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Electrophoretic variations in *Amaranthus*

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Abstract. Total seed proteins of some *Amaranthus* species were electrophoretically analysed on SDS-PAGE. Chromosome number and mean chromosome length were also determined. Numerical analysis (cluster and principal coordinate analysis) of the electrophoretic data show a heterogeneity between the samples of some taxa. This heterogeneity was discussed in the light of the controversy over the taxonomic position of these species. Natural hybridization within the genus and the dibasic chromosome number within the species were also discussed.

Key words: *Amaranthus*; Chromosome number; Cluster analysis; Electrophoretic patterns; Principal coordinate analysis; Seed proteins.

Introduction

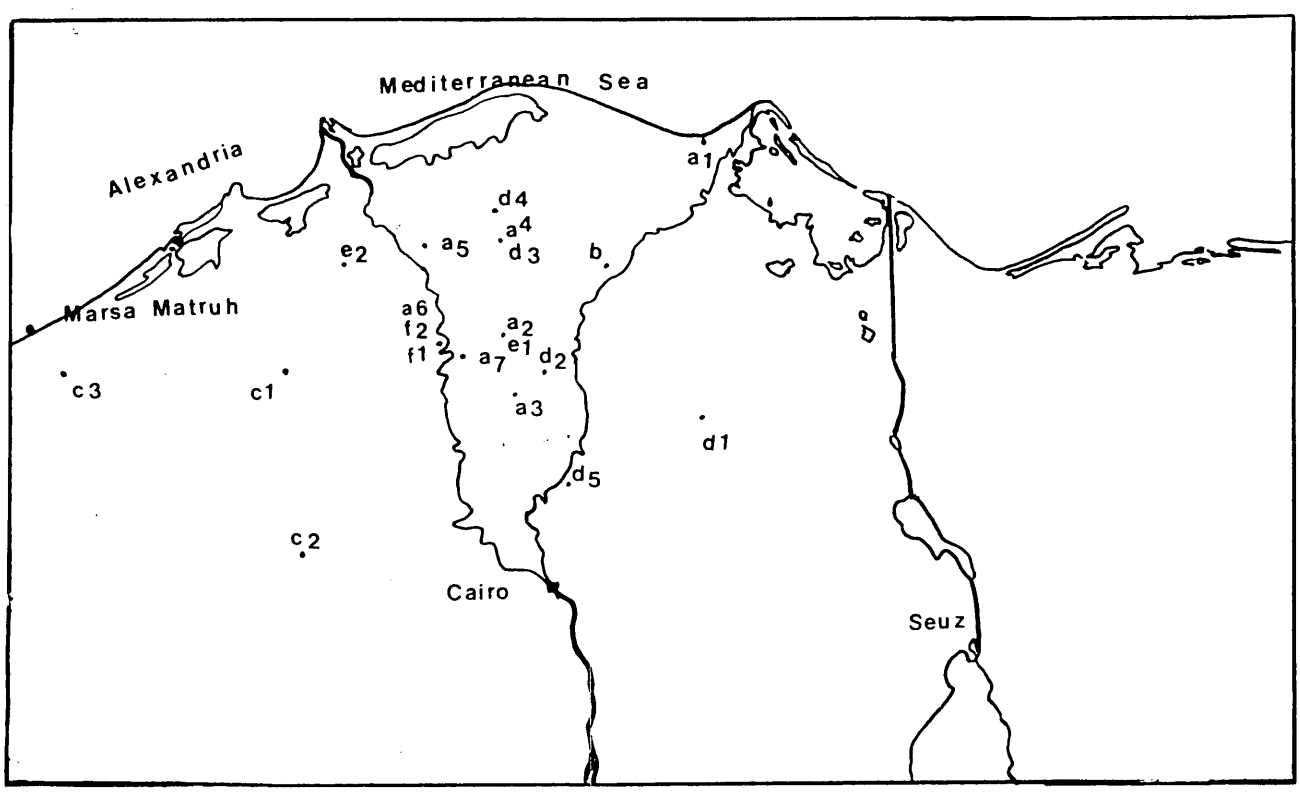
Genus *Amaranthus* belongs to sub-tribe Amarantinae which in turn belongs to tribe Amaranteae, subfamily Amarantiodeae, family Amaranthaceae. This

driacus L. as subspecies under *A. hybridus,* and *A. graecizans* and *A. sylvestris* (Desfi) Vill as subspecies under *A. graecizans*.

