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TAXONOMIC STUDIES ON SOME SPECIES OF FAMILY CHENOPODIACEAE (GOOSEFOOT).

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ABSTRACT

Macromorphological characters of roots, stems, leaves, fruits and seeds, in addition to the micromorphological features of seed surface sculptures performance by Scanning Electron Microscope (SEM), as well as electrophoretic identification of 4 species belongs to 3 genera of the family Chenopodiaceae (Goosefoot) were studied. These species were; *Chenopodium quinoa* Willd (Quinoa), *Spinacia oleracea* L. (Spinach), *Beta vulgaris* var. *cicle* L. (Chard) and *Beta vulgaris* L. (Beet).

Because the species *Chenopodium quinoa* introduced and cultivated quite recently in Egypt and considered a new additive product to wheat cereal to produce a good bread quality,a new high protein crop (50 % more than wheat), so it is a pseudocereal rather than a true cereal. The objective of this study was raised up to distinguish between this species and some of the more popular species of the same family using some micro and macromorphologyical characters. In addition, electrophoretic identification of the investigated species was under consideration.

The brief results obtained from the macromorphologyical studies on roots, stems, leaves, fruits and seeds indicated that the species *Chenopodium quinoa* has characters varied compared with the other species. The macromorphologyical results on seed surface indicated that the species *Chenopodium quinoa* has falsifoveate seed surface pattern, while these patterns were; reticulate - rugose in *Spinacia oleracea*; reticulate - areolate in *Beta vulgaris* var. *cicle* and scalariform in *Beta vulgaris*. Data on biochemical analysis indicated that polyacrylamide gel electrophoresis separation of total soluble proteins can be used as a genetic finger print for identification, differentiation and comparison among the four studied species of the family Chenopodiaceae.

Keywords: Taxonomy, Chenopodiaceae, Macromorphology, Micromorphology, Seed surface, Scanning Electron Microscope (SEM), Electrophoretic identification.

INTRODUCTION

Family Chenopodiaceae (Goosefoot family) is herbs and shrubs (very rare small trees). The family comprise some 100 genera and 1500 species. Chenopodiaceae plants widely distributed especially in arid and semi-desert regions, often in alkaline or saline habitats of North and South Africa, Asia, Australia, Europe and North and South America (Scott, 1978a and b). Economically, the plants of the family are often intimately involved with the daily life of people around the world (Kuhn, 1993), for example, *Beta vulgaris* is one of the most important sources for sugar, *Chenopodium quinoa* is a new high protein crop and grain widespread acceptance as cereal crop, *Spinacia oleracea* and *Beta vulgaris* are common edible vegetables. Many other species are used as medicine treatments, insecticide, animal forage, windbreaks, soil binders and as biological reconditioning of the desert.

The recent investigation was carried out to clarify the relationships between *Chenopodium quinoa* and some common species of

Chenopodiaceae by studying some micro and macromorphological features of root, stem, leaf, fruit and seed, in addition to the sculpture patterns of seed surface by using the SEM. Moreover, biochemical analysis was done, in seeds of each studied species, using SDS-polyacrylamide gel electrophoresis separation of total soluble protein method for genetic identification and differentiation among the studied species of the family Chenopodiaceae.

MATERIALS AND METHODS

In this study, 4 species belongs to the family Chenopodiaceae were studied (Table 1). Seeds of *Chenopodium quinoa* obtained by Non-Governmental National Organization from Denimark and grown on the sandy soil at Janaklees Farm, Ministry of Agriculture, Nobaria, Egypt. Seeds of the other species were obtained through the curtsey of the staff of vegetable Research Institute, Agriculture Research Center, Giza, Egypt. These seeds were sown at September 2007 in the experimental station, Faculty of Agriculture, Cairo University, Giza, Egypt. The plants were harvested at January, 2008.

Table (1): Scientific and English names and habit of the studied species

Scientific name	English name	Habit
1- *Chenopodium quinoa* Willd	Quinoa	Cultivated
2- *Spinacia oleracea* L.	Spinach	Cultivated
3- *Beta vulgaris* var. *cicle* L.	Chard	Cultivated
4- *Beta vulgaris* L.	Beet	Cultivated

Macromorphological characters of roots, stems, leaves and fruits of the fresh plants were examined using the Binocular Stereo-Microscope, while the detailed seed surface scan features were examined by using Scanning Electron Microscope with different magnifications. The SEM micrographs were taken after mounting of the dry seeds with SPI supplies on copper stubs and coated with a thin layer of gold palladium in Edwards Sputter Coater Unit, S150B. Scanning was carried out by JEOL-JSMT 100 Model, National Research Center, Giza, Egypt.

