

EVALUATION OF VEGETATIVE GROWTH AND GENETIC IDENTIFICATION OF SOME *Cassia* SPECIES GROWN IN EGYPT

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ABSTRACT

The present investigation was carried out at the Wire-House of Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing seasons of 2004 and 2005 to evaluate the vegetative growth of some *Cassia* species grown in Egypt; i.e., *C. glauca* Lam., *C. corymbosa* Lam., *C. occidentalis* L. and *C. sophera* L. In addition, electrophoretic identification of the investigated *Cassia* species was under consideration. SDS-polyacrylamide gel electrophoresis separation of total soluble proteins in seeds of the investigated *Cassia* species was done at Central Laboratory of Agricultural Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

The obtained results revealed that the four investigated species of the genus *Cassia* showed significant differences in their vegetative growth characters (plant height, stem diameter, number of developed leaves per plant, shoot fresh weight per plant and shoot dry weight per plant) in both studied seasons due to the effect of species and *Cassia sophera* exceeded all other investigated species in this respect followed by *Cassia occidentalis*. 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 different species of the genus *Cassia* under investigation.

Keywords: *Cassia*, Evaluation, Vegetative Growth, Genetic Identification, Electrophoresis.

INTRODUCTION

Cassia, the subject of the present investigation, is a well known and largest genus of the family *Caesalpinaceae* with about 250 species (Cronquist, 1981). *Cassia* species are annual or perennial herbs, shrubs or trees, rarely scrambling or climbing, unarmed. Recently, the genus *Cassia* was split into three genera; namely, *Cassia*, *Chamecrista* and *Senna* (Irwin and Barneby, 1982). The work in molecular biology by Whitty *et al.* (1994) has supported this separation. At the same time, Luckow (1996) illustrated the diagnostic characters for each genus and for major cultivated groups within the largest genus *Senna* and organized artificial key based on morphological characters for the identification and separation of the three genera (*Cassia*, *Chamecrista* and *Senna*). Moreover, Tucker (1992 and 1996) investigated the floral ontogeny among the three segregated genera of *Cassia* and supported their separation.

In Egypt, *Cassia* species are trees, shrubs or herbs, cultivated as ornamentals, rarely wild. Täckholm (1974) stated that *Cassia* in the flora of Egypt is represented by four species; namely, *C. holosericeae*, *C. italica*, *C.*

occidentalis and *C. senna*. In this connection, Ibrahim (1996) organized artificial key based on macromorphological characters for the identification and separation of 8 selected species of *Cassia*, L. in Egypt.

Some species of *Cassia* are cultivated as a source of several sennas; i.e., purgatives (Tomlinson, 1980). Others are used to provide good amount of fodder, and is gaining significant importance as fuelwood, yielding plants having successful role in combating the crisis of fuelwood in many developing countries (Tiwari, 1983). *Cassia* species are also grown as shade tree and for ornamental.

Recently, electrophoretic profiles of seed proteins have been used in systematic studies. The importance of electrophoretic evidence in plant systematics has been discussed at length by Boulter and Derbyshire (1971); Gottlieb (1977) and Ladizinsky and Hymowitz (1979). They presented a more recent brief discussion of seed protein electrophoresis in taxonomic and evolutionary studies. They suggested that protein profiles are often species-specific. In this respect, Das and Chatterjee (1994) carried out electrophoretic study of seed protein of three species of *Cassia* (*C. fistula*, *C. siamea* and *C. spectabilis*) in order to show the variation in their banding patterns. They found that *C. siamea* and *C. spectabilis* are closely related species in respect to their similarity percentage of protein bands and Jaccard's similarity coefficient values.

The present investigation was conducted to evaluate vegetative growth of four available *Cassia* species in Egypt; i.e., *C. glauca* Lam., *C. corymbosa* Lam., *C. occidentalis* L. and *C. sophera* L. In addition, biochemical analysis in seeds of each investigated species was done using SDS-polyacrylamide gel electrophoresis separation of total soluble protein method for genetic identification and differentiation among *Cassia* species under investigation.

