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MORPHOLOGICAL AND GENETIC DIVERSITY IN ELEVEN SAFFLOWER GENOTYPES.

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ABSTRACT

Morphological and genetic diversity of eleven safflower genotypes were screened and evaluated, via Principal Component Analysis (PCA) and Random Amplified polymorphic DNA (RAPD) methods. Genetic diversity for nine agronomic traits : 50% flowering, plant height, number of branches, seed weight/capsule , number of capsules / plant ,100 seed weight , seed weight / plant, seed yield/fed and seed oil content, were studied. The combined analysis showed that line- 1 was the earliest in flowering, while variety Giza -1 was the latest. Variety Giza-1 had the tallest plants ,while line -2 had the shortest ones . Dem -137 gave the highest number of branches ,but line -2 had the lowest value . The combined analysis showed also that line- 4 gave the highest weight of seed yield /plant while mutant- 1 was the lowest with white flowers. Seed weight / capsule , was the highest for line-1, while mutant-1 recorded the lowest capsule weight . Concerning number of capsules , line -4 was superior, while line -1 was inferior in this respect. The combined analysis showed that line- 1 gave the highest weight of 100 seeds, while line -3 gave the lightest one. Also, seed yield/fad , was maximal with line -4 and variety Giza -1 , whereas it was minimal with line- 3 and mutant- 1 . Line -2 showed the highest oil content (32.75%) , while line -350 was the lowest(27.91%) ..

Molecular analysis using fourteen RAPD primers to detect polymorphism among the eleven genotypes, indicated total of 46 reproducible fragments. Nineteen were monomorphic and the rest revealed polymorphic banding patterns. Polymorphism reached 0.59%.

Key words: ***Safflower ,Carthamus tinctoris, PCA, RAPD.***

INTRODUCTION

Safflower *(Carthamus tinctorious* L) is one of the oil seed crops attracting attention due to its highly valued cholesterol free oil (Balamurgan *et al* 2004). It is an important oil seed crop (27.91-32.75 % oi)l of high quality in Egypt. It is basically a self pollinated crop, although some out crossing occurs, mainly through insects.

Success in any breeding programme depends on the amount of genetic variability present for a specific character in breeding populations. The genetic coefficient of variation gives an idea about the extent of variability present in the breeding material. In Egypt there are few safflower lines in addition to some mutant lines. Detailed information is known about

their agronomic traits and yield potentiality. Safflower is also an annual herb usually used as medicinal materials (Lee 1980).It is cultivated as an oil crop and for other important uses. Its flowers contains carthamin ($C_{12}H_{22}O_{11}$) which inhibits platelet coagulation and delays bleeding time, (An and Yuk, 1975 and Huang 1993). Safflower seed contains several important fatty acids such as oleic and, linoleic acids which cause markable reduction in blood cholesterols (Kim *et al* 1999). The cultivated safflower belongs to family Asteracea, 2n=2x=24. It is characterized with a strong central branched stem and varying number of branches. Safflower has a wide range of related species within the genus of Carthamus. The genus contains more than 20 species divided into 4 sections (Knowles 1988). Section one (2n=20) contains oxyantha and palaestinu. Section two (2n=24) (tinctorius, alexandrius glaucus syriacus and tenuis). Section three (lanatus 2n=44) while section four (baeticus 2n=64). Sections number one and two are diploids, the third is a tetraploid while the fourth section consists of hexaploid species (Khidir 1969). Safflower is a drought tolerant annual oil crop and this gives it an advantage over other oil crops. It is known world wide as a source of high quality vegetable oil. In the past, safflower germplasm was identified and characterized entirely via morphological features. Recently, biochemical characters was used which do not necessarily reflect genetic diversity (Fernandez -Martinz *et al* 1993). The environment has a strong influence on morphological traits (especially quantitative traits). Studies have also shown that there are no sufficient number of morphological markers to provide detailed knowledge of most genomes (Shawla 2002). Hence, selection of genotypes based on molecular markers will be highly reliable and more effective. It is more effective to use RAPD markers to detect genetic diversity in safflower accessions. Experiments were conducted to get basic information on the clustering and affiant of several agronomic characteristic for the identification of imported and domestic safflower by RAPD analysis and principal component analysis (Williams *et al* 1990 and Cooper and Delacy 1994)

The aim of this study, is to evaluate the genetic diversity among some introduced safflower accessions in addition to two local induced mutants via agro morphological and biotechnological approaches , and to assess the potentiality of using it for germplasm identification and classification, and my be for improving safflower .

