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Genetic Diversity of Achene Heteromorphism in Egyptian *Calendula micrantha* Tineo et Guss

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Abstract: The present investigation is carried out in order to gain insight into the genetic variability which correlates with seed polymorphism in *Calendula micrantha* Tineo et Guss. based on evidence obtained from karyotype analysis and electrophoretic patterns of seed proteins. Six distinct morphs of achene heteromorphism have been reported in *C. micrantha* collected from the Mediterranean desert of Egypt. These morphs are balloon-shaped lacking spines, balloon shaped with spines, insect leg, winged, ring-like and curved achenes. All of these morphs were observed on every infructscence. All achene forms of *C. micrantha* has a diploid chromosome number of 2n = 44. Cytological analyses include nucleic acid content DNA and RNA (mg g^{-1}), total chromosome length (TCL), mean chromosome length (MCL), total chromosome volume (TCV), mean arm ratio (Mr value), intra chromosomal index (A_1), inter chromosomal index (A_2), total form percent (TF%) and karyotype formulae carried out for all the studied samples. Karyotype characteristics are given here for the first time. The karyotype formulae revealed that all of them had symmetrical karyotype except in insect leg form. The electrophoretic analysis of total seed protein extracts using discontinuous SDS-PAGE gel for the studied samples are recorded. The total number of recorded bands were 26. Such bands are not always expressed in all samples. Each sample exhibited a distinctive electrophoretic pattern. From this study, it could be suggested that the genetic differentiation in *C. micrantha* might be the consequence of an adaptive process.

Key words: *Calendula micrantha* Tineo et Guss., achene heteromorphism, karyotype analysis, protein electrophoresis

Introduction

The existence of heteromorphic fruits has been well described in a number of families including Asteraceae, Brassicaceae, Poaceae and Chenopodiaceae (Tanowitz *et al*., 1987). The production of heteromorphic seeds has been shown for many plant species inhabiting unpredictable environments, such as frequently disturbed habitats (Harper, 1977) and arid and semi-arid environments (Venable and Lawlor 1980; Ellner and Shmida, 1984).

Achene polymorphism in Asteraceae most likely spreads germination out in space and time and thereby increases the number of safe sites an individual percent can exploit in disseminating off springs (McEvoy, 1984). Seed heteromorphism has been assumed to increase adaptation in highly variable environments because different seed types have been shown to function differentially in dispersal (e.g. *Gymnarrhena micrantha*, koller and Roth 1964*; Picris echioides*, Sorensen 1978; *Crepis sancta*, Imbert *et al*., 1996), within-or among year time of germination (e.g. *Plantago coronopus*, Schat 1981*; Senecio jacoboea*, Mc Evoy 1984; *Heterotheca latifolia*, Venable and Levin 1984; *Hemizonia increscens*, Tanowitz *et al*., 1987 and *Cakile edentula*, Zhang, 1993).

Heteromorphic achenes have been reported in *Calendula micrantha* (Asteraceae) (Zohary 1950, Täckholm 1974 Syn: *C. arvensis* L., *C. aegyptiaca* Pers., Bolous 1995). The environment of the Egyptian Mediterranean desert is highly unpredictable in time and space (El-Keblawy *et al*., 1997). It seems that *C. micrantha* may survive such conditions by producing different achenes capable of success in different habitats and different times.

El-Keblawy (1999) described six distinct morphs for *C. micrantha* collected from the Mediterranean desert of Egypt. The different morphs of *C. micrantha* differ in size, shape, dispersal and total germination percentage. He found that there are positive relationship between achene size and germination. Also, the variation in achene shape and colour was associated with variation in achene mass. *Calendula arvensis* (Syn. *C. micrantha*) is important for its economic value as it used as anti-inflammatory agents for skin disease (Graf, 2000), calendula cream for grazes and scalds (Kaplan, 1994). Also, it is used as antiviral agent (Bogdanova *et al*., 1970). Also, Elias *et al*. 1990 and Chemli *et al*. 1990 reported that the species contains saponins, as well as calendic acid (Chisholm and Hopkins, 1967 and Fritsche *et al*., 1999) which are used in the treatment of skin diseases.

However, no study, to my knowledge, examined the genetic variability which correlates with seed polymorphism in *C. micrantha*. This investigation is carried out in order to gain insight into its genetic variation based on evidence obtained from karyotype analysis and electrophoretic patterns of seed proteins.

Materials and Methods

Calendula micrantha is an annual monoecious plant, flowering from early winter (Nov., or Dec.) to spring (April). It grows in a variety of habitats ranging from sand to clay and loamy compact soils. It is observed to be insect pollinated.

Mature fruits were collected from about 40 plants from the Mediterranean desert near El-Arish, North Sinai, Egypt. Achenes were separated into six morphs described as follows: balloon-shaped achenes lacking spines at the back of the pericarp, balloon-shaped with spines, insect leg, large achenes with big wide wings, ring-like and curved achenes (Fig. 1). All of these morphs were observed in every infructscence.