MATERIALS AND METHODS

The research work presented in this paper was carried out at the Wire-House of Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing seasons of 2004 and 2005 in order to evaluate the vegetative growth of certain *Cassia* species grown in Egypt. In addition, electrophoretic identification of the investigated *Cassia* species was under consideration. SDS-polyacrylamide gel electrophoresis separation of total soluble proteins in seeds of the investigated *Cassia* species was done at Central Laboratory of Agricultural Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

Source of seeds and experimental work:

Seeds of *Cassia glauca* Lam., *Cassia occidentalis* L. and *Cassia sophera* L. were obtained from El-Orman Botanic Garden, Ministry of Agriculture, Giza, Egypt. Whereas, seeds of *Cassia corymbosa* Lam. were secured from Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt. Seeds of each investigated species were treated by boiling water and then soaked in tap water for 12 hours before sowing. Seeds were sown on fourteenth March, 2004 in the first season and replicated on eighteenth March, 2005 in the second one to provide the experimental plant

materials. Seeds were sown in plastic trays, 40x60 cm, filled with peatmoss and clean sand at the ratio of 1:1 by volume. One month from sowing date, the emerged seedlings were transplanted to plastic pots, one seedling per pot, (25 cm diameter) filled with clay and sand at the ratio of 1:1 by weight. The experiment was made in a randomized complete design with four replicates. The replicate contained 40 pots, each 10 pots were assigned for one species. At the age of eight months from sowing date (seven months from transplanting), plants were lifted from pots for recording the characters of vegetative growth. For each genotype, the recorded data were:

- 1-Plant height (cm).
- 2-Stem diameter (mm).
- 3-Number of developed leaves per plant.
- 4-Fresh weight of shoot (g) per plant.
- 5-Dry weight of shoot (g) per plant.

Data on vegetative growth characters were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The least significant difference (L.S.D.) at 0.05 level was calculated for each investigated character.

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 shaken for one hour. The extract was centrifuged at 3000 rpm for 15 min. The obtained supernatant, which containing total soluble proteins (albumins and globulins), was stored at 20 °C 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 pH8.3, TEMED and freshly prepared ammonium per sulphate solution. Extracts of seed proteins 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.

Data of the comparative chemical studies of protein in the seeds of investigated species of the genus *Cassia* were subjected to regenerate similarity coefficients according to Jaccard (1908). The similarity coefficients were used to construct a dendrogram through the clustering method of UPGMA (Unweighed Pair-Group Method with Arithmetical Averages) using NTSYS-PC (Rohlf, 1993).

RESULTS AND DISCUSSION

I - Evaluation of vegetative growth characters:

The amount of vegetative growth of four *Cassia* species throughout eight months from sowing date in two successive seasons was followed up. Investigated characters included: plant height, stem diameter, number of developed leaves per plant, shoot fresh weight per plant and shoot dry weight per plant. Data on vegetative characters are given in Table (1).

1-Plant height:

It is obvious from Table (1) that the maximum height was recorded by *Cassia sophera* (234.7 cm in the first season and 226.1 cm in the second one) which showed significant difference with any of the other three species of the genus

Table (1): Vegetative growth characters, at the age of 8 months from sowing date, of the four investigated species of the genus *Cassia* in two successive seasons (2004 and 2005)

First season of 2004					
Characters	Investigated species				L.S.D. (0.05)
	<i>Cassia glauca</i>	<i>Cassia corymbosa</i>	<i>Cassia occidentalis</i>	<i>Cassia sophera</i>	
Plant height (cm).	151.3	115.7	212.4	234.7	21.07
Stem diameter (mm).	8.2	6.3	19.8	21.1	1.82
Number of leaves/plant.	28.4	24.2	70.2	72.8	6.11
Shoot fresh weight (g)/plant.	102.9	55.2	259.1	309.2	26.83
Shoot dry weight (g)/plant.	47.3	24.9	125.5	144.1	14.40
Second season of 2005					
Plant height (cm).	144.2	119.3	198.8	226.1	19.25
Stem diameter (mm).	8.5	6.7	18.9	20.3	1.66
Number of leaves/plant.	27.1	25.3	66.9	71.5	5.72
Shoot fresh weight (g)/plant.	113.7	59.6	224.3	281.7	24.58
Shoot dry weight (g)/plant.	49.5	28.2	119.1	135.2	13.94

Cassia in both studied seasons. On the other hand, the minimum height was detected by *Cassia corymbosa* (115.7 cm in the first season and 119.3 cm in the second one), being significantly lower when compared with any of the other investigated species in both seasons. The descending order of plant height was 234.7, 212.4, 151.3 and 115.7 cm for *C. sophera*, *C. occidentalis*, *C. glauca* and *C. corymbosa*; respectively in the first season. It is realized that the four studied species of the genus *Cassia* showed the same trend of plant height in the second season, the records were 226.1 cm for *C. sophera*, 198.8 cm for *C. occidentalis*, 144.2 cm for *C. glauca* and 119.3 cm for *C. corymbosa*.