MATERIALS AND METHODS

The materials of the present work comprised eleven safflower genotypes nine of them, were obtained from the Oil Crops Research Section, Field Crops Research Institute (FCRI), Agriculture Research

Center (ARC) , and two mutants were obtained from the International atomic energy (IAE) in Egypt. Evaluation was performed during the winter seasons of 2007-2008 and 2008-2009, at Giza Research Station ARC, Giza Egypt. The description of materials is presented in Table (2). The general architectural type of safflower germplasm was spine and spineless. The predominant color was yellow orange except for, one mutant which had white flower. All genotypes were branchy with erect growth habit and lancelet leaves. To asses the similarity and/or diversity of molecular markers, extracted DNA from the eleven genotypes were tested against fourteen arbitrary chosen 10 mere RAPD primers. The universal names and sequence of tested primers are presented in Table (1)

Seeds from all studied genotypes were planted in 2007-2008 and 2008-2009 seasons. Phenological measurements were made during the growth period. The measured traits were days to 50% flowering, plant height (cm), number of branches, number of capsules/plant, seed weight/capsule (g), 100 seed weight (g), seed weight/plant (g), seed yield/fed (kg) and seed oil content . The entries were evaluated in

A randomized compete block design with three replications. Each entry/plot consisted of five rows 4 m long. Spacing between rows and plants within the row was at 60 cm and 15 cm, respectively. Thinning was done at one plant/hill. Means were compared by using Duncans, Multiple Range Test as outlined by Steel and Torrie (1980).

A modified CTAB (hexadecyl trimethyl ammonium bromide) was used to obtain genomic DNA. The procedure is based on the protocol suggested by (Sue–Porebsk *et al* 1997). Random amplified polymorphic DNA (RAPD) has been carried out on the eleven genotypes. Genomic DNA was used as template for polymerase chain reaction (PCR) amplification as described by (William *et al* 1990). A set of 14 arbitrary primers (Table 1) were synthesized by Bioron, Germany, to produce distinct marker profiles for the studied genotypes. PCR reaction was performed in 50_{UL} sample, using 50_{ng} DNA as template, 4_{Mm} MgL_2, 10_{Mm} Tris HCL, 1_{Mm} EDTA, pH8.4, 200_{UM} each of dNTPs, 40_{p} moles of each primer, and 2.5_{U} Taq DNA polymerase PCR was programmed for 5 min at 94C° for one cycle. 1 min 94C°, 1.5 min 36C°, 2min 72C°, during 35 cycle, and 10 min end, extension at 72C°. Then followed by soaking at 4C°. Amplified products were separated by electrophoresis on 1.2 % agrose gel in 1XTBE buffer.

Table 1. Sequence of the random (10- mer) RAPD primers

No	Primer	Sequence	GC%
1	A-02	5¯-TGCCGAGCTG-¯3	70
2	A-06	5¯- GGTCCCTGAC-¯3	70
3	B-08	5¯- GTCCACACGG-¯3	70
4	B-11	5¯- GTAGACCCGT-¯3	60
5	C-16	5¯-CACACTCCAG-¯3	60
6	E-06	5¯-AAGACCCCTC-¯3	60
7	E-10	5¯CACCAGGTGA-¯3	60
8	G-01	5¯-CTACGGAGGA-¯3	60
9	J-14	5¯-CACCCGGATG-¯3	70
10	L-17	5¯AGCCTGAGCC-¯3	70
11	M-04	5¯-GGCGGTTGTC-¯3	70
12	N-12	5¯-CACAGACACC-¯¯3	60
13	O-20	5¯-ACACACGCTG-¯3	60
14	Z-10	5¯- CCGACAAACC-¯3	60

