



F-AFLP GENOME SCAN AMONG FOURTEEN GENOTYPES OF SUNFLOWER (*Helianthus annuus* L.)

[183]

Rizk^{1,2*} M.S., Abd El-Twab¹ F.M., Abo Doma¹ A., Amar² M.H. and Magdy¹ M.

1- Genetics Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt

2- Genetics Resource Dept., Desert Research Center, P.O. Box1, Mathaf El-Matariya St 11753 El-Matariya, Cairo, Egypt

*Corresponding author: drmokhtarsaid@yahoo.com

Received 6 June, 2018

Accepted 2 July, 2018

ABSTRACT

Sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae. The current study used the fluorescent amplified fragment length polymorphism (F-AFLP) technique for evaluating the genetic diversity among 14 sunflower genotypes including eight agronomical traits. Highly significant difference between genotypes was observed for all studied traits ($p \leq 0.01$). The results of correlation between the studied traits ($p \leq 0.05$) revealed that positive correlations were observed between most of the traits. Six F-AFLP combination were succeeded in the development of 178 molecular markers within a total of 1007 polymorphic bands, with 97.42% polymorphic percentage. Concerning the molecular markers associated traits, 73 were observed related to morphological and yield component traits. These results may facilitate the simultaneous selection of several economic traits and can thus improve the efficacy of selection based on F-AFLP in the sunflower breeding programs.

Keywords: Sunflower, F-AFLP, Marker trait associations

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop that belongs to family Asteraceae (Composite) originated in North America (Bukhsh et al 2011). The wild sunflowers from the genus *Helianthus* are branched annuals and perennials of family Asteraceae and is a diploid species ($2n = 2x = 34$) (Aboki et al 2012). Globally,

sunflower is one of the most important crop used for oil production all over the world. Seeds contain 40-50% oil and 23% protein and constitute an excellent source of unsaturated fats, crude protein, fibers and important nutrients like many vitamins such as (A, D and E) which support the immune system. Vitamin A is antioxidant and prevents damage to body cells and organs and the appearance of premature aging. While, vitamin D is needed for the growth of bones and teeth. However, vitamin E is required to activate blood circulation (El-Wakil, 2014). In Egypt, sunflower represents the best choice for increasing local production of edible oils to overcome the shortage of its production (Kandil et al 2016). To overcome the problems associated with morphological traits, molecular genetic markers have many advantages over morphological traits including higher information content, which is of primary importance for systematization and registration of collection specimens (Mahmoud and Abdel-Fatah 2012). Molecular markers played a major role in identifying variation in genomic DNA sequences. Polymorphism at the molecular level in different crop species have contributed to assessment of genetic diversity, reconstruct accurate phylogenetic relationships and transferring of desired traits (Suresha et al 2017). The AFLP technique (Vos et al 1995) is widely used to characterise genetic diversity within and among populations, particularly in non-model organisms for which no prior DNA sequence information is available. Such wide multi-locus screening (also known as a genome scan) allows the identification of the so-called outlier loci for which alleles are highly differentiated among

populations, and hence are potentially linked to adaptive divergence (Black et al 2001; Luikart et al 2003; Storz 2005 and Magdy et al 2016).

The overall aim of the present investigation was to assess the genetic diversity among fourteen genotypes of sunflower using some important morphological traits and F-AFLP molecular marker systems.

MATERIALS AND METHODS

Sunflower genotypes were obtained from different sources, their names and sources are presented in Table (1). Sunflower genotypes were evaluated at the molecular level using the fluorescence-based AFLP (F-AFLP) technique.

