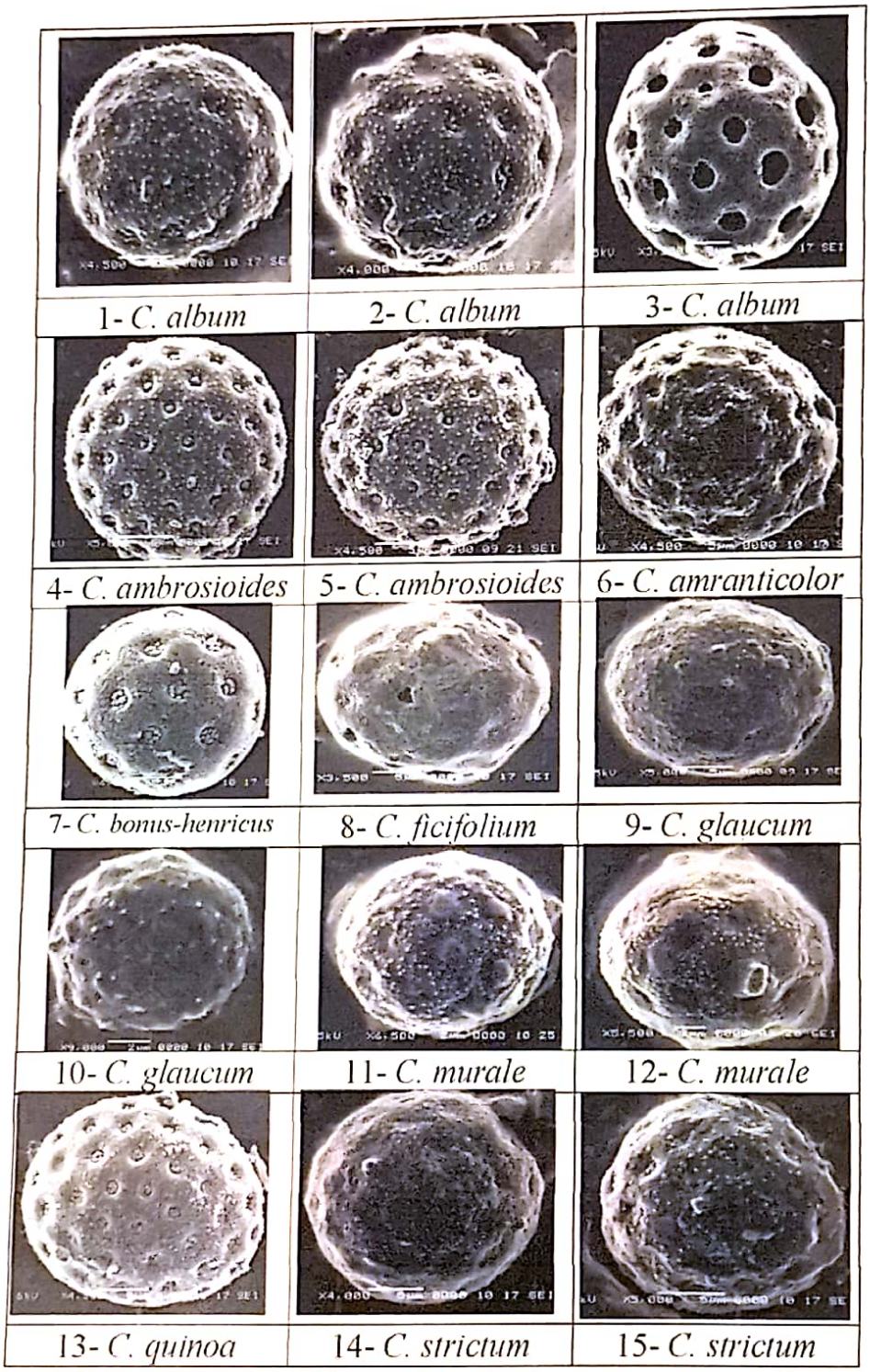

Plate I (1-15). SEM photomicrographs of pollen grains of the studied taxa of *Chenopodium* L.