RESULTS AND DISCUSSION

Agronomic characteristics of the eleven safflower genotypes are presented in (Table 2).Five genotypes were spiny , and six were spineless. The predominant flower color was yellow orange, except mutant-1 (white flower). All genotypes were branchy . Growth type was erect. and leaf type was lance late. These results agree with, *Kang et al., (2004).* Results in Table (3) revealed that, line- 1 was the earliest in flowering in the two season (141.3 3 and 138.67 days)., but , lines-350 and Giza-1 were the latest (147) in 2007/2008 season, while line-4 (145.33 days) was the latest in 2008-2009 season . Also the combined analysis showed that line - 1 was the earliest in flowering (140 days) , while Giza -1, was significantly the latest. These results differed in the two years due to the interaction between genotypes and environments. Regarding plant height, in season 2007-2008 Giza- 1. had the tallest plants (149 cm), while the shortest plants were shown by line- 2 (136.67cm).In season 2008-2009, the local cultivar Giza- 1 had also the tallest plants (147.67 cm) followed by line -4 (147cm), while the shortest plants were shown by line -2 (136.67cm)

Mean while the combined analysis revealed that Giza -1 had the tallest plants (148.78cm), while the shortest plants were shown by line- 2 (136.67cm)

Table 2 Description of the eleven safflower genotypes.

Genotype	Spine	Color of flower petal
Mutant 1	Spine	White
Mutant 2	Spineless	Yellow orange
Line 2	Spineless	Yellow orange
Line 6	Spineless	Yellow orange
Dem 137	Spineless	Yellow orange
Line 350	Spineless	Yellow orange
Line 5	Spine	Yellow orange
Line 1	Spine	Yellow orange
Giza -1	Spineless	Yellow orange
Line 3	Spine	Yellow orange
Line 4	Spine	Yellow orange

Concerning number of branches, in season 2007/2008 Dem-137gave the highest number (10.10), while line-2 had the lowest one (6.60), also in season 2008/2009 Dem -137 had the highest number (10.33) followed by variety Giza -1 (10), while line -2 had the lowest value (6.67). Also in the combined data Dem- 137 reached (10.22), while line -2 had the lowest number (6.63).

With respect to seed weight/plant, line- 4 in season 2007-2008 gave the highest value for seed weight /plant which reached (34.33g), while the lowest was line- 5 (29.33g) followed by the while flower mutant-1 (29.40g). In season 2008-2009 line- 4 gave the highest value for seed weight /plant (33.9 g) followed by Giza -1 (33.3g) and line -3 (33.27g), while the lowest was mutant -1 (28.4 g).In the combined data line -4 gave the highest value for seed weight /plant (34.12g) ,while the lowest was mutant- 1 (28.9 g).

As for seed weight/capsules line -1 was the highest (88.67g), while the lowest seed weight/capsule was shown by mutant-1 (52.23g), in season 2007/2008. In season2008/2009 line -1 was the highest (93.33g)), while mutant- 1 gave the lowest seed weight /capsule (55.33g). The combined analysis showed that line- 1 was the highest (91 g), while mutant -1 was the lowest (53.78g).

For number of capsules /plant, line -4 was the highest (57.90) followed by, line- 5, (57.23) and Dem -137 (57) while line -2 gave the lowest capsule number (53.13) in season 2007/2008 .However in season 2008/2009 line- 3 gave the highest capsule number (57), while line -1 was the lowest (50.67) .The combined analysis revealed that line- 4 showed the highest number of capsules (56.95) followed by Dem -137 (56.33),

Table 3. Mean of flowering date, plant height , No of branches/plant, seed weight capsule, No of capsules , 100 seed weight, seed yield/plant, seed yield/fedan , and % of oil.