Table 1. Names of the fourteen sunflower genotypes and their sources

Code No.	Common Name	Source
S1	Sakha53	OCRD –FCRI-ARC
S2	Qena	(NGB)- ARC
S3	Al Buhayrah	(NGB)- ARC
S4	The new Valley	(NGB)- ARC
S5	The new Valley	(NGB)- ARC
S6	Qena	(NGB)- ARC
S7	Bani Sweif	(NGB)- ARC
S8	Aswan	(NGB)- ARC
S9	Sohag	(NGB)- ARC
S10	Sohag	(NGB)- ARC
S11	Luxor	(NGB)- ARC
S12	Luxor	(NGB)- ARC
S13	Luxor	(NGB)- ARC
S14	Luxor	(NGB)- ARC

Sakha*53 Local variety, obtained from Oil Crops Research Department, Field Crops Res., Inst., Agricultural Res. Center, Giza, Egypt, while genotypes from S2 to S14 are accessions from the National Gene Bank, Agricultural Research Center, Giza, Egypt.

Morphological traits

A field experiment was conducted to evaluate fourteen sunflower genotypes for their morphological, yield and yield components traits. The studied yield related traits are: plant height (cm), number of leaves, stem diameter (cm), head diameter (cm), head weight (g), seeds weight (g), number of seeds/head and 1000 seeds weight (g).

Statistical analysis

The collected data were statistically analyzed using Costat program and correlations between eight yield related-traits were estimated. The differences among means were compared using Duncan's new multiple ranged test (Duncan 1955). The results were considered significant at $P < 0.01$.

Genomic DNA Extraction

Leaves samples of the fourteen sunflower genotypes were collected and were stored in liquid nitrogen for DNA extraction. Genomic DNA was extracted using Gene JET™ Plant Genomic DNA Purification Kit (Thermo Scientific, Lithuania).

AFLP-polymerase chain reaction (PCR)

The original protocol of Vos et al (1995) was followed using fluorescent primers instead of radioactive agents. All primers and adaptors were synthesized (Invitrogen, UK) and prepared as recommended (Table 2). Six different selective PCR combinations (3Eco+NNN×2Mse+NNN primers) were amplified using the original PCR program.

Table 2. Sequence 5- 3' of primers and adaptors used to establish the AFLP-PCR technique

Primer/ Adaptor	5' sequence 3'
EcoRI - A1	CTCGTAGACTGCGTACC
EcoRI - A2	AATTGGTACGCAGTC
Mse I - A1	GACGATGAGTCCTGAG
Mse I - A2	TACTCAGGACTCAT
Eco + A	GACTGCGTACCAATTCA
Mse + C	GATGAGTCCTGAGTAAC
Eco + ACA	FAM-GACTGCGTACCAATTCACA
Eco + AGG	HEX-GACTGCGTACCAATTCAGG
Eco + ATA	CY3-GACTGCGTACCAATTCATA
Mse + CAA	GATGAGTCCTGAGTAACAA
Mse + CTC	GATGAGTCCTGAGTAAGTC

AFLP Analysis

Automated AFLP scoring was performed using two programs Peak Scanner™ for peak calling and Raw Geno V2 for automated scoring, according to the software's manuals. The analysis of the AFLP data was based on the band-binary criterion (*i.e.* codifying the detected bands to, 1 when present and 0 when absent) and processed according to Bonin et al (2007).

Marker trait association analysis

RESULTS AND DISCUSSION

Marker trait association of the AFLP markers using 1000 seeds weight (1000W), number of seeds per head (NSH), seeds weight (SW), head weight (HW), stem diameter (SD), head diameter (HD), number of leaves (NL) and plant height (PH) were analyzed by Power marker, using f-statistical analysis.

Analyses of variance among the 14 sunflower genotypes were investigated to evaluate some important morphological traits. The results indicated that significant differences among studied genotypes were obtained. Significant differences were observed among all studied genotypes ($p \leq 0.01$), as shown in **Table (3)**.