From the aforementioned results, it could be stated that plant height was significantly affected by species and *C. sophera* exceeded significantly all other species in this respect and followed by *C. occidentalis* in both studied seasons.

2- Stem diameter:

Data presented in Table (1) clearly show that the maximum diameter of the main stem at its median portion was obtained by *Cassia sophera* (21.1 mm in the first season and 20.3 mm in the second one) which in turn being statistically indifferent with that of *Cassia occidentalis* (19.8 mm in the first season and 18.9 mm in the second one) and showed significant difference with any of the other two species in both studied seasons. By contrast, the minimum diameter was recorded by *Cassia corymbosa* in both studied seasons. It was 6.3 mm in the first season and 6.7 mm in the second one, being significantly lower when compared with any of the other investigated species of the genus *Cassia*. The descending order of stem diameter was 21.1, 19.8, 8.2 and 6.3 mm for *C. sophera*, *C. occidentalis*, *C. glauca* and *C. corymbosa*; respectively in the first season. It is clear that the same order was also observed in the second season, the records were 20.3 mm for *C. sophera*, 18.9 mm for *C. occidentalis*, 8.5 mm for *C. glauca* and 6.7 mm for *C. corymbosa*.

As inferred earlier, it could be stated that stem diameter at its median portion was significantly affected by species and *C. sophera* as well as *C. occidentalis* exceeded significantly the other two species in this respect.

3- Number of developed leaves per plant:

It is clear from Table (1) that the highest number of leaves was recorded by *Cassia sophera* (72.8 leaves in the first season and 71.5 leaves in the second one) which in turn being statistically indifferent with that of *Cassia occidentalis* (70.2 leaves in the first season and 66.9 leaves in the second one) and showed significant increase over any of the other two investigated species of the genus *Cassia* in both studied seasons. On the contrary, the lowest number of leaves was obtained by *Cassia corymbosa* (24.2 leaves in the first season and 25.3 leaves in the second one) which in turn being statistically indifferent with that of *Cassia glauca* (28.4 leaves in the first season and 27.1 leaves in the second one) and showed significant decrease below any of the other two species in both studied seasons. The descending order of number of leaves was 72.8, 70.2, 28.4 and 24.2 leaves for *C. sophera*, *C. occidentalis*, *C. glauca* and *C. corymbosa*; respectively in the first season. It is worthy to note that the same order was also observed in the second season, the records were 71.5 leaves for *C. sophera*, 66.9 leaves for *C. occidentalis*, 27.1 leaves for *C. glauca* and 25.3 leaves for *C. corymbosa*.

From the above mentioned results, it could be stated that the number of leaves was significantly affected by species and *Cassia sophera* as well as *Cassia occidentalis* exceeded significantly the other two *Cassia* species in this respect in both studied seasons.

4- Shoot fresh weight per plant:

Results in Table (1) clearly show that the differences in shoot fresh weight per plant among the four investigated species of the genus *Cassia* proved significant in both studied seasons. The maximum fresh weight was achieved by *Cassia sophera* (309.2g in the first season and 281.7g in the second one) which showed significant increase over any of the other investigated species

in both studied seasons. In contrast, the minimum fresh weight of shoot per plant was recorded by *Cassia corymbosa* (55.2g in the first season and 59.6g in the second one), being significantly lower when compared with any of the other investigated species in both studied seasons. The descending order of shoot fresh weight was 309.2, 259.1, 102.9 and 55.2g for *C. sophera*, *C. occidentalis*, *C. glauca* and *C. corymbosa*; respectively in the first season. The descending order of shoot fresh weight in the second season was similar to that observed in the first season, the records were 281.7g for *C. sophera*, 224.3g for *C. occidentalis*, 113.7g for *C. glauca* and 59.6g for *C. corymbosa*.