UPGMA phenogram (Fig.1) illustrated two groups, the first included accession 7, 8, 9 10, 12, 14 and 15, the second included accession 1, 2, 3 , 4, 5, 6 ,11 and 13. In the first group, *C. glaucum* accession No (9), *C. glaucum* (10), and *C. strictum* (14), linked together at taxonomic level of 65% of dissimilarity level. Similarly, *C. bonus-henricus* (7), and *C. ficifolium* (8), correlated closely at dissimilarity level of 65%. Such two species belongs two different sections; *Agathophyton* and *Chenopodium*, respectively. The obtained results agreed with that of Münevver and Özden, 1999; in their studies on *Chenopodium* in Turkey. On the other hand the second group comprises three accessions of *Chenopodium album*, two accessions of *C. ambrosioides*, one of each *C. murale* from Egypt, *C. quinoa* from Italy and *C. amranticolor* from Italy. Our results of pollen grains agree with the previous studies in morphological data including dimensions and shapes. The exine surface often develops various forms of sculpturing and ornamentation, including various types and numbers of apertures. The numbers of pores on periporatae pollen has been used as a diagnostic character for taxonomic value of this genus; such pores differed in the studied taxa. Differences in numbers of pores used as good marker between several genera such as pollen in the *Caryophllaceae* (Faegri & Iversen 1975). On the other hand, Pollen grain of *Chenopodium* L. species examined are radially symmetrical; isopolar; pantopolyporate and spheroidal. There exine structure is similar. MŰnevver; & Özden (1999), Malekloo *et al.*, (2008) Hamdi *et al.*, (2009).

II- Electrophoretic results:

1- Seed proteins:

Fifteen bands ranged between 0.40:9.40 (migration distances) were found. Bands clear that some bands are considered in a large number of taxa, while others are in a few accessions. Band No. 38 with migration distance of 7.40 was detected in 10 taxa, *C. album* from (India), C. *ambrosioides* from (Egypt and Italy), *C. amranticolor* from (Italy), *C. ficifolium* from (Egypt), *C. glaucum* from (U.S. Lowa), *C. murale* (12), from (Portugal), *C. quinoa* (13) and *C. strictum* from (India and Germany). Also, band No. 40 with migration distance of 7.80 was estimated in 8 taxa, *C. ambrosioides* (4), *C. amranticlor* (6), *C. ficifolium* (8), *C. glaucum* (9&10), *C. murale* (12), *C. quinoa* (13), C. *strictum* (14). On the other hand, band No. 32 with migration distance of 6.40 was recorded in 7 taxa, *C. album* (2&3), *C. ambrosioides* (5), *C. amranticlor* (6), *C. glaucum* (9&10) and *C. quinoa* from Italy (Plate II).

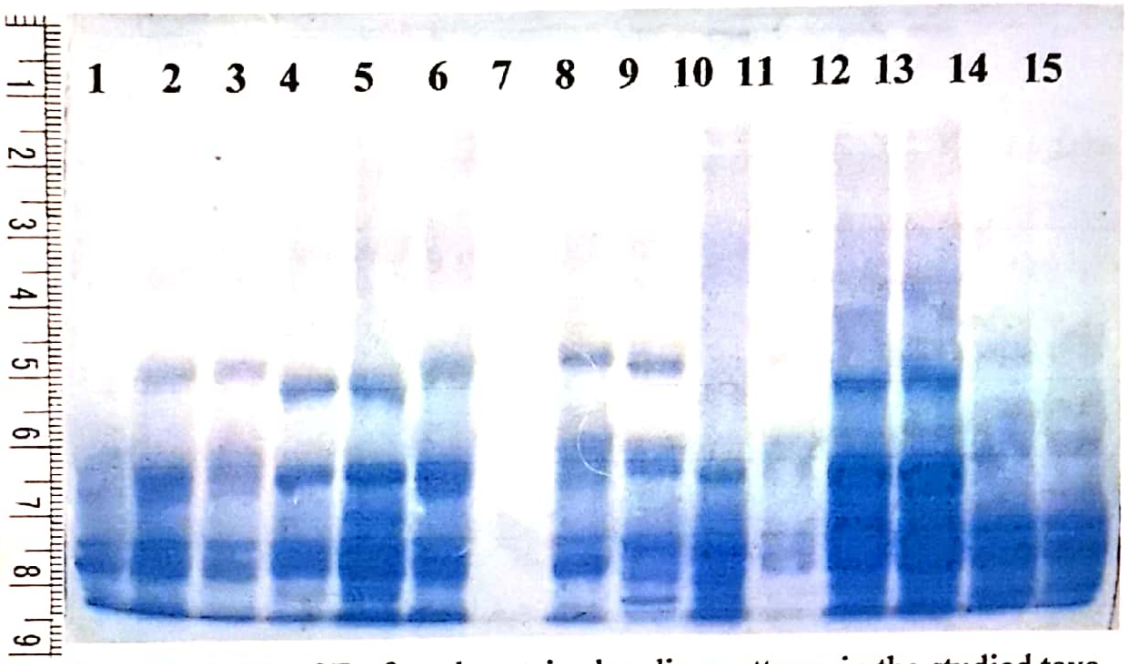

Plate II. SDS PAGE of seed proteins banding patterns in the studied taxa of the genus *Chenopodium*, numbered as in Table 1.