Table 3. Mean values of some morphological traits for fourteen sunflower genotypes

Genotypes	Plant height Cm	No. of leaves	Head diameter cm	Stem diameter cm	Head weight g.	Seeds weight g.	No. of seeds per head	1000 Seeds weight g.	X ²
S1	147.8 ^{cdef}	25.8 ^{ab}	13.8 ^{ab}	5.6 ^{abc}	255.6 ^a	116.7 ^a	532.2 ^a	220.3 ^{ab}	164.7
S2	141.9 ^{ef}	23.8 ^{ab}	12.6 ^{ab}	5.8 ^{abc}	141.1 ^{abc}	60.6 ^{bcd}	401.7 ^{ab}	162.0 ^{abc}	118.7
S3	131.1 ^f	22.6 ^{ab}	8.9 ^b	4.8 ^{abc}	58.3 ^c	23.3 ^d	270.3 ^b	110.8 ^{abc}	78.8
S4	147.1 ^{def}	26.3 ^a	13.0 ^{ab}	5.8 ^{abc}	136.7 ^{abc}	71.1 ^{bc}	384.4 ^{ab}	190.4 ^{abc}	121.9
S5	155.2 ^{bcdef}	22.8 ^{ab}	14.8 ^a	6.6 ^a	158.3 ^{abc}	57.2 ^{bcd}	440.6 ^{ab}	128.7 ^{abc}	123.0
S6	176.1 ^{abcd}	19.4 ^{bc}	12.8 ^{ab}	5.7 ^{abc}	145.6 ^{abc}	42.2 ^{bcd}	352.0 ^{ab}	131.5 ^{abc}	110.7
S7	180.1 ^{ab}	20.8 ^{abc}	14.9 ^a	6.4 ^{ab}	187.8 ^{ab}	56.7 ^{bcd}	520.0 ^a	104.6 ^{bc}	136.4
S8	193.3 ^a	25.2 ^{ab}	12.6 ^{ab}	5.6 ^{abc}	172.2 ^{abc}	74.4 ^{bc}	344.9 ^b	267.6 ^{ab}	137.0
S9	152.8 ^{bcdef}	21.8 ^{abc}	9.6 ^{ab}	4.7 ^{bc}	113.9 ^{bc}	33.9 ^{cd}	271.7 ^b	142.6 ^{abc}	93.9
S10	165.7 ^{abcde}	15.1 ^c	14.0 ^{ab}	4.6 ^c	211.1 ^{ab}	77.2 ^{ab}	313.9 ^{ab}	241.8 ^a	130.4
S11	178.3 ^{abc}	26.6 ^a	11.0 ^{ab}	5.1 ^{abc}	114.4 ^{bc}	36.7 ^{bcd}	440.0 ^{ab}	82.9 ^c	111.9
S12	156.1 ^{bcdef}	19.3 ^{bc}	13.4 ^{ab}	4.7 ^{bc}	144.4 ^{abc}	35.6 ^{cd}	396.7 ^{ab}	103.0 ^{bc}	109.2
S13	155.6 ^{bcdef}	20.3 ^{abc}	13.2 ^{ab}	5.1 ^{abc}	238.9 ^{ab}	62.8 ^{bcd}	364.1 ^{ab}	233.3 ^{ab}	136.7
S14	147.8 ^{cdef}	24.7 ^{ab}	10.4 ^{ab}	4.7 ^{bc}	134.4 ^{abc}	39.4 ^{bcd}	375.9 ^{ab}	101.4 ^{bc}	104.8
X ²	159.2	22.5	12.5	5.4	158.1	56.3	386.3	158.6	159.2
LSD 0.01	30.99	6.72	5.7	1.9	125.3	41.0	235.1	134.6	

Means followed by the same letter (s) are not significantly different by Duncan's new multiple range test at ($p \leq 0.01$).