Protein extraction and polyacrylamide gel electrophoresis:

Total soluble proteins from seeds of each genotype were isolated according to the method described by **Harborne (1984)**.

The dried seeds were ground to a fine powder. Finely ground sample (0.1g) was mixed with 10.0 ml of tris-HCl buffer solution (0.1M, pH 8.1) and then mechanically shaked for one hour. The extract was centrifuged at 3000 rpm for 15 min. The obtained supernatent, which containing total soluble proteins (albumins and globulins), was stored at 20 ºC for subsequent polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis method was used to detect the protein fractionations (Weber and Osborn, 1969). The gel contained 7.5 % acrylamide, 0.2 M tris citric acid buffer pH 8.3. TEMED and freshly prepared ammonium per sulphate solution. Extracts of seed protein were saturated with sucrose crystals and 0.05 ml samples were put on the tops of the gel

tubes using a micropipette. Electrophoresis was performed for 10 min., at 2.5 mA/ tube, then continued at 12 mA for 6 hours. The gels were gently extruded by water surrounding using a syringe and stained with 7% Amido-Black solution for 10 min. Stained gels were transferred into the destaining solution (7.5% acetic acid) for 10 min. With several changes until the background gels became clear. The position of protein bands on the gel tubes is expressed as the Rf value calculated as the distance migrated by the band/ total length of the gel tube of each sample.

RESULTS AND DISCUSSION

Macro-morphological characters of roots, stems, leaves, fruits and seeds, in addition to seed surface scan of the studied species are presented in the forms of cumulative Tables and Plates in order to facilitate the relationships between these species.

I - Macromorphological studies:

1- Root colour:

Data in Table (2) and Fig (1) indicated that, the dark violet colour of root was characterized only *Beta vulgaris*, while the root colour of the other species was yellowish-white.

Table (2): Morphological and seed surface characters of the studied species

Characters \ pecies	*Chenopodium quinoa*	*Spinacia oleracea*	*Beta vulgaris* var. *cicle*	*Beta vulgaris*
1-Root colour	Yellowish-white	Yellowish-white	Yellowish-white	Dark violet
2-Stem habit	Aerial	Aerial	Aerial	Subterranean
3-Stem shape	Erect	Erect	Erect	Disk-Napiform
4-Blade shape	Ovatus	Runcinatus	Undulatus	Ovatus
5-Blade tip shape	Obtusus	Acutus	Acutus	Mucronatus
6-Blade base shape	Breve angustatus	Saggitatus(triangular)	Truncatus	Truncatus
7- Blade margin	Sinuate	Deltoides	Repandum	Venosum
8-Veins colour	Yellowish-green	Yellowish-green	Yellowish-green	Dark violet
9-Phyllotaxy	Alternate	Alternate	Alternate	Whorld
10-Petiole length (cm)	3-4	9-14	11-20	12-28
11-Petiole colour	Yellowish-green	Yellowish-green	Yellowish-green	Dark violet
12-Stomatal shape	Anomocytic	Anomocytic	Anomocytic	Anomocytic
13-Fruit colour	Pale brown	Yellowish-green	Pale brown	Brown
14-Fruit shape	Oblong	Ovate	Squar with 4 furrows	Squar with 4 furrows
15-Seed shape	Clavatus	Reniformis	Ovatus	Rectangularis vel oblongus
16-Seed colour	Bage	Brown	Dark red	Dark red
17-Seed sculpture plate patterns	Falsifoveate	Reticulate-rugose	Reticulate areolate	Scalariform