Referring to the aforementioned results it could be stated that the fresh weight of plant shoot was significantly affected by species and *C. sophera* exceeded significantly all other investigated species in this respect and followed by *C. occidentalis* in both studied seasons.

5-Shoot dry weight per plant:

Data presented in Table (1) clearly show that the differences in shoot dry weight per plant among the four investigated species of the genus *Cassia* proved significant in both studied seasons. The maximum dry weight was obtained by *Cassia sophera* (144.1g in the first season and 135.2g in the second one), being significantly higher when compared with any of the other three species of the genus *Cassia* in both studied seasons. On the other hand, the lowest dry weight was recorded by *Cassia corymbosa* (24.9g in the first season and 28.2g in the second one), being significantly lower when compared with any of the other three investigated *Cassia* species in both studied seasons. The descending order of shoot dry weight per plant was 144.1, 125.5, 47.3 and 24.9g for *C. sophera*, *C. occidentalis*, *C. glauca* and *C. corymbosa*; respectively in the first season. It is realized that the four investigated species of the genus *Cassia* showed the same trend of shoot dry weight in the second season, the records were 135.2g for *C. sophera*, 119.1g for *C. occidentalis*, 49.5g for *C. glauca* and 28.2g for *C. corymbosa*.

As inferred earlier, it could be stated that the dry weight of plant shoot was significantly affected by species and *Cassia sophera* exceeded significantly all other investigated species of the genus *Cassia* in this respect in both studied seasons.

From the aforementioned results concerning the vegetative growth characters of the four investigated species of the genus *Cassia*, it could be stated that the four studied genotypes of the genus *Cassia* showed significant differences in their vegetative growth attributes due to the effect of species. Similar results were also recorded by Sun and Dickinson (1997) as well as by Reda et al. (2001) on Mahoganies. Likewise, Abdel-Dayem (1998) reached to similar conclusion on Poplars.

II-Electrophoretic identification of seed protein in *Cassia* species:

Seeds of *C. glauca*, *C. corymbosa*, *C. occidentalis* and *C. sophera* are photographed in Figure (1). An electrophoretic separation of total soluble proteins in these seeds using SDS-PAGE was performed (Figure.2).

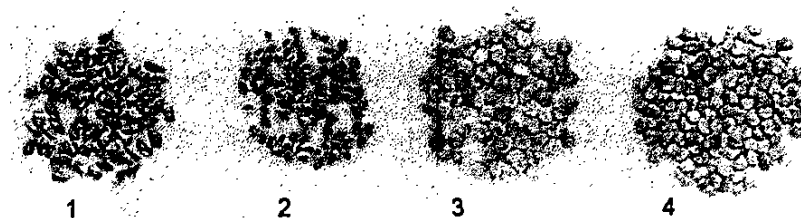


Fig. (1): Mature seeds of :
1 - *Cassia glauca* Lam. 2 - *Cassia corymbosa* Lam.
3 - *Cassia occidentalis* L. 4 - *Cassia sophera* L.

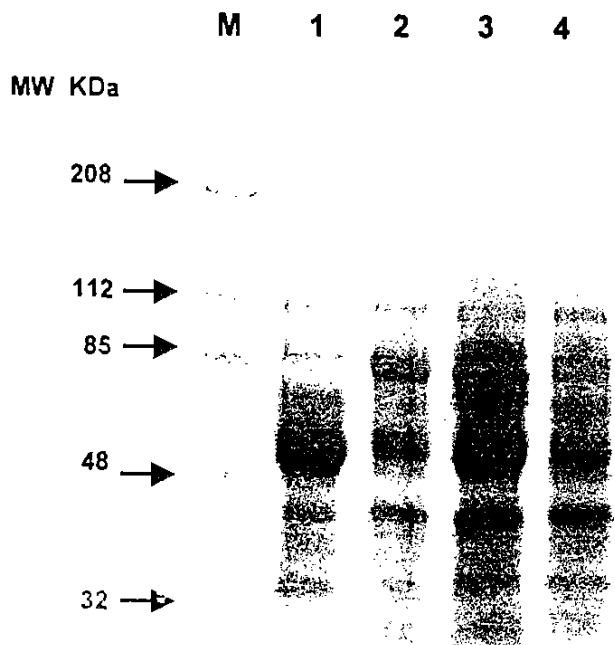


Fig. (2): SDS-PAGE separation of total soluble proteins in seeds of the four investigated species of the genus *Cassia*.
M = Low molecular weight protein marker.
1 = *Cassia glauca* Lam. 2 = *Cassia corymbosa* Lam.
3 = *Cassia occidentalis* L. 4 = *Cassia sophera* L.