Trait	50% flowering date		Combined	Plant height(cm)		Combined	No of branches		Combined	Seed yield /plant(g)		Combined
Genotye	2007-2008	2008-2009		2007-2008	2008-2009		2007-2008	2008-2009		2007-2008	2008-2009	
Mutant 1	145.33*	140.67	143.00*	140.60	138.00	139.30	9.20*	9.33*	9.278	29.40	28.40	28.90
Mutant 2	144.00*	140.33	142.17	143.33	140.67*	142.00*	8.30*	8.00	8.15*	31.93	32.37*	32.15*
Line 2	145.00*	141.33	143.17*	136.67	136.67	136.67	6.60	6.67	6.63	31.93*	32.03*	31.98*
Line7	143.67*	144.00*	143.83*	145.86*	143.33*	144.60*	7.60	8.33*	7.97	31.63*	32.83*	32.23*
Dem137	145.67*	144.67*	145.17*	140.73*	145.00*	142.87*	10.10*	10.33*	10.22*	31.37*	31.13*	31.25*
Line 350	147.00*	144.33*	145.67*	145.33	144.67*	145.00*	7.17	7.67	7.42	31.93*	32.70*	32.32*
Line5	145.67*	142.33*	144.00*	141.13	144.00*	142.57*	7.33	8.00	7.67	29.33	29.83	29.58
Line1	141.33	138.67	140.00	141.57	141.00*	141.28	7.43	7.67	7.55	30.40*	30.80*	30.60*
Giza1(check)	147.00*	144.67*	145.83*	149.00	147.67*	148.78*	9.57*	10.00*	9.78*	32.33*	33.30*	32.82*
Line3	144.33*	143.33*	143.83*	146.17*	145.33*	145.75*	9.20*	8.66*	8.93*	32.27*	33.27*	32.77*
Line -4	144.67*	145.33*	145.00*	146.03*	147.00*	146.52*	8.23*	8.67*	8.45*	34.33*	33.90*	34.12*
L.S.D5%	3.37	2.16	2.83	4.82	8.27	6.77	1.22	1.54	1.39	2.06	2.52	2.82

Table 3. Cont.

Trait / Genotype	Seed weight/capsule (g)			No of capsules/plant			100 seed weight(g)			Seed yield /fed (kg)			% of oil		
	2007-2008	2008-2009	Combined	2007-2008	2008-2009	Combined	2007-2008	2008-2009	Combined	2007-2008	2008-2009	Combined	2007-2008	2008-2009	Combined
Mutant -1	52.23	55.33	53.78	55.67*	53.33*	54.50*	5.69*	5.39*	5.54*	543.33	559.67	551.55	32.13*	29.19*	30.66*
Mutant -2	62.00	61.00	61.50	54.00*	54.33*	54.17*	5 85*	5.55*	5.70*	571.32	559.07	565.19	29.58*	29.71*	29.78*
Line -2	63.87	63.00	63.43	53.13*	54.00*	53.57*	5.52*	5.35*	5.44*	554.50	584.53	569.52	32.93*	32.56*	32.75*
Line -7	66.67*	65.00	65.83	56.40*	54.67*	55.53*	5.52*	5.83*	5.68*	545,56	557.33	551.45	29.88*	29.77*	29.82*
Dem 137	65.77*	67.00	66.38	57.00*	55.57*	56.33*	5.79*	5.75*	5.77*	551.44	560.40	555.92	28.35*	28.29*	28.32*
Line 350	73.33*	80.33*	76.83*	54.10*	54.67*	54.38*	5.52*	5.64*	5.58*	574.72	577,33	576.03	27.89*	27.92	27.91*
Line 5	79.00*	82.00*	80.50*	57.23*	55.00*	56.12*	5.53*	5.61*	5.57*	575.56	573.97	574.77	29.00*	29.13*	29.07*
Line 1	88.67*	93.33*	91.00*	54.00*	50.67*	52.33*	6.38*	5.74*	6.06	552.02	548.63	550.33	28.14*	27.92	28.03*
Giiza-1	70.00*	80.33*	75.17*	55.33*	54.33*	54.83*	5.52*	5.69*	5.61*	612.34*	626.43*	619.39*	30.23*	30.31*	30.27*
Line -3	70.20*	73.67*	71.93*	55.40*	57.00*	56.20*	5.41*	5.25 *	5.38*	573.00	549.40	561.20	30.75*	27.59	29.17*
Line -4	75.20*	72.00	73.60*	57.90*	56.00*	56.95*	5.56*	5.51*	5.54*	660.45*	636.17*	648.31*	30.32*	30.30*	30.32*
LSD5%	5.59	6.95	6.31	4.06	4.49	3,97	0.49	0.47	0.48	31.08	31.09	31.09	2.96	2.07	2.82