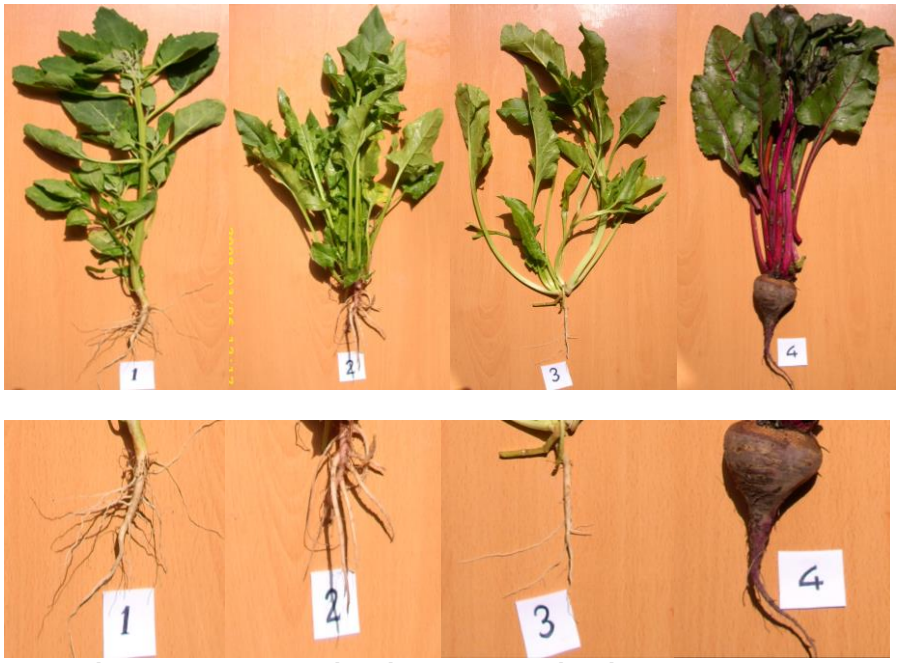
2- Stem characters:

Only *Beta vulgaris* was the unique of the studied species that has subterranean stem and disk-napiform shape, while the other species have aerial and erect stem.

3- Leaf characters:

It is obvious from Table (2) and Fig. (2) that the *Chenopodium quinoa* has leaf characters vary comparing with the other species. It has an ovatus blade shape, obtusus leaf apix, breve augustatus base, sinuate margin and short petiole.

It is worthy to mention that each of the studied species has a unique leaf characteristic, except sharing in few, which could be used to differeniate between species and another (Table 2 and Fig. 2). Also, all the studied species have the same stomata shape; anomocytic, the only exception was
the square shape of guard cells of spinach stomata, while the other species have stomata with oblong guard cells (Fig. 3).



1- Quinoa 2- Spinach 3- Chard 4- Beet

Fig. 1: The morphological apperance of the whole plants and the root shape of the studied species.