Cytologically, *Amaranthus* has been most well studied (Grant, 1959a-d, Darlington and Wylie, 1955; Sharma and Banik, 1965; Mittra, 1971; Pale, 1972). It

Table 1. *Cytological features and number of protein bands of the studied taxa of genus* ***Amaranthus***

Species	Sample location	Chromosome number Somatic (2n)	Chromosome number Basic (n)	Chromosome measurements Mean Length μm±SE	Chromosome measurements Mean Arm Ratio ±SE	Number of protein bands −ME	Number of protein bands +ME
A. viridis	a1	34	17			15	17
A. viridis	a2	34	17			15	17
A. viridis	a3	34	17			15	17
A. viridis	a4	34	17	0.65±0.02	1.21±0.05	15	17
A. viridis	a5	34	17			15	17
A. viridis	a6	34	17			15	17
A. viridis	a7	34	17	0.47±0.01	1.26±0.07	15	17
A. hypochondriacus	d1	32	16			13	14
A. hypochondriacus	d2	32	16	0.92±0.04	1.19±0.04	21	21
A. cruentus	e1	34	17	1.04±0.04	1.14±0.04	11	13
A. cruentus	e2	34	17	0.78±0.05	1.13±0.05	11	13
A. hypochondriacus	d3	32	16	0.89±0.02	1.13±0.06	10	13
A. hypochondriacus	d4	32	16	0.90±0.02	0.26±0.04	10	13
A. hypochondriacus	d5	32	16			10	13
A. chlorostachys	f1	32	16	1.03±0.05	1.13±0.05	9	13
A. chlorostachys	f2	32	16			9	13
A. sylvestris	b	32	16	0.84±0.02	1.20±0.05	11	12
A. graecizans	c1	34	17	0.87±0.03	1.43±0.05	11	16
A. graecizans	c2	34	17			11	16
A. graecizans	c3	34	17	0.81±0.04	1.15±0.05	11	16



Agriculture Research Center and Cairo University.

Mature seeds representing 20 samples of six species belonging to genus *Amaranthus* L. were freeze-dried for 2-3 days, then ground to a fine powder using a Jank and Kunkel water cooled mill.

100 mg of the seed meal of each sample was extracted with 400 μl tris/HCl buffer (pH 6.8) containing 2% SDS and free of or mixed with 2-ME (2-mercaptoethanol) in a tube for 24 h at 4°C and centrifuged for 20 minutes at 10,000 xg. The supernatants were used for electrophoresis.

Sample application and 17% SDS-PAGE preparation were carried out and run according to the method of Laemmli (1970).

Transferin (76.7 KiloDalton (KD)), bovin serum albumin (68 KD), albumin egg (43 KD), Chymotrypsinogen (25.7 KD), Myoglobin (16.2 KD), and cytochrome c (12.7 KD) were used as marker proteins.

Chromosome counting were determined in root tips pretreated with 0.05% colchicine solution and fixed in 3:1 alcohol : acetic acid for 24 hours. Slide were prepared using Feulgen squash technique.

Electrophoretic profiles were compared for band positions using the following numerical technique : a basic data matrix was constructed for the 8 operational taxonomic units (OTUs) and the 28 protein bands or characters. Cluster analysis was carried out from Jaccard coefficent (Crisci and Loppez Armengol, 1983). Principal coordinate analysis adopted here are mostly those used by Small (1981). Both cluster analysis and principal coordinate analysis were determined using computer program STATITCF.

Results and Discussion

The total seed proteins of samples of *Amaranthus*

ptides with molecular weights ranging between 50 KD and 55 KD. In the second group the electrophoretic pattern of *A. graecizans* has a unique band with molecular weight 20 KD (denoted with arrow Fig. 2). The great similarity in the electrophoretic pattern between *A. sylvestris* and *A. graecizans* supports their classification as subspecies under *A. graecizans* (Pale, 1972; El - Hadedi, 1980). However, the data in Table 1 indicate

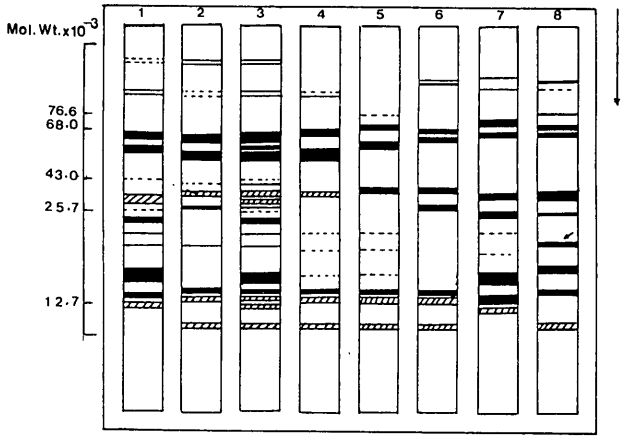
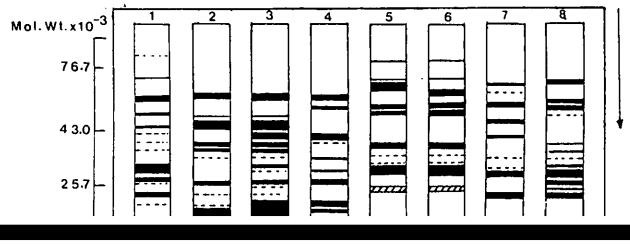