Genotype S8 recorded the highest plant height (193.3 cm), whereas genotype S₃ revealed the lowest one (131.1 cm). Two genotypes S₁ and S₈ recorded the highest values of number of leaves/plant 25.8 and 25.2, respectively, whereas only one genotype S₁₀ revealed the lowest number of leaves/plant value (15.1). While, genotype S₇ showed the highest head diameter/plant value (14.9 cm), S₃ recorded the lowest head diameter values: 8.9 cm. Regarding to the stem diameter at harvest, genotype S₇ displayed the highest diameter value (6.4 cm), meanwhile the three genotypes: S₉, S₁₂ and S₁₄ exhibited the lowest diameter value

(4.7 cm). With respect to the head weight/plant, there were wide differences within the 14 sunflower genotypes, it was 58.3g in S₃ genotype and reached 255.6g in S₁ genotype, respectively. S₁ genotype recorded the highest number of seeds/head value (532.2), meanwhile, the two genotypes S₃ and S₉ recorded the lowest value in this respect with mean of 270.3 and 271.7, respectively. The results of seeds weight/plant varied from 23.3g in S₃ genotype to 116.7g in S₁ genotype, respectively. In the context, the seed index (weight of 1000 seeds/plant) were recorded among the studied genotypes and the results showed sig-

nificant differences varied from 82.9 g in S₁₁ genotype to 267.6 g in S₈ genotype, respectively. Our results were in agreement with those reported by (Razzaq et al 2014) who evaluated ten sunflower genotypes using the morphological traits and genetic variability. However, (Purwati and Herwati 2016) estimated low variation values in some quantitative traits and the qualitative traits. Overall, the results indicated that some traits could be used as selection criteria in sunflower breeding programs for appropriate screening of parental genotypes included in hybridizing process aiming at increasing plant productivity. More recently, (Riaz et al 2017) studied the seed yield and related components in sunflower using diallel crossing. The results were recorded on quantitative traits analysis of variance and combining abilities, and indicated that the genotypes were significant in general combining ability effects for days to maturity, plant height, stem diameter, head diameter, number of leaves, filled seed, seed per head and seed yield per plant.

Correlation among yields-related traits

Correlations between eight yields-related traits were evaluated among 14 genotypes of sunflower

(Table 4). The results indicated positive correlations between head diameter and the four traits including: stem diameter, head weight, seeds weight and number of seeds per head, which recorded 0.6147, 0.7247, 0.6025 and 0.5453, respectively. Additionally, a significant correlation was observed between head weight and both of seeds weight (0.8177) and 1000 seeds weight (0.6816). However, seed weight/plant recorded highly significant and positive correlation with 1000 seeds weight (0.7640). The results of correlation between the yield-related traits of sunflower were in agreement with (Purwati and Herwati 2016) who examined three genotypes of sunflower and found that seed size and seed thickness showed significant positive correlation with 100 seeds weight. More recently, (Chambó et al 2017) served eight sunflower genotypes to estimate the correlation values for nine agronomic traits. In both tests, there were significant ($p < 0.05$) positive correlation values between seed yield & head diameter, head diameter, both of number and diameter of seeds per head and first and last count of germination. The path analysis, in both pollination tests, indicated that head diameter had the highest direct effect on seed yield.

Table 4. Correlation between the eight yields-related traits of sunflower

Traits	Plant height cm	No. of leaves	Head diameter cm	Stem diameter cm	Head weight g.	Seeds weight g.	No. of seeds per head
No. of Leaves	-0.1042	1					
Head diameter cm	0.3487	-0.2833	1				
Stem diameter cm	0.2508	0.2672	0.6147*	1			
Head weight g.	0.2513	-0.2191	0.7247*	0.2161	1		
Seeds weight g.	0.0933	0.1443	0.6025*	0.3263	0.8177*	1	
No. of seeds per head	-0.0495	0.1912	0.5453*	0.5084	0.4029	0.3505	1
1000 seeds weight g.	0.0802	-0.1322	0.3409	-0.0254	0.6816*	0.7640*	-0.2477

*= Correlation significant test at ($p \leq 0.05$).