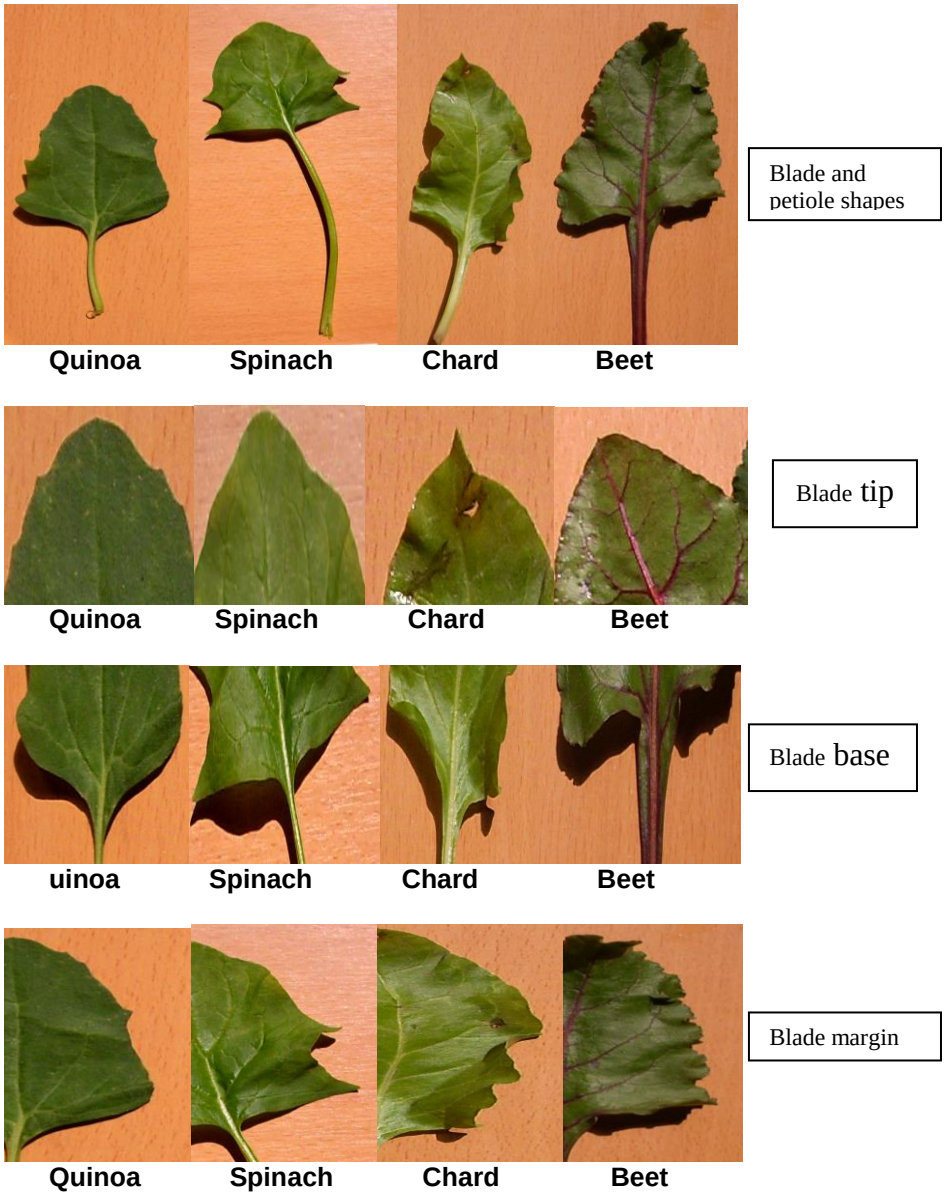


Fig. 2: The morphological leaf shapes of some Chenopodiaceae plants.

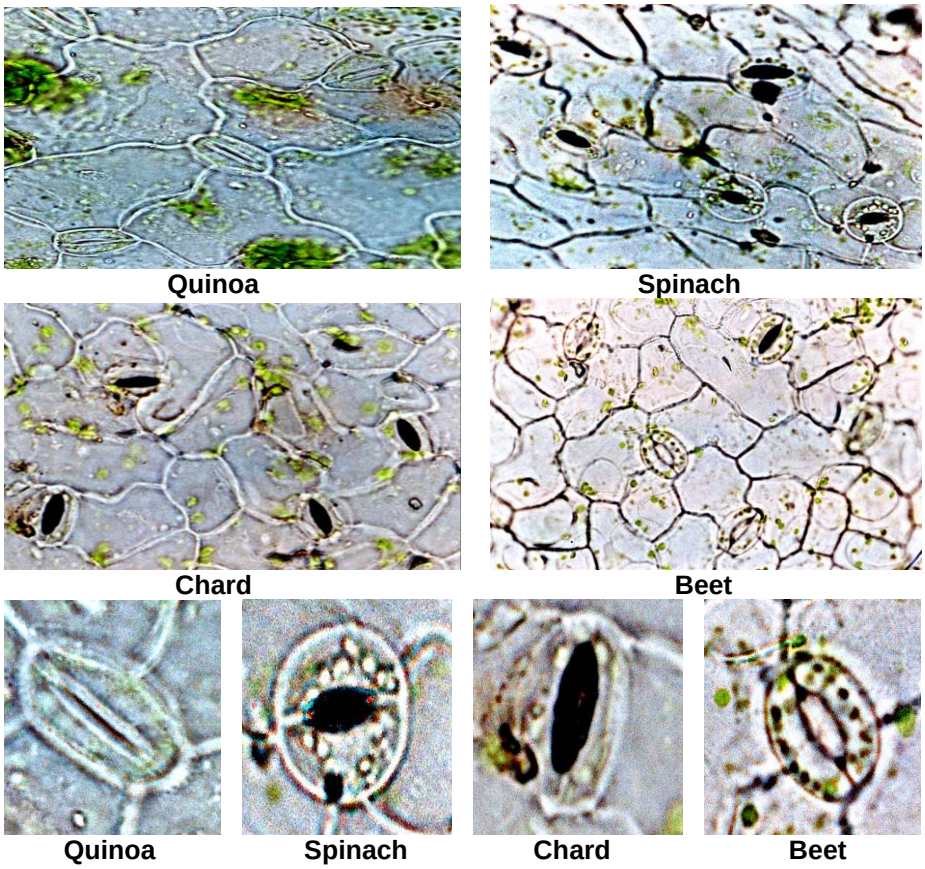


Fig. 3: The epidermal cells and stomata shapes of the studied species.