Seeds of the studied samples were germinated. Actively growing root tips were pretreated for 2-4 h in 0.002 M 8-hydroxyquinoline (Tjio and Levan, 1950), fixed in 3:1 (absolute ethanol: acetic acid), hydrolysed for 5 min. in 1N HCl at 60°C and stained by using modified carbol fuchsin (for 2 h.) (Koa, 1975 aandb) followed by aceto orcein stain. Well spread metaphase plates were selected and photographed. Determination of karyotype parameters was carried out using image and working with photomicrographs. Mean descriptive values for karyotypes were calculated from a minimum five scattered metaphase plates measured in each sample. Karyograms were drawn, lengths of long arm (L) and short arm (S) were measured for karyotype analysis. The nomenclature used for the description of chromosome morphology is that proposed by Abraham and Prasad (1983). Mean chromosome length (MCL) in μ and mean arm ratio (Mr value) in μ were determined. The total chromosome volume (TCV) was estimated using the formula: TCV=2 (Π x r^2 x TCL) where r is the average radius of the chromatid and TCL the total chromosome length. To estimate karyotype asymmetry, two numerical parameters were used according to Romero Zarco (1986): A_1 (intra chromosomal asymmetry index) and A_2 (inter chromosomal asymmetry index). Also, the total form percent (TF%) i.e. the average degree of symmetry over the whole karyotype was calculated according to Huziwara (1962). The values of analysed criteria were expressed by calculating the standard error (SE) of these parameter.

For nucleic acids extraction, the method based on that of Shibko *et al.* (1967). DNA was estimated by diphenylamine colour reaction described by Burton (1968). RNA was determined followed the method of Dische (1962).

For protein electrophoretic analysis, the method for discontinuous SDS-PAGE techniques was based on that of Laemmli (1970). For the determination of the molecular weight a mixture of the marker proteins are used. The banding profile in gel was photographed. The number of bands for each sample was scored. The analysis percentage of the bands were carried out using Gel Doc 2000, Bio Rad Densitometer scanner.

Results

All the samples under study were diploid. In the analyzed samples 44 chromosomes were observed in somatic cells (Fig. 2).

Cytological analyses include nucleic acid content DNA and RNA (mg g^{-1}), total chromosome length (TCL), mean chromosome length (MCL), total chromosome volume (TCV), mean chromosome arm ratio (Mr value), intra chromosomal index (A_1), inter chromosomal index (A_2), total form percent (TF%) and karyotype formula were carried out for all the studied samples, data are summarized (Table 1). Karyograms of the studied samples illustrated in (Fig. 3).

The electrophoretic analysis of total seed protein extracts using discontinuous SDS-PAGE gel for the studied samples are recorded in Fig. 4. The scanning of SDS-PAGE gel of the different samples, their molecular weight (MW), relative front (RF), number of bands and average optical density (OD) are shown in Fig.5 and Table 2, 3.

Discussion

Seed polymorphism is a common phenomenon associated with discrete and (or) continuous morphological or physiological variation among individual seeds produced by a plant or population (Venable, 1985; Silvertown 1984). Nevo *et al.* (1998) indicated that micro climatic conditions generate both protein and DNA patterns of polymorphism that parallel macroscale environmental pattern. They also emphasized that natural selection appears to be a major differentiating and orienting force of regional evolutionary change, maintaining genetic polymorphism under conditions of environmental heterogeneity and stress. Fruit heteromorphism in *C. micrantha*, as in most species of Asteraceae, represents a form of bet-hedging in the face of environments that may vary significantly in time (Venable, 1985), or space (Schoen and Lloyed, 1984).

DNA localized in cell nuclei codes most of the genetic information of an organism (Dolezel *et al.*, 1998). From

Table 1: Cytological data [Nucleic acid content DNAandRNA (mg g^{-1}), Total chromosome length (TCL), Mean chromosome length ± SE (MCL), Total chromosome volume (TCV), Mean chromosome arm ratio ± SE (Mr value), Intra chromosomal index (A_1), Inter chromosomal index (A_2) Total form percent (TF%) and karyotype formula of the different achene morphs of *Calendula micrantha.*

							Asymmetry Values			Karyotype Formula					
Achene morph	DNA mg g^{-1}	RNA mg g^{-1}	TCL (µm)	MCL±SE (µm)	TCV (µm^3)	Mr value (µm)±SE	A_1	A_2	TF%	M	nm	nsm(-)	SM	nsm(+)	nst(-)
Balloon- like	0.435	0.848	52.71	1.20±0.005	45.32	1.360±0.009	0.212	0.190	43.089	14	20	10	-	-	-
Balloon - like with spines	2.904	1.704	50.21	1.14±0.006	87.75	1.848±0.014	0.399	0.241	35.846	2	18	22	2	-	-
Insect leg	1.423	2.262	71.14	1.62±0.009	130.17	2.038±0.022	0.401	0.254	34.639	4	14	18	2	4	2
Winged	0.094	0.619	65.36	1.49±0.010	90.85	2.039±0.019	0.417	0.283	33.530	4	14	18	2	6	-
Ring- like	2.381	7.721	39.71	0.90±0.004	24.42	1.651±0.015	0.315	0.212	38.489	8	20	14	-	2	-
Curved	3.333	3.529	48.43	1.10±0.006	24.74	1.875±0.014	0.406	0.245	36.283	4	16	22	-	2	-