Data of SDS-PAGE of total soluble proteins in seeds of the four investigated species of the genus *Cassia* are shown in Table (2) and Figures (2 and 3).

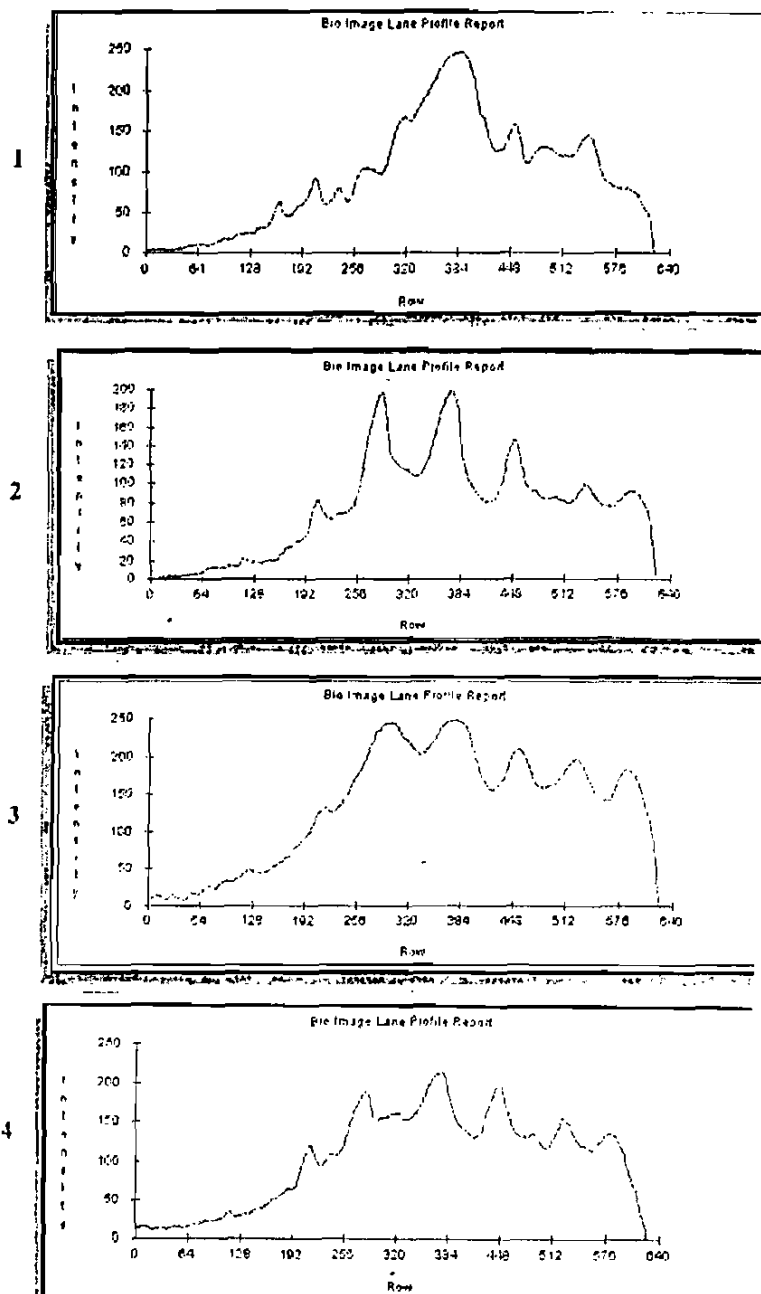


Fig. (3): Scanning curves of SDS-PAGE separation of total soluble proteins in seeds of the four investigated species of the genus Cassia.

1-Cassia glauca Lam. 2-Cassia corymbosa Lam.
 3-Cassia occidentalis L. 4-Cassia sophera L.