4- Fruit characters:

The fruit colour of most of the studied species is ranged between pale brown to brown, except in *Spinacia oleracea* where the colour is yellowish-green. The fruit shape was square with furrows the same in both species of genus Beta; *Beta vulgaris* var. *cicle* and *Beta vulgaris*, while it was oblong in *Chenopodium quinoa* and ovate in *Spinacia oleracea* (Table 2 Fig. 4).

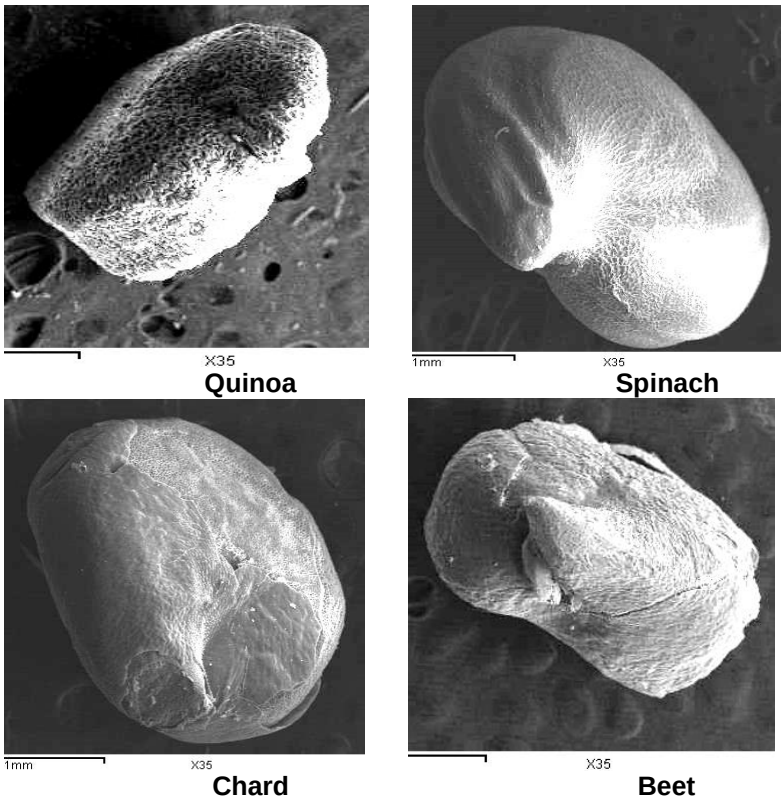
5- Seed characters:

Both species of Beta**;** *Beta vulgaris* var. *cicle* and *Beta vulgaris* have seed testa of dark red colour, while this colour was bage in *Chenopodium quinoa* and brown in *Spinacia oleracea.* There are four shapes of seeds, each characterized every one of the studied species; clavatus for *Chenopodium quinoa;* reniformis for *Spinacia oleracea;* ovatus for *Beta vulgaris* var. *cicle* and rectangularis vel oblongus for *Beta vulgaris* (Table 2 Fig. 5).

The previous obtained results were in accordance with those stated by Gallardo *et al.* (1997) and Prego *et al.* (1998).

Quinoa **Spinach** **Chard** **Beet**

Fig. 4: The morphological fruit shapes of some Chenopodiaceae plants.