M: Metacentric, SM: SubMetacentric, nm: nearly metacentric, nsm(+): nearly submetacentric (+), nsm(-): nearly submetacentric (-), nst (-): nearly subtelocentric (-)

Table 2: Protein electrophoretic analysis of different achene morphs of *Calendula micrantha*: curved (1), Balloon-like (2), Balloon-like with spines (3), winged (4), insect leg (5), and ring like (6)

Lane number	Band number	Relative front	Mol. Wt. Kda	Average OD
1	1	0.330	155.894	0.238
1	2	0.351	141.824	0.250
1	3	0.475	99.455	0.371
1	4	0.579	69.965	0.312
1	5	0.595	66.200	0.416
1	6	0.631	59.672	0.366
2	1	0.332	154.062	0.314
2	2	0.353	140.157	0.434
2	3	0.462	101.955	0.274
3	1	0.331	155.286	0.410
3	2	0.352	141.236	0.554
3	3	0.456	103.250	0.279
3	4	0.560	74.932	0.176
4	1	0.332	154.066	0.316
4	2	0.351	141.737	0.275
4	3	0.450	104.335	0.211
4	4	0.555	76.252	0.152
5	1	0.350	142.244	0.166
5	2	0.442	105.974	0.184
5	3	0.553	76.889	0.158
6	1	0.327	157.817	0.298
6	2	0.351	141.647	0.230
6	3	0.446	105.206	0.345
6	4	0.512	88.856	0.176
6	5	0.549	77.938	0.260
6	6	0.594	66.468	0.200

Table 3: Comparative analysis of relative concentrations and molecular weights (M.Wt) of extracted proteins of different achene morphs of *Calendula micrantha* separated using SDS-PAGE technique

	Band %						
Band No.	Curved	Balloon like	Balloon like with spines	Winged	Insect leg	Ring like	M.Wt. (KDa)
1	...	...	...	...	...	8.143	157.817
2	1.091	...	...	...	...	...	155.894
3	...	...	2.131	...	...	...	155.286
4	...	...	...	6.558	...	...	154.066
5	...	1.317	...	...	...	...	154.062
6	...	...	...	...	3.567	...	142.244
7	1.837	...	...	...	...	...	141.824
8	...	...	...	6.719	...	...	141.737
9	...	...	...	...	...	6.842	141.647
10	...	...	2.302	...	...	...	141.236
11	...	2.727	...	...	...	...	140.157
12	...	...	...	...	4.701	...	105.974
13	...	...	...	...	...	9.829	105.206
14	...	...	...	4.880	...	...	104.335
15	...	...	5.215	...	...	...	103.250
16	...	4.881	...	...	...	...	101.955
17	10.217	...	...	...	...	...	99.455
18	...	...	...	...	...	2.722	88.856
19	...	...	...	...	...	6.799	77.938
20	...	...	...	...	0.847	...	76.889
21	...	...	...	0.741	...	...	76.252
22	...	...	0.914	...	...	...	74.932
23	2.293	...	...	...	...	...	69.965
24	...	...	...	...	...	6.167	66.468
25	7.637	...	...	...	...	...	66.200
26	8.061	...	...	...	...	...	59.672
Total	6	3	4	4	3	6	

Table 1 it is clear that, the nuclear DNA contents ranges from 0.094 mg g^{-1} to 3.333 mg g^{-1}. The maximum value of 3.333 mg g^{-1} was recorded in curved achene and the minimum value of 0.094 mg g^{-1} was recorded in winged achene. The RNA content ranges from 0.619 mg g^{-1} to 7.721 mg g^{-1}. The highest content value was found in ring-like achene of 7.721 mg g^{-1}, followed by curved form the RNA content found (3.529 mg g^{-1}) while the lowest calculated content was found in winged form (0.619 mg g^{-1}).

Cytological features used to determine the degree of karyotype variation of the studied samples are summarized in Table 1. All achene forms of *C. micrantha* has a diploid chromosome number of 2n= 44 (Fig. 2). This diploid chromosome number confirmed the previous reports by Hazra (1970), Strother (1972), Nordenstam (1972), Powell *et al.* (1974), Heyn *et al.* (1974), Van Loon (1974) and Borgen (1975).

The karyotype characteristics are given here for the first time (Table 1, Fig. 3).