Fig. 2. SDS-PAGE of the seed proteins, extracted with Tris/HCl buffer containing 2% SDS, of some species of genus *Amaranthus*. 1, *A. viridis*; 2, *A. hypochondriacus*. d1; 3, *A. hypochondriacus* d2; 4, *A. cruentus*; 5, representative of the other samples of *A. hypochondriacus*; 6, *A. chlorostachys*; 7, *A. sylvestris*; 8, *A, graecizans*.



that the somatic chromosome numbers of *A, sylvestris* and *A. graecizans* are 34 and 32 respectively. These data are in a good agreement with the work of Pale (1972). Since these species can hybridized naturally (Sauer, 1957) and they have queit similar electrophoretic pattern, their classification under one species have a solid basis (Pale, 1972; El-Hadedi, 1980). The prevailing two basic chromosome numbers in this species can be interprated on the basis that 16 is the original basic chromosome number as addition of a chromosome can be tolerated easily than loss (Grant, 1959a-d).

Analysis of the total seed protein extracts of *Amaranthus* species extracted with Tris/HCl (pH 6.8) containing 2% SDS and 1% 2-ME on 17% SDS-PAGE displayed that all *Amaranthus* species had a few disulphide bonded polypeptides (legumin-like proteins) (Fig. 3 and Table 1). However the slight increase in the number of bands on extraction in the presence of 2-ME could be due the role of 2-ME in releasing of the proteins bound to membrane by S-S-links or in the splitting of intermolecular S-S-linkages of the disulphide bounded proteins (Shah and Stegemann, 1983). As shown in Fig. 3, there is a close similarity in the electrophoretic patterns among *A. cruentus, A. chlorostachys,* and *A. hypochondriacus*. The variation was located only in the movining mobility of a few number of bands in both the upper and middle parts of the gel. The electrophoretic analysis of the seed proteins of the samples of *A. hypochondriacus* showed three different electrophoretic patterns; one represented to sample d1 (lane 2), the second to sample d2 (lane 3) and the third to the other samples (lane 5). It was very interested to notice that the electrophoretic pattern of sample d2 has the comparable bands of *A. viridus* and sample d1 of *A. hypochondriacus*. This finding confirms the postulation that the natural hybridization is abundant in genus *Amaranthus* and even between the species of different basic chromosome numbers (Covas, 1950; Sauer, 1950, 1967; Tucker and Sauer, 1958; Singh, 1961).

The dendrogram produced from the cluster analysis (using Jaccard coefficient) based on the protein bands derived from the electrophoretic analysis of the

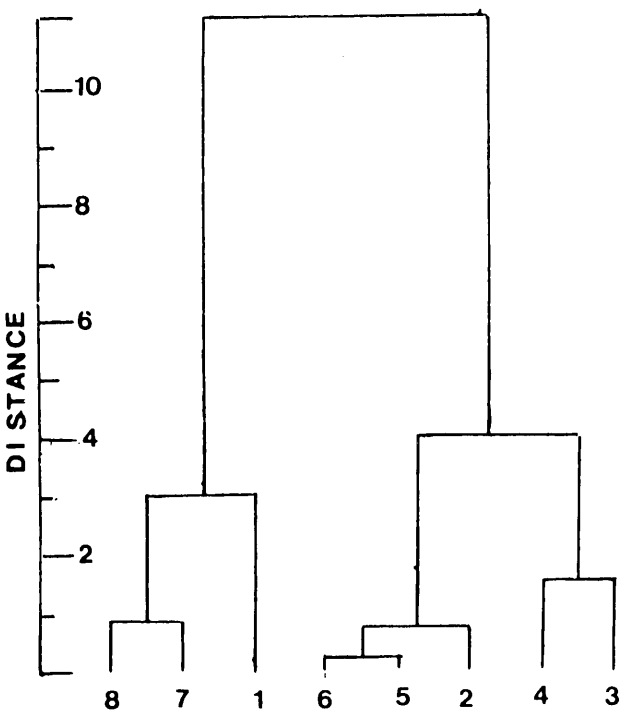


Fig. 4. Dendrogram showing relations of *Amaranthus* species indicated by seed proteins extracted with Tris/HCl buffer containing 2% SDS and 1% ME and analysed on 17% SDS-PAGE. 1, *A. viridis*; 2, *A. hypochondriacus* d1; 3, *A. hypochondriacus* d2; 4, *A. cruentus*; 5, representative of the other samples of *A. hypochondriacus*; 6, *A. chlorostachys*; 7, *A. sylvestris*; 8, *A. graecizans*.