AFLP genomes scan

Six primers combination of F-AFLP were used to identify the genetic diversity among 14 genotypes of sunflower. In general, the total positive unique markers recorded 178 positive markers which ranged in size from 50 to 659 bp, as presented in Table (5) and Figures (1, 2 and 3). A

total positive unique marker were exhibited by C1-Blue combination Figure (1) and Table (5) which recorded (34) markers ranged from 90 to 526 bp in size. S₁₃ genotype scored the highest positive unique marker (28) and recorded the highest four molecular size 526,446,435 and 434 bp. However, S₈ and S₁₂ genotypes showed the lowest molecular size (90 and 95 bp), respectively.

Table 5. Summary of positive unique F-AFLP markers via fourteen sunflower genotypes across the C1 and C4 Blue primers combination

Genotype	Unique positive markers						Total grand markers
	Combinations name	Amplicon size (bp)	Total number of markers	Combinations name	Amplicon size (bp)	Total number of markers	
S1	C1-Blue	113	1	C4-Blue	122	1	2
S2		-	-		77	1	1
S3		122	1		-	-	1
S4		-	-		376,151,61	3	3
S5		161	1		-	-	1
S6		415	1		242	1	2
S7		-	-		111,61	2	2
S8		90	1		-	-	1
S9		-	-		-	-	-
S10		-	-		-	-	-
S11		-	-		125	1	1
S12		95	1		-	-	1
S13		526, 446, 435, 434, 383, 345, 343, 310, 304, 299, 280, 265, 256, 255, 242, 240, 228, 221, 218, 215, 209, 194, 174, 167, 164, 152, 150, 118	28		440, 410, 401, 362, 343, 338, 284, 239, 210, 187, 185, 168, 89	13	41
S14		-	-		494, 472, 236, 186, 74	5	5
Total			34		27	61	

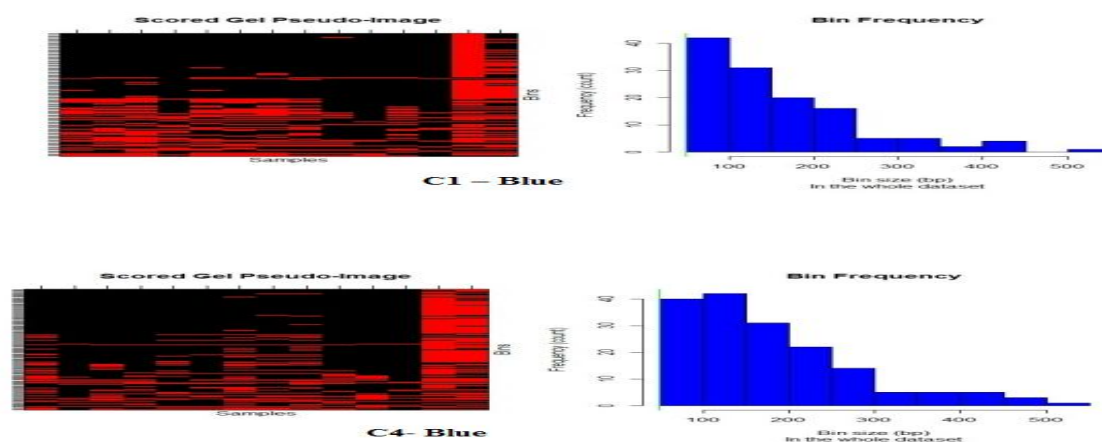


Fig. 1. F-AFLP profiles of 14 sunflower genotypes using two pairs of primers combination C1 and C4 Blue, where Sakha53, Qena (S1), Al-Buhayrah (S2), New Valley (S3-S4), Qena (S5), Bani Sweif (S6), Aswan (S7), Sohag (S8-S9) and Luxor (S10-S14).