The TCL (total chromosome length) at mitotic metaphase varies between 39.71 and 71.14 µm. The TCV (total chromosome volume) shows differences among the studied achene forms, being lower in ring-like (24.42 µm^3) and curved form (24.74 µm^3) than in insect leg form (130.17 µm^3) (Table 1).

Karyotypic differences were observed between different achene forms with regard to chromosomal morphology and chromosome length. The chromosomes of insect leg

Fig. 1: Shapes and relative size of different achene morphs of *Calendula micrantha*: Baloon-like (1), Baloon like with spines (2), Insect-leg (3), Winged (4), ring-like (5) and Curved (6)

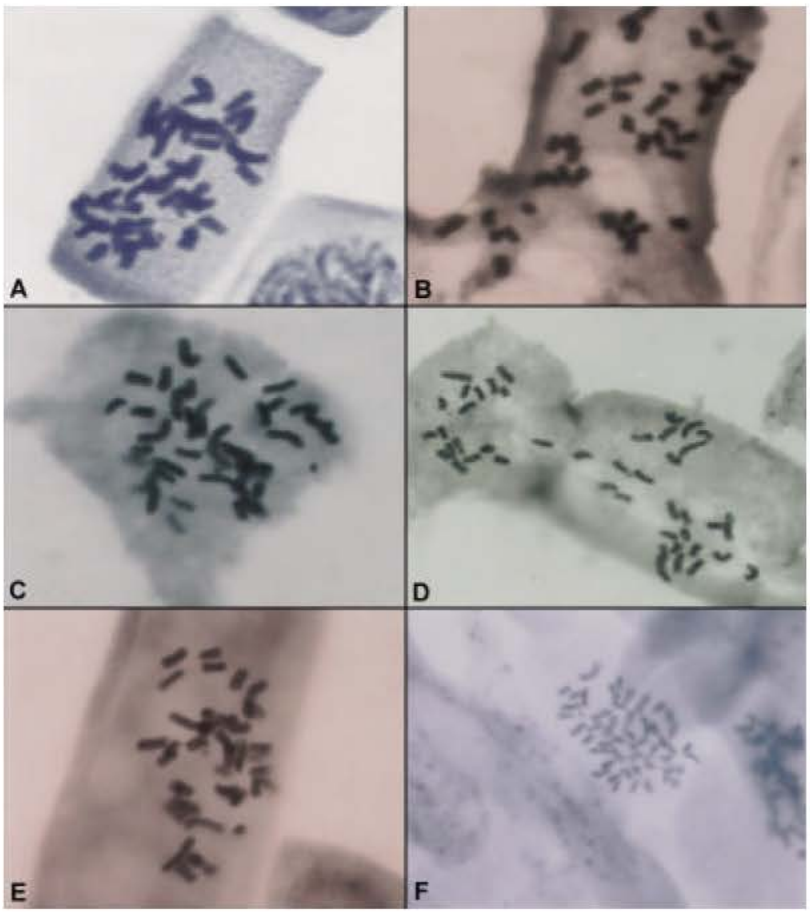

Fig. 2: Somatic chromosomes of different achene morphs of *Calendula micrantha*: Balloon-like (A), Balloon like with spines (B), Insect-leg (C), Winged (D), ring-like (E) and Curved (F) (X=1000)

form falls in 6 groups (Metacentric, nearly metacentric, nearly submetacentric (-), Sub Metacentric, nearly submetacentric (+) and nearly subtelocentric (-) types) where balloon-like achene had 3 groups of chromosomes (Metacentric, nearly metacentric and nearly submetacentric (-) (Table 1).

The karyotype formulae of the studied samples revealed that all of them had symmetrical karyotype except in insect leg form where nearly subtelocentric (-) chromosomes appeared and thus gave the asymmetric affinity. The total form percentages (TF%) of all the studied samples ranged from 33.530 to 43.039%. Taxa with asymmetric karyotype tend to have low TF% (Huziwara, 1962), therefore, insect leg (TF% = 34.639%) and winged (TF% = 33.530%)