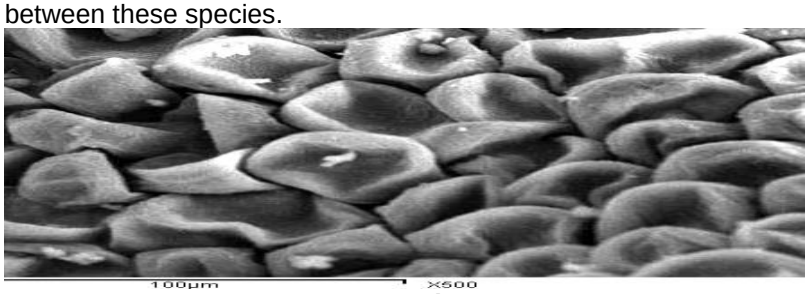
Quinoa **Spinach**

Chard **Beet**

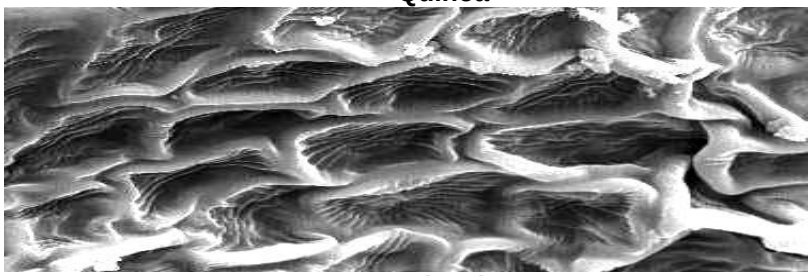
Fig. 5: Seed shapes of the four studied species.

II- SEM results:

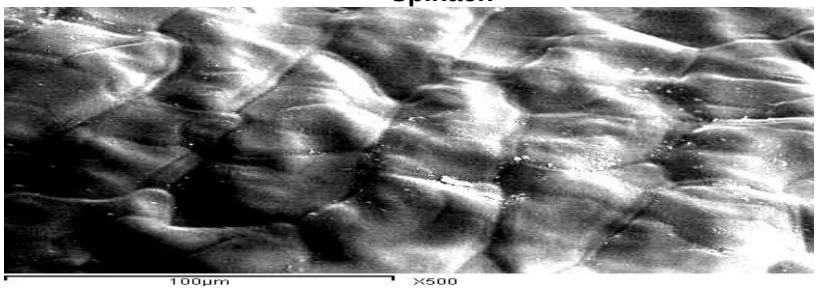
Data in Table (2) and Plate (1) indicated that there are four patterns of seed surface among the studied species. These patterns were; falsifoveate in *Chenopodium quinoa;* reticulate-rugose in *Spinacia oleracea;* scalariform in *Beta vulgaris* var. *cicle* and reticulate areolate in *Beta vulgaris.* This result could be used for further studies as a taxonomic evidence to distinguish between these species.



Quinoa



Spinach



Chard

Beet

Plate 1: Seed surface sculpture as shown by SEM for the studied species.

III- Numerical analysis:

Data obtained from the micro- and macro-morphological characters were applied in clustering analysis using Single Linkage Cluster Analysis Technique producing a dendrogram. The represented dendrogram (Fig. 6) show that the highest average distance of similarity among the studied species was at 0.50. At that level the studied species split into two clusters. The first distinguished at that level (0.50), includes the species *Chenopodium quinoa.* The second cluster divided into two sub-clusters at similarity level 0.33. The first sub cluster includes only the species of *Spinacia oleracea,* where link with the other sub-cluster which includes both species of genus Beta; *Beta vulgaris* var. *cicle* and *Beta vulgaris.* The last two species were more close to each other than to the other studied species and link together earlier at 0.2 similarity level.

Seed surface sculptures appearance; stem habit; blade shape, tip, base and margin; fruit and seed shapes are considered the most diagnostic taxonomic characters to distinguish among the studied species.

The present results of numerical analysis were in accordance with those reported by Yossef *et al*. (2003) and El-Sagai (2006) on some species of Poaceae and Khattab *et al*. (2007) on some legume species.