*Significant at 0.05 probability level

line- 3 (56.20) and line -5 (56.12). However, line- 1 had the lowest number of capsules (52.33). The 100 seed weight of line -1 was the heaviest (6.38g). While the lowest 100 seed weight was shown by line- 3 (5.41g) in season2007/2008. In season 2008/2009, line -7 had the heaviest weight of 100 seeds (5.83g), while the lowest 100 seed weight was shown by line -2 (5.35g) followed by mutant-1 . The combined analysis for 100 seed weight showed that line -1 had the heaviest seed (6.06g) while line -3 had the lightest seed (5.38g).

Results showed also that mutant -1, gave the lowest seed yield / fed (543.33kg), while line 4 gave the highest seed yield/fed (660.45kg) in season 2007/2008. However, in season 2008/ 2009 line -1 gave the lowest yield (548.63kg) while line -4 gave the highest yield (636.17kg) followed by the cultivar Giza -1 (626.43 kg) .Similarly combined analysis showed that line -4 gave the highest seed yield (648.31 kg), while line - 1 gave the lowest yield (550.33kg).

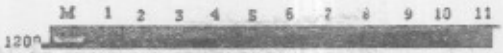
Results in season 2007/2008 indicated that seed oil content varied from 32.93-27, 89, while in season 2008/2009, it varied from 32.56-27.59%. However, combined analysis showed that oil content varied from 32.75- 27.91%. Differences in results of the two seasons may be due to the genotype x environment interaction

RAPD fingerprinting

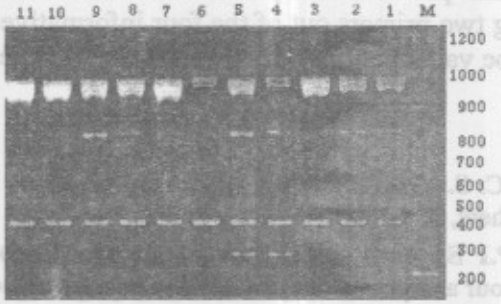
Fourteen RAPD primers were tested to generate amplified DNA fragments, four primers generated polymorphic profiles (A-02, E-06, l-17 and Z-20). Three primers did not show any amplification (A-06-G-01 and 0-20) while, the remaining generated monomorphic profiles. The polymorphism was scored as presence or absence of a specific band in samples. A total number of 46 reproducible fragments were generated (Table 4 and Fig 1), from which nineteen were monomorphic and the rest revealed polymorphic banding patterns. Polymorphism reached 0.59%.Number of polymorphic fragments/primer varied and ranged from ten to thirteen polymorphism ratio .It also varied

Table 4. Levels of polymorphism and unique genotype specific markers based on RAPD analysis.

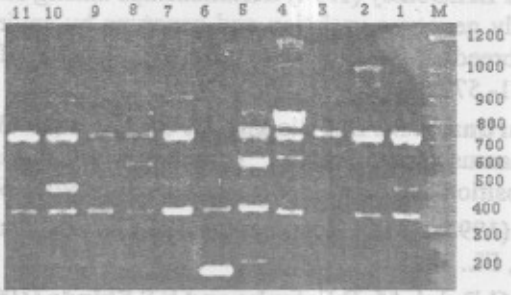
Fig/ primer	Total fragments	Polymorphic fragments	Polymorphism%	Unique genotype	Fragments modal size
A-02	13	10	76.9	-	-
E-06	11	2	18.81	6	(-) 890
L-17	12	11	91.66	1	(-)360
	-	-	-	5	200
	-	-	-	6	120