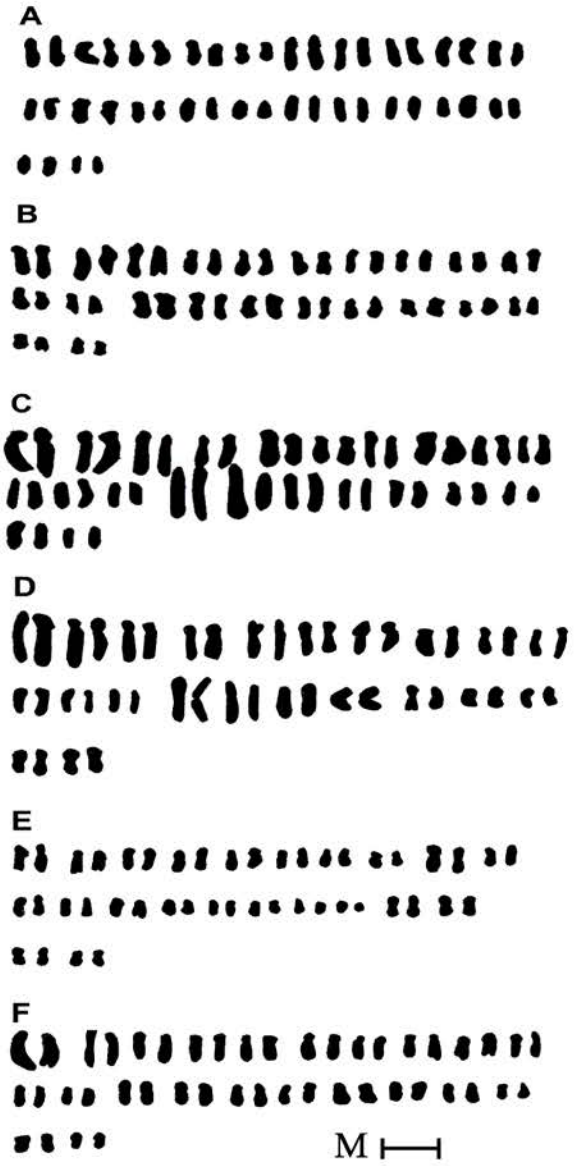

Fig. 3: Karyograms of different achene morphs of *Calendula micrantha*: Balloon-like (A), Balloon like with spines (B), Insect-leg (C), Winged (D), ring-like (E) and Curved (F)

achenes are presumed to have more asymmetrical karyotype than other forms.

The symmetry of the chromosomes within the samples is expressed by interchromosomal index (A_2) for each of the studied samples. The lower A_2 value (0.190) was recorded in balloon like form and the higher value (0.283) in winged form.

Karyotypic variations exist not only between different species but also within the same species. This karyologic differences within different cytotypes of the same species

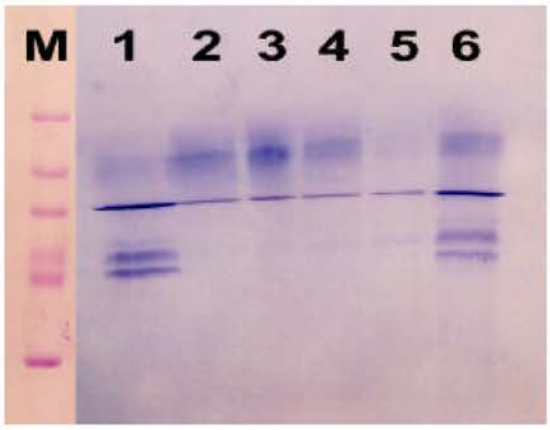

Fig. 4: Polyacrylamid gel illustrating protein bands of different achene morphs of *Calendula micrantha*: Curved (1), Balloon-like (2), Balloon like with spines (3), Winged (4), Insect-leg (5) and Ring-like (6)

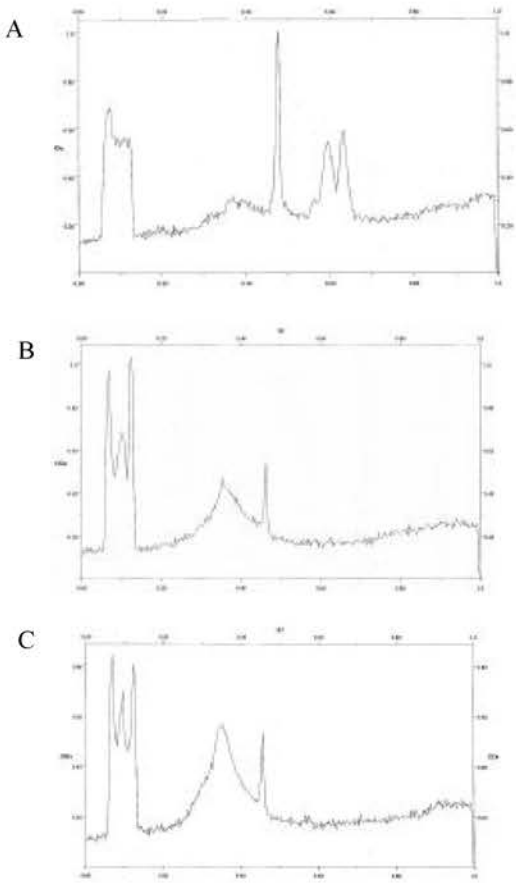

Fig. 5: The densitometer scanning pattern of extracted protein of different achene morphs of *Calendula micrantha*: Curved (A), Balloon-like (B), Balloon-like with spines (C)