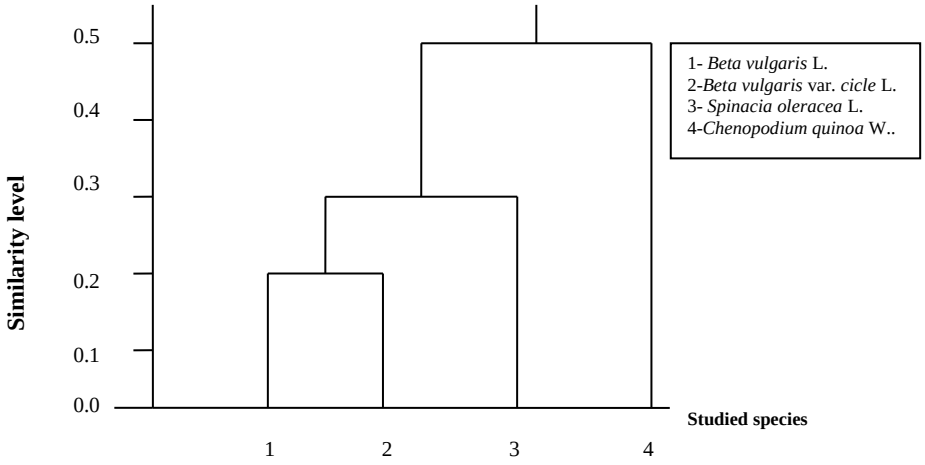


Fig.6: Dendrogram representing the similarity among the studied species.

The following proposed key was designed based on some posterior micro and macro- morphological characters to identify the studied species:

A- Stem aerial, root yellowish, alternate leaves

b- Blade shape ovatus, blade tip obtusus, blade margin sinuate, fruit brown……..…*Chenopodium quinoa* W.

bb- Blade shape vary, blade tip aculatus, blade margin and fruit vary…….

c- Blade shape runciuatus, blade base saggitatus, margin deltoids, fruit ovate……..……*Spinacia oleracea* L.

cc- Blade shape undulates, blade base runcatus, margin repandum, fruit squire with 4 furrows…*Beta vulgaris* var. *cicle* L.

AA- Stem subterranean, root dark violet, whorld leaves…....*Beta vulgaris* L.

IV- Electrophoretic identification of seed protein:

Data of SDS-PAGE of total soluble proteins in seeds of the four investigated species of the family Chenopodiaceae are shown in Figure (7) and Table (3).

It is realized that of 11 bands, Quinoa, Spinach, Chard and Beet recorded 4, 3, 5 and 5 bands; respectively. The four investigated species shared one band at the molecular weight 10 KDa. It is clear that Chard and Beet shared another four bands at the molecular weight 94, 45, 30 and 27 KDa and this proves that Chard and Beet are genetically very close to each other because they showed strong relationship and high similarity. Likewise, Spinach shared and Beet another two bands at molecular weight 45 and 30 KDa and this proves that these three species (Spinach, Chard and Beet) are genetically close to each other and they had not monomorphic bands. However, Quinoa had three monomorphic bands at molecular weight 48, 35 and 25 KDa and this prove that such species, genetically, showed weak relationship with the other three species (Spinach, Chard and Beet) of the family Chenopodiaceae.

From the aforementioned results, it could be concluded thatsuch method of analysis (SDS-polyacrylamide gel electrophoresis separation of total soluble proteins) can be used for identification, differentiation and comparison among the different investigated species of family Chenopodiaceae. Similar results were also reported by Lagercrantz *et al*. (1988) on Norway spruce. Likewise, these results agree also with those of Das and Chatterjee (1994) as well as of Reda *et al*. (2006) on *Cassia* species. Also, these results are in harmony with these reported by Abdel-Dayem (1998) on Poplar species and of Reda *et al.* (2001) on Mahogany species as well as of Reda and Ismail (2008) on *Salix* species.