It is evident that of 17 bands, *C. glauca*, *C. corymbosa*, *C. occidentalis* and *C. sophera* recorded 12, 9, 8 and 11 bands; respectively. The four investigated species shared four bands at the molecular weights 158.37, 61.47, 44.88 and 39.69 KDa and relative front 0.170, 0.586, 0.724 and 0.778; respectively. *Cassia glauca* shared *C. corymbosa* one band at molecular weight 32.23 KDa, and relative front 0.869. However, *C. glauca* and *C. occidentalis* shared two bands at the molecular weight 110.85 and 35.13 KDa, and relative front 0.326 and 0.832; respectively. At the same time, *C. glauca* and *C. sophera* shared four bands at the molecular weight 102.06, 88.82, 74.72 and 35.13 KDa, and relative front 0.363, 0.424, 0.500 and 0.832; respectively and this proves that *C. glauca* and *C. sophera* are genetically close to each other because they showed high similarity. In this respect, *C. corymbosa* shared *C. occidentalis* two bands at the molecular weight 85.54 and 27.42 KDa, and relative front 0.441 and 0.941. Also, *C. corymbosa* shared *C. sophera* two bands at molecular weight 114.23 and 27.42 KDa, and relative front 0.314 and 0.941. Likewise, *C. occidentalis* shared *C. sophera* two bands at molecular weight 35.13 and 27.42 KDa and relative front 0.832 and 0.941. It is worthy to mention that there were 2, 1, 0 and 1 monomorphic bands in case of *C. glauca*, *C. corymbosa*, *C. occidentalis* and *C. sophera*; respectively.

Table (2): Densitometer analysis of total soluble proteins SDS-PAGE in seeds of four species of the genus *Cassia* showing band number, relative front (Rf) and molecular weight (MW)

Band No.	Rf	MW (KDa)	Investigated species			
			<i>Cassia glauca</i>	<i>Cassia corymbosa</i>	<i>Cassia occidentalis</i>	<i>Cassia sophera</i>
1	0.170	158.37	+	+	+	+
2	0.251	131.75	+	-	-	-
3	0.314	114.23	-	+	-	+
4	0.326	110.85	+	-	+	-
5	0.363	102.06	+	-	-	+
6	0.424	88.82	+	-	-	+
7	0.441	85.54	-	+	+	-
8	0.500	74.72	+	-	-	+
9	0.586	61.47	+	+	+	+
10	0.637	54.71	-	-	-	+
11	0.663	51.52	+	-	-	-
12	0.724	44.88	+	+	+	+
13	0.778	39.69	+	+	+	+
14	0.802	37.58	-	+	-	-
15	0.832	35.13	+	-	+	+
16	0.869	32.23	+	+	-	-
17	0.941	27.42	-	+	+	+

Similarity index of the four investigated species of the genus *Cassia* based on SDS-PAGE of total soluble proteins in the seeds is given in Table (3). This is also illustrated dendrographically in Figure (4). Two main clusters were recorded the first one included both *C. glauca* and *C. sophera*. Whereas, the second cluster included both *C. corymbosa* and *C. occidentalis*.

Table (3): Similarities among the four investigated species of the genus *Cassia* based on SDS-PAGE of total soluble proteins

<i>Cassia</i> species	<i>C. glauca</i>	<i>C. corymbosa</i>	<i>C. occidentalis</i>	<i>C. sophera</i>
<i>C. glauca</i>	1.00000			
<i>C. corymbosa</i>	0.31250	1.00000		
<i>C. occidentalis</i>	0.42857	0.54546	1.00000	
<i>C. sophera</i>	0.53333	0.42857	0.46154	1.00000