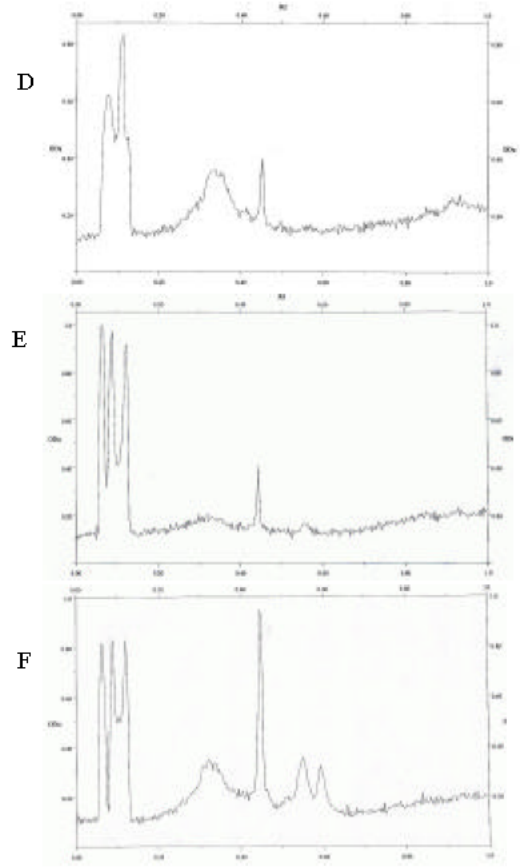

Fig. 5: Continued:
Winged (D), Insect-leg (E) and Ring-like (F)

have been observed previously in some species such as *Amaryllis balladonna* (Guha, 1979) and *Astragalus* (Ashraf and Gohil, 1988).

The DNA content is generally known to increase as the number of chromosomes increase (Ramachandran and Narayan, 1985), but there are many complicated factors affecting the amount of the DNA content, such as total chromosome length (TCL), total chromosome volume (TCV) (Furuta and Nishikawa, 1991), chromosome folds and V-shaped chromosomes Nishikawa *et al*. (1979).

Nishikawa, *et al*. (1979) observed the greater number of V-shaped chromosomes in the complement of *Lycoris*, is accompanied by the greater DNA content. He assumed that, this may be due to the presence of V-shaped chromosomes carried an extra segment on both sides of centromere. This may be applied to both curved and balloon like with spines achene characterized by greater number of nearly submetacentric (-) (22) as well as the highest DNA content.

The use of seed protein electrophoresis has provided valid evidence concerning the genetic variability which could help in understanding the mechanisms controlling the biosynthesis of the different protein fractions and the source of information for genetic and systematic relationships of several plant groups (Stegemann, 1984; Evans 1985, Sammour, 1987). It was applied to *Trifolium* (Badr, 1995), *Gossypium barbadense* L. cultivars in Egypt (Sammour, 1990) and Khalifa *et al*. 1998 on Solanaceae. Jones and Luchsinger (1987) reported that proteins represent direct products of DNA code. Seed proteins are usually considered to be more stable than many other characters used in classification (Larsen, 1967 and Larsen and Coldwell, 1968). So using the electrophoretic techniques provide the information about genetic relationships among plant species.

Electrophoretic analysis of seed protein showed variations among the studied samples of *C. micrantha*. Each sample exhibited a distinctive electrophoretic pattern. The observed changes were both qualitative and quantitative and may be illustrated by the disappearance of some bands, changes in band intensity, changes in band relative mobility or changes in some fractionation of some bands (Fig. 3). From, Table 2 and 3 it is obvious that the total number of recorded bands were 26. Such bands are not always expressed in all samples. Thus, the total number of bands in the studied samples ranges between 3 (in balloon-like and insect leg form) to 6 (in curved and ring form) bands. There is no common bands in all samples. However, there are a manifested band at approximate M wt 141.611 KDa recorded in all samples, except in balloon like with spines and insect leg form.

Protein variation in natural populations *Hordeum spontaneum* was observed by Nevo (1997) who indicated that the pattern of protein diversity was often associated with ecological factors. El-Monayeri *et al*. (2001) revealed that ecological factors (climatic, edaphic and vegetational) play the major roles in the genetic diversity in *Zilla spinosa* natural populations.

From this study, it could be suggested that the genetic differentiation in *C. micrantha* might be the consequence of an adaptive process.

References

Abraham, Z. and P.N. Prasad, 1983. A system of chromosome classification and nomenclature. Cytologia, 48: 95-101.

Ashraf, M. and R.N. Gohil, 1988. Studies on the cytology of Legumes of Kashmir Himalaya III. Interpopulation differences in the karyotypes of 3 species of *Astragalus* L. Cytologia, 53: 543-549.

Badr, A., 1995. Electrophoretic studies of seed proteins in relation to chromosomal criteria and the relationships of some taxa of *Trifolium*. Taxon, 44: 183-191.

Bogdanova, N.S., I.S. Nikolaeva, L.I. Shcherbakova, T.I. Tolstova, N.I. Moskalenko and G.N. Pershin, 1970. Study of antiviral properties of *Calendula officinalis*. Farmakol Toksikol, 33: 349-355.

Boulos, L., 1995. Flora of Egypt. Checklist. Al Hadara Publishing, Cairo, Egypt.