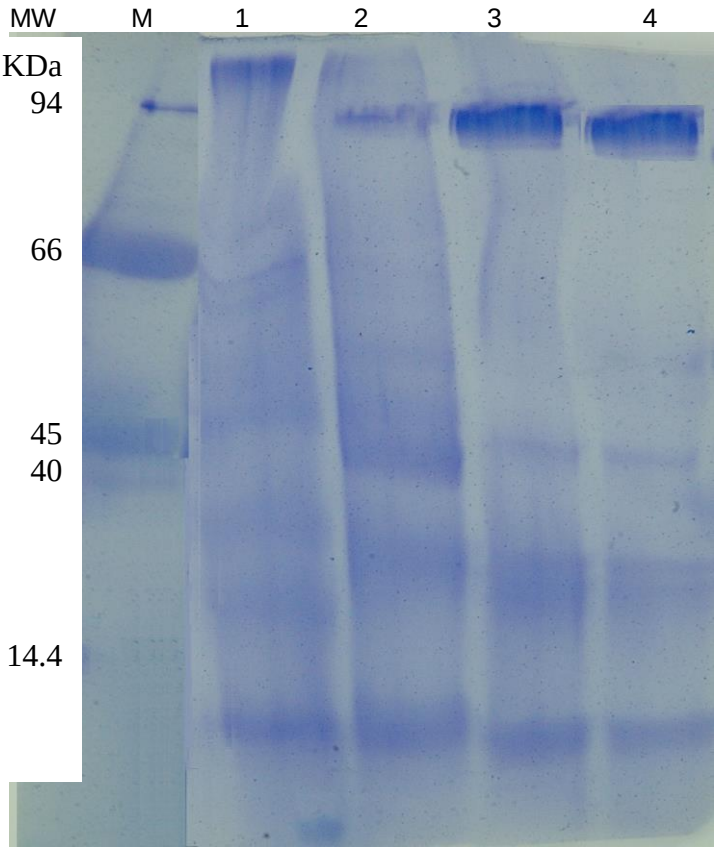


Fig. (7): SDS-PAGE separation of total soluble proteins in seeds of the four investigated species of the family Chenopodiaceae.
M = Low molecular weight protein marker.
1 = *Chenopodium quinoa* Willd (Quinoa).
2 = *Spinacia oleracea* L. (Spinach).
3 = *Beta vulgaris* var. *cicle* L. (Chard).
4 = *Beta vulgaris* L. (Beet).

Table (3): Densitometer analysis of total soluble proteins SDS-PAGE in seeds of four species of the family Chenopodiaceae showing band number and molecular weight (MW)

Band No.	MW (KDa)	Protein marker	Investigated species			
			Quinoa	Spinach	Chard	Beet
1	94	+	-	-	+	+
2	66	+	-	-	-	-
3	48	-	+	-	-	-
4	45	+	-	+	+	+
5	40	+	-	-	-	-
6	35	-	+	-	-	-
7	30	-	-	+	+	+
8	27	-	-	-	+	+
9	25	-	+	-	-	-
10	14.4	+	-	-	-	-
11	10	-	+	+	+	+

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دراسات تقسيمية على بعض أنواع من الفصيلة الرمرامية

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أجريت دراسة على أربعة أنواع نباتية تنتمى الى ثلاثة أجناس من الفصيلة الرمرامية وهى الكينوا , السبانخ , السلق وبنجر المائدة وتناولت تلك الدراسة مقارنة للصفات المورفولوجية للجذور , السوق ، الأوراق , الثمار والبذور لهذه الأنواع بالاضافة الى استخدام الميكروسكوب الالكترونى الماسح لاظهار الاختلافات المورفولوجية الدقيقة لزخارف سطح البذور للأنواع تحت الدراسة. وكذلك تم تعريفها باستخدام التفريد الكهربائى للبروتينات الذائبة الكلية فى بذور هذه الأنواع.

وحيث أن نبات الكينوا قد تمت زراعته حديثاً فى مصر ونظراً لإحتوائه على نسبة مرتفعة من البروتين (أكثر من القمح بنسبة ٥٠ %) تضاف الى دقيق القمح فى صناعة الخبز و المكرونه و البسكويت و المخبوزات المختلفة والى أغذية الأطفال وكبار السن لسهولة هضمه كان من الضرورى معرفة الفروق التقسيمية بينه وبين الأنواع الاكثر شيوعاً التى تتضمنها نفس الفصيلة وذلك باستخدام بعض الصفات المورفولوجية للجذور والسوق والأوراق والثمار والبذور التى أظهرت بعض الاختلافات الواضحة بين تلك النباتات. كما أوضحت الدراسة بالميكروسكوب الالكترونى الماسح أن زخارف سطح بذرة نبات الكينوا تتميز بالشكلfalsifoveate بينما سطح البذرة فى نبات السبانخreticulate - rugose وفى نبات السلق - reticulate areolate أما نبات بنجر المائدة فتميز سطح البذرة بالشكل scalariform . كما أمكن عن طريق التفريد الكهربائى للبروتينات الذائبة الكلية فى البذور من تحديد البصمة الوراثية للأنواع تحت الدراسة و بالتالى يمكن استخدام هذه الطريقة فى التمييز و تحديد صلة القرابة بين هذه الأنواع.