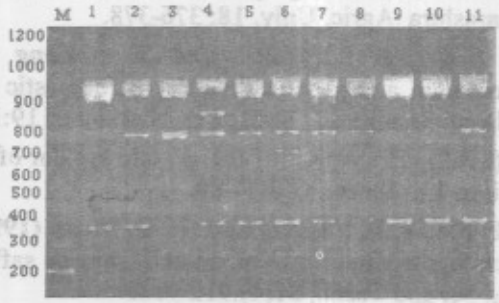
(A-02)5'-TGCCGAGCTG-3'



(E-06) 5'-AAGACCCCTC-3



(L-17) 5'-AGCCTGAGCC-3'



(Z-10) 5'-CCGACAAACC-3

Fig. 1. Profiles of eleven genotypes as reveled by different RAPD primers

from one primer to the other and ranged from 20% for Z-10 to91.6 for l-17 with an average of 51.84%. Some genotypes showed unique fragments that could be used as specific markers to discriminate the respective genotypes, while others were not be able to be distinguished through the tested primers. For instance, genotype -6 was characterized by the absence of band no -4 with a molecular size of 890 bp when tested against primer E-06. Means while, primer L-17 was more informative and generated three specific markers to distinguish genotype-1 (negative markers of 360bp), genotype -5 positive markers of 200 bp and genotype- showed (positive markers of 120bp. From the previous data, it is clear that four genotypes could be identified using two primers out of the four informative ones .Further primer testing would be valuable in fingerprinting the whole set of genotypes.

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التنوع المورفولجي والوراثي لاحدى عشر تركيبا وراثيا من القرطم.

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يهدف هذا البحث إلى استخدام الوراثة الجزيئية في تقييم التنوع الوراثي بين بعض التراكيب الوراثية المختلفة من القرطم وتطبيقاتها في تعريف الجيرمبلازم وتقسيماتها وانتخاب بعض التراكيب الوراثية المبنية على بعض المعلمات الجزيئية وأوضحت بعض التجارب أن بعض التراكيب الوراثية باستخدام طريقة التكبير العشوائي لجزيئيات الحامض النووي (RAPD) أظهرت اختلافات وراثية بين بعض التراكيب الوراثية المختلفة، ولقد أظهرت النتائج أن السلالة (١) كانت مبكرة في الإزهار بينما الصنف جيزة (١) كان متأخرا في الازهار (١٤١.٦٧). أما صفة ارتفاع النبات فقد وجد أن السلالة (٣) أعطت أعلى نتائج في صفة ارتفاع النبات، بينما السلالة (٤) أعطت أقل طولا في صفة ارتفاع النبات وقد اعطت السلالة رقم (٥) أعلى عدد من الأفرع، بينما اعطت السلالة رقم (٧) أقل عدد من الأفرع . و بالنسبة لوزن الكبسولات وجد ا ن السلالة رقم (٧) أعطت أعلى وزن للكبسولات بينما السلالة رقم (٥) أقل وزن للكبسولات . و قد أعطت السلالة رقم (١) أعلى وزن مائة بذرة بينما كان الصنف جيزة (١) الاقل في وزن المائة بذره . وبالنسبة لبذور النبات فكانت السلالة رقم (١) الأعلى في محصول ا لبذور، بينما السلالة الطفرية الصفراء كانت الاقل في محصول النبات. وبالنسبة لمحصول الفدان فكانت

السلالة (١٣٧) أعلى محصول، بينما اعطت السلالة البيضاء الطفرية أقل محصول للفدان، و بالنسبة لمحتوى الزيت فكانت السلالة رقم (٢) هى الاعلى فى محتوى الزيت ، بينما السلالة رقم (٥) كانت هى الاقل في النسبة المئوية في الزيت.

ولقد أظهرت نتائج التحليل الجزئيي للمادة الوراثية باستخدام طريقة التكبير العشوائي للجزيئات الحامض النووي وجود اختلافات بين السلالات ، ولكن بعض السلالات لم تعطى اختلافات واسعة أو كبيرة