Borgen, L., 1975. Chromosome numbers of vascular plants from Macaronesia. Norw. J. Bot., 22: 71-76.

Burton, K., 1968. A study of the condition and mechanism of the diphenylamine reaction for the colourimetric estimation of DNA. Biochem. J., 62: 315-323.

Chemli, R., A. Toumi, S. Oueslati, H. Zouaghi, K. Boukef and G. Balansard, 1990. *Calendula arvensis* L. Impact of saponins on toxicity hemolytic effect and anti-inflammatory activity. J. Pharm Belg., 45: 12-16.

Chisholm, M.J. and C.Y. Hopkins, 1967. Calendic acid in seed oils of the genus *Calendula*. Can. J. Biochem., 45: 251-254.

Dische, Z., 1962. Color reactions of pentoses. In Whistler, R.L. and Wolfrom, M.L. (eds.) "Methods in carbohydrate Chemistry". Academic Press. New York, pp: 484-488.

Dolezel, J., J. Greihuber, S. Lucretti, A. Meister, M.A. Lysak, L. Nardi and R. Obermayer, 1998. Plant Genome size estimation by flow cytometry: Interlaboratory comparison. Annals of Botany, 82: 17-26.

Elias, R., M. De Meo, E. Vidal-Ollivier, M. Laget, G. Balansard and G. Dumenil, 1990. Antimutagenic activity of some saponins isolated from *Calendula officinalis* L., *C. arvensis* L. and *Hedera helix* L. Mutagenesis, 5: 327-331.

El-Keblawy, A., 1999. Hyper-variable seed heteromorphy in Egyptian *Calendula micrantha* Tineo et Guss: Effects on Achene dormancy and progeny traits. J. Union Arab Biol. Cairo., 9: 351-370.

El-Keblawy, A., K.H. Shaltout, J. Lovett-Doust and A. Ramadan, 1997. Population dynamic of an Egyptian desert shrub, *Thymelaea hirsuta* Can. J. Bot., 75: 2027-2037.

Ellner, S. and A. Shmida, 1984. Seed dimorphism in relation to habitat in genus *Picris* (Compositae) in Mediterranean and arid regions. Isr. J. Bot., 33: 25-39.

El-Monayeri, M.O., Z. Abo-El Khier and D. Abd El Raof, 2001. Genetic variation in *Zilla spinosa* natural populations using molecular markers in response to ecological diversity. Al-Azhar Bull. Sci., 12: 263-285.

Evans, N.J., 1985. The use of electrophoresis in the separation of two closely related species of terrestrial slugs. Biochem. Sys. and Ecol., 13: 325-328.

Fritsche, K., E. Hornung, N. Peitzsch, A. Renz and I. Feussner, 1999. Isolation and characterization of a calendic acid producing (8,11)-Linoleoyl desaturase. FEBS Lett., 462: 249-253.

Furuta, Y. and K. Nishikawa, 1991. Variation in nuclear and individual chromosomal DNA content and its role in evolution of plants. In: Gupta, P.K. and T. Tuschiya (eds.) Chromosome engineering in plants genetics, breeding, evolution. Part A. New York, pp: 71-85.

Graf, J., 2000. Herbal anti-inflammatory agents for skin disease. Skin Therapy Lett., 5: 3-5.

Guha, S., 1979. Cytological studies in the genus *Amaryllis*. Bull. Bot. Surv. India, 21: 18-21.

Harper, J.L., 1977. Population biology of plants, Academic Press, New York.

Hazra, R.R., 1970. Chromosome studies in *Calendula*. Bull. Bot. Soc. Bengal, 24: 95-100.

Heyn, C., O. Dagan and B. Nachman, 1974. The annual *Calendula* species: taxonomy and relationships. Israel J. Bot., 23: 169-201.

Huziwara, Y., 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of *Aster*. Amer. J. Bot., 49: 116-119.

Imbert, E., J. Escarre, and J. Lepart, 1996. Achene dimorphism and among-population variations in some biological traits in *Crepis sancta* (Asteraceae). Int. J. Plant Sci., 157: 309-315.

Jones, S. and A. Luchsinder, 1987. Plant systematics. McGraw-Hill Book Company, New York.

Kaplan, B., 1994. Homoeopathy: 3. Every day uses for all the family. Prof Care Mother Child; 4: 212-213.

Koa, K.N., 1975a. A nuclear staining method for plant protoplasts. In plant tissue culture methods Ch. 10, O.L. Gamborg and L.R. Wetter (eds.) National Research Council of Canada, Ottawa, Ontario.

Koa, K.N., 1975b. A chromosomal staining method for cultured cells. In plant tissue culture methods Ch. 10, O.L. Gamborg and L.R. Wetter (eds.), National Research Council of Canada, Ottawa, Ontario.

Khalifa, S.F., A. Badr, A.I. Aboel-Atta and M.M. Abou El-Enain, 1998. Electrophoretic studies of seed proteins and their impact on the taxonomic relationships in the Solanaceae. J. Union Arab Biol., Cairo 5(B), Botany, pp: 171-180.