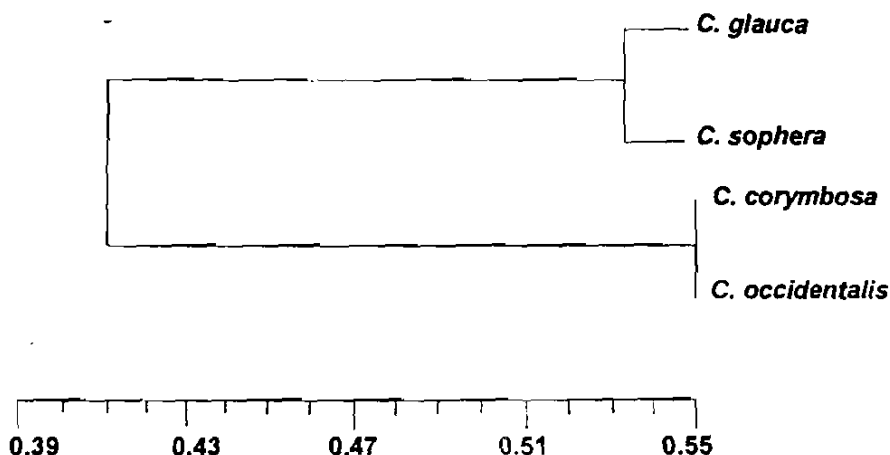


Fig. (4): Dendrogram of Jaccard similarity obtained by the UPGMA method showing relationship among the four studied species of the genus *Cassia* based on SDS-PAGE of total soluble proteins.

Results of SDS-PAGE of total soluble proteins in seeds of the four studied species of the genus *Cassia* proved that *C. glauca* was more related to *C. sophera* with similarity of 53.3%. Likewise, *C. corymbosa* was more related to *C. occidentalis* with similarity of 54.5%.

From the aforementioned results, it could be concluded that such 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 the genus *Cassia*. In this respect, Das and Chatterjee (1994) carried out electrophoretic study of seed protein of three species of *Cassia* (*C. fistula*, *C. siamea* and *C. spectabilis*) in order to show the variation in their banding patterns. They found that *C. siamea* and *C. spectabilis* are closely related species in respect to their similarity percentage of protein bands and Jaccard's similarity coefficient values. Such results supported the use of electrophoretic identification of seed protein of *Cassia* species. Similar results were also reported by Lagercrantz *et al.* (1988) on Norway spruce. Likewise, these results agree also with those of Cheliak and Pitel (1984) as well as of Abdel-Dayem (1998) on Poplar species and of Reda *et al.* (2001) on Mahogany species.

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تقييم النمو الخضري لبعض أنواع جنس الكاسيا النامية في مصر وتعريفها وراثيا
فاتن محمد رضا* ، حسن رمضان حسن رمضان** و داليا محمد عبد العزيز نصار
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أجرى هذا البحث في الصوبة السلوكية التابعة لقسم النبات الزراعي بكلية الزراعة جامعة القاهرة
بالجيزة خلال موسم النمو ٢٠٠٤ و ٢٠٠٥ بهدف تقييم النمو الخضري لبعض أنواع جنس الكاسيا النامية
في مصر وهي:

- 1- *Cassia glauca* Lam. 2- *Cassia corymbosa* Lam.
3- *Cassia occidentalis* L. 4- *Cassia sophera* L.

كما تم تعريف هذه الأنواع وراثيا باستخدام طريقة التفريد الكهربائي للبروتينات الذاتية الكلية في
البذور وذلك في المعمل المركزي التابع لقسم الكيمياء الحيوية بكلية الزراعة جامعة القاهرة بالجيزة.
أوضحت النتائج المتحصل عليها ظهور اختلافات معنوية في جميع صفات النمو الخضري المدروسة
(ارتفاع النبات - قطر الساق - عدد الأوراق المتكونة على النبات - الوزن الرطب للمجموع الخضري
للنبات - الوزن الجاف للمجموع الخضري للنبات) بين الأربعة أنواع من جنس الكاسيا تحت الدراسة مما
يؤكد تأثير النوع على هذه الصفات مع تفوق النوع *Cassia sophera* L. ثم يليه النوع *Cassia*
occidentalis L. على بقية الأنواع الأخرى في صفات النمو الخضري في كلا موسمي الدراسة، لذا
يوصى بإكثار ونشر هذين النوعين للاستفادة منهما في مصر لسرعة نموها كاشجار زينة وأيضا للحد من
مشكلة نقص الأخشاب خصوصا أخشاب الوقود وأيضا لأهمية هذين النوعين من الناحية الطبية. كما أمكن
عن طريق التفريد الكهربائي للبروتينات الذاتية الكلية في البذور من تحديد البصمة الوراثية لأنواع جنس
الكاسيا الأربعة التي تم دراستها وبالتالي يمكن استخدام هذه الطريقة في التمييز وتحديث صلة القرابة بين
الأنواع تحت الدراسة.