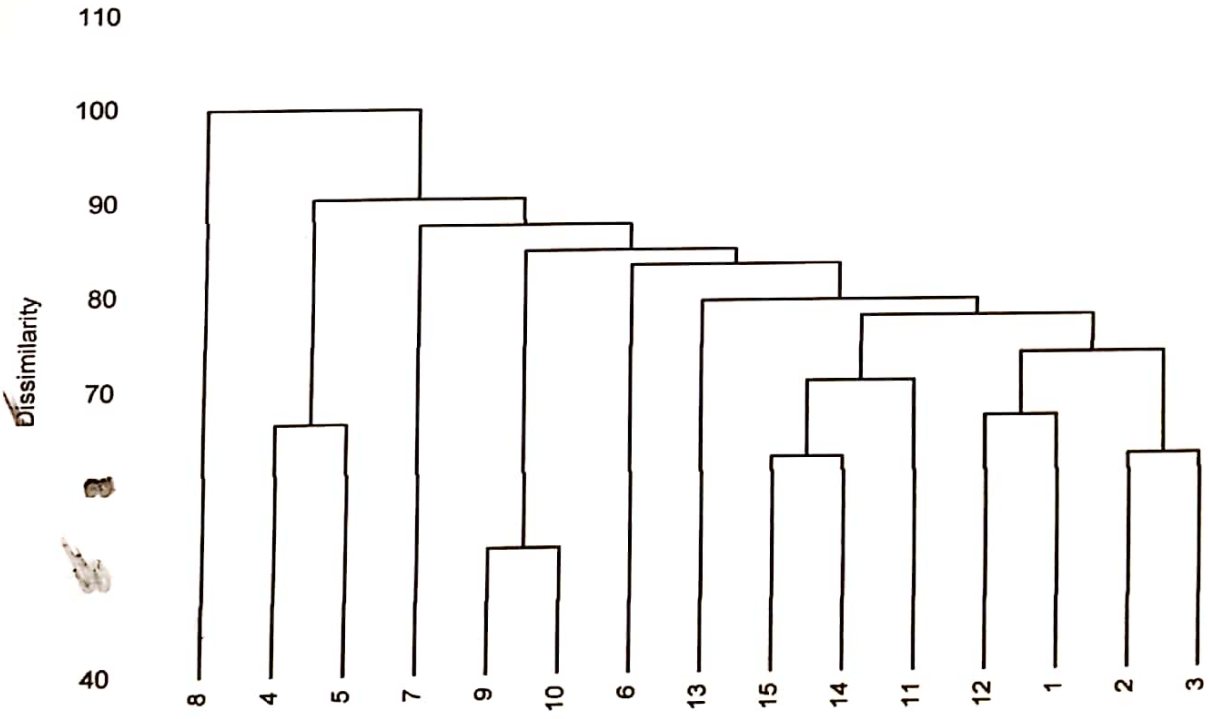

Fig. 2. Phenogram constructed by the UPGMA (average linkage) using 50 Protein data of the 15 taxa of the genus *Chenopodium* L.

The tree of the SDS-PAGE results (Fig. 2), showed that the studied taxa of genus *Chenopodium* have an average distance of dissimilarity levels of 100.0.At this level *C. amranticolor* (Italy), *C. bonus-henricus* (Germany),*C. ficifolium*(Egypt) and *C. quinoa* (Italy) in different taxonomical levels of 100.0, 87.0, 85.0 and 77.0, respectively. The rest of the studied taxa were classified into three groups, the first included the two species of *C. ambrosioides* (Italy and Egypt) at taxonomic levels of 67.0 such two accessions characterized with a distinct pollen grain characters; the second group comprised of *two accessions of C. glaucum* (Denmark and U.S.Iowa) which correlated with *C. bonus-henricus* (Germany) such clusters related to the same section of *Agathophyton*, hence agreed with the earlier results of Münever and Özden, 1999; Hamdi *et al.*, (2009). In the last group the studied taxa of subgenus *Chenopodium* were grouped into two subgroups, the first included three accessions of *C.album* and *C. murale* (Poutgal) the second subgroup comprised of different taxa of *C. murale* (Egypt) and two accessions of *C. strictum* (India & Germany) such three taxa of section *Chenopodium* shared in five protein bands.

It is clear from the above study that seed protein data is congruent with taxonomic position, crossability relationships and other biochemical characters.

Acknowledgements

The authors gratefully indebted to Prof. Dr. Mohamed M. A. Abou Zaid professor of Aquatic Biology Marine Biology & Fish Science, Zoology Dept., Faculty of Science Al-Azhar University for his valuable guidance during the practical work of this study.

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