Koller, D. and N. Roth, 1964. Studies of the ecological and physiological significance of amphicarpy in *Gymnarrhena micrantha* (Compositae). Am. J. Bot., 51: 26-35.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature London, 227: 680-685.

Larsen, A.L., 1967. Electrophoresis differences in seed proteins among varities of Soya bean, *Glycine max* L., Morrill. Crop Sci., 7: 311-313.

Larsen, A.L. and B.E. Coldwell, 1968. Inheritance of certain protein in Soya bean s eed. Crop Sci., 8: 474-476.

Mc-Evoy, P.B., 1984. Dormancy and dispersal in dimorphic achenes of Tansy ragwort, *Senecio jacobaea*. Oecologia, 61: 160-168.

Nevo, E., 1997. Evolution in action across phylogeny caused by microclimatic stresses at "Evolution Conyon", Theor. Pop. Biol., 52.

Nevo, E., B. Baum, A. Beiles and D.N. Johnson, 1998. Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the fertile crescent. Genet. Resour. and Crop Evol., 45: 151-159.

Nishikawa, K., Y. Furuta and H. Endo, 1979. Consideration of the chromosome evolution on the basis of nuclear DNA content and total chromosome length in *Lycoris*, Jap. J. Genet., 54: 387-396.

Nordenstam, B., 1972. Chromosome numbers in some compositae from Egypt. Bot. Notiser, 125: 393-396.

Powell, A.M., D.W. Kyhos and P.H. Raven, 1974. Chromosome numbers in Compositae. X. Amer. J. Bot., 61: 909-913.

Ramachandran, C. and R.K.J. Narayan, 1985. Chromosomal DNA variation in *Cucumis*. Theor. Appl. Genet., 69: 497-502.

Romero Zarco C., 1986. A new method for estimating karyotype asymmetry. Taxon, 35: 526-530.

Sammour, R.H., 1990. Using SDS-PAGE in identification of certain *Gossypium barbadense* L. cultivars. Egypt. J. Bot., 33: 169-174.

Sammour, R.H., 1987. Chemical constituents and electrophoresis of seed proteins of some wild species of *Vicia*. FABIS Newsletter, 18: 30.

Schat, H., 1981. Seed polymorphism and germination ecology of *Plantago coronopus* L. Acta Ecologica/Ecologia Plantarum, 4: 367-380.

Schoen, D.J. and D.G. Lloyed, 1984. The selection of clistogamy and heteromorphic diaspores. Biol. J. Linn. Soc., 23: 303-322.

Shibko, S., P. Koivistoven, C.A. Tratnyek, A.R. Newhall and L. Friedman, 1967. A method for sequential quantitative separation and determination of protein, RNA, DNA, Lipid and glycogen from a single liver homogenate or from subcellular fraction. Annal. Biochem., 19: 415-528.

Silvertown, J.W., 1984. Phenotypic variety in seed germination behaviour: the ontogeny and evolution of somatic polymorphism in seeds. Am. Nat., 124: 1-16.

Sorensen, A.A., 1978. Somatic polymorphism and seed dispersal. Nature, 276: 174-176.

Stegemann, H., 1984. Retrospect on 25 years of cultivar identification by protein patterns and prospects for future. In Draper, S.R. and Cooke, R.J. (eds.) "Biochemical tests for cultivar identification" The International Seed Testing Association, Zurch, Switzerland, pp: 20-31.

Strother, J.L., 1972. Chromosome studies in western North American Compositae. Amer. J. Bot., 59: 242-247.

Täckholm, V., 1974. Students' flora of Egypt. Cairo University, Egypt.

Tanowitz, B.D., P.F. Salopek and B.E. Mahall, 1987. Differential germination of ray and disk achenes in *Hemizonia increscens* (Asteraceae). Am. J. Bot., 74: 303-312.

Tjio, J.H. and A. Levan, 1950. The use of oxyquinoline in chromosome analysis. Anales de La Estac. Exper. De Aula Dei, 2: 21-64.

Van Loon, J., 1974. A cytological investigation of flowering plants from the Canary islands. Acta Bot. Neerl, 23: 113-124.

Venable, D.L., 1985. The evolutionary ecology of seed heteromorphism. Am. Nat., 126: 577-595.

Venable, D.L. and L. Lawlor, 1980. Delayed germination and dispersal in desert annuals: escape in space and time. Oecologia, 46: 272-282.

Venable, D.L. and D.A. Levin, 1984. Ecology of achene dimorphism in *Heterotheca latifolia*. 1. Achene structure, germination and dispersal. J. Ecol., 73: 133-145.

Zhang, J., 1993. Seed dimorphism in relation to germination and growth of *Cakile edentula*. Can. J. Bot., 71: 1231-1235.

Zohary, M., 1950. Evolutionary trends in the fruiting heads of compositae. Evolution, 4: 1403-1409.