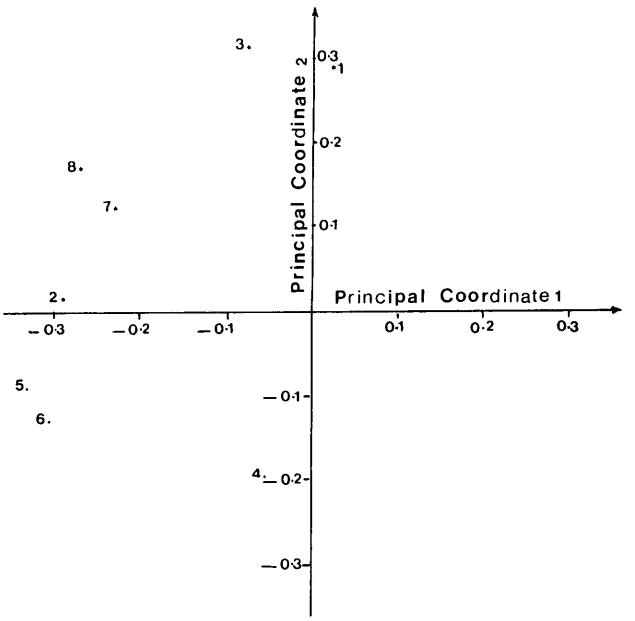


Fig. 5. Principal coordinate analysis showing relations of *Amaranthus* species indicated by seed proteins extracted with Tris/HCl buffer containing 2% SDS and 1% ME and analysed on 17% SDS-PAGE. 1, *A. viridis*; 2, *A. hypochondriacus* d1; 3, *A. hypochondriacus* d2; 4, *A. cruentus*; 5, representative of the other samples of *A. hypochondriacus*; 6, *A. chlorostachys*; 7, *A. sylvestris*; 8, *A. graecizans*.

total seed proteins extracted in the presence of 2-ME and analysed on SDS-PAGE (Fig. 4) showed that *A. chlorostachys* and *A. hypochondriacus* (samples 3-5) formed one cluster. This support their classification as two varities of subspecies *hybridus*. The dendrogram also displayed a great similarity between *A. graecizans* and *A. sylvestris*. The highest degree of similarity in genome products (seed proteins) in a combination with the prevailing of natural hybrididation between them (Covas, 1950; Sauer, 1950, 1967; Tucker and Sauer, 1958; Singh, 1961) support their classification as subspecies under *A. graecizans* (El-Hadedi, 1980). The split of *A. cruentus* from *A. chlorostachys* and *A. hypochondriacus* (samples 3-5) at distance 3.9 could be due to their variation in basic chromosome number.

Principal coordinate analysis results in a similar grouping of samples to that produced by the hierarchical analysis (Fig. 5). However *A. hypochondriacus* d2 which was proposed to be the offspring of *A. viridus* and *A. hypochondriacus* d1 is more closer to *A. viridus* than to *A. hypochondriacus* d1 and *A. hybochondriacus* d3-d5. The variation between the samples of *A. hypochondriacus* needs more study.

It can be concluded that *A. cruentus, A. chlorostachys, A. hypochondriacus, A. graecizans,* and *A. sylvestris* should be classified as *A. hybridus* subsp. *cruentus, A. hybridus* subsp. *hybridus* var *hybridus, A. hybridus* subsp. *hybridus* var *erythrostachys, A. graecizans* subsp. *graecizans, A. graecizans* subsp. *sylvestris* respectively. Cluster and principal coordinate analysis confirmed the previous conclusion. The data also confirmed that natural hybridization is abundant in *Amaranthus* and the dibasic chromosome numbers prevails within the species. The prevailing chromosome numbers within *Amaranthus* are 34 and 32. It can also found that seed proteins of *Amaranthus* contain a few amount of legumin-like protein, the protein responsible for the nutritional value of seed proteins.

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莧屬植物之電泳變異性分析

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本報告利用一些莧屬植物進行其全蛋白質之十二烷硫酸鈉聚丙烯醯胺膠體電泳分析並測定其染色體數及平均染色體長。由其電泳資料進行數值分析(集群分析及主對等分析)顯示一些類屬的樣品間呈現有異質性之情形。本報告並討論此些異質性與這些種屬在分類上矛盾處之關係。此外，屬內天然雜交情形及種內雙基數染色體數之情形亦加以討論。