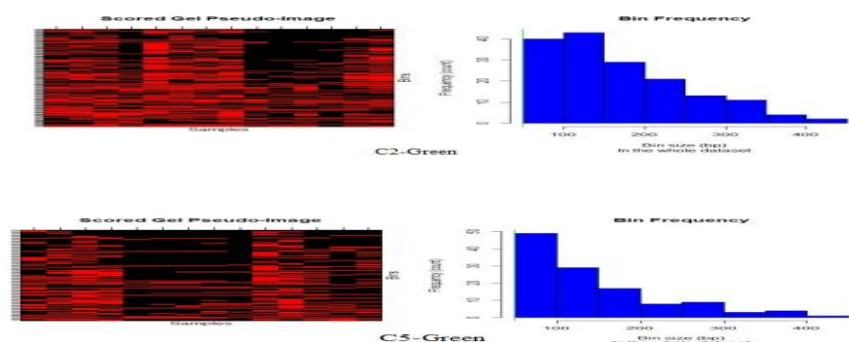


Fig. 2. F-AFLP profiles of 14 sunflower genotypes using two pair of primers combination C2 and C5-Green, where Sakha53, Qena (S1), Al-Buhayrah (S2), New Valley (S3-S4), Qena (S5), Bani Sweif (S6), Aswan (S7), Sohag (S8-S9) and Luxor (S10-S14).

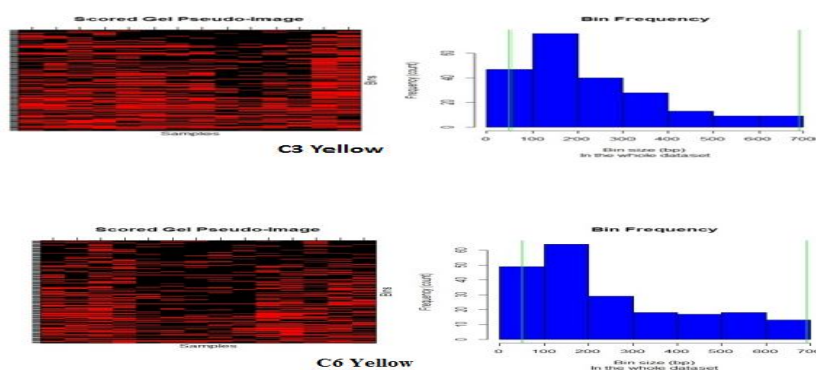


Fig. 3. F-AFLP profiles of 14 sunflower genotypes using two pair of primers combination C3 and C6-Yellow where Sakha53, Qena (S1), Al-Buhayrah (S2), New Valley (S3-S4), Qena (S5), Bani Sweif (S6), Aswan (S7), Sohag (S8-S9) and Luxor (S10-S14).

For the C4- Blue combination, 27 positive unique marker were recorded and ranged from 61 to 494 bp in size. S₁₃ genotype scored the highest positive unique marker (13), meanwhile, S₁₄ genotype counted the highest two molecular size 494 and 472 bp. On the other hand, S₄, S₇, S₁₄ and S₂ genotypes exhibited the lowest molecular size (61, 61, 74 and 77 bp), respectively. In C2- Green combination, 19 positive unique marker were recorded and ranged from 56 to 400 bp in size as shown in **Figure (2) and Table (6)**. S₂ genotype scored the highest positive unique marker (4), while, S₁₄, S₁₀, S₂ and S₁₁ genotypes counted the highest four molecular size (400, 339, 334 and 328 bp) respectively. However, S₁₁, S₁₂, S₁₃ and S₁₄ genotypes recorded the lowest molecular size: 56, 56, 83 and 95 bp, respectively. The results of C5- Green combination, exhibited a total of positive

unique marker (23) ranged from 78 to 431 bp in size, as shown in **Figure (2) and Table (6)**. S₇ and S₁₁ genotypes recorded the highest number of positive unique marker (6 and 5), however S₁ genotype counted the two highest molecular size 431 and 385, as well as, S₇ and S₁₀ recorded the highest molecular size 382 and 377 bp. S₉, S₁₁, S₁ and S₂ genotypes presented the lowest molecular size 78, 80, 93 and 98 bp, respectively. In the same context, C3- Yellow combinations, 31 positive unique marker were recorded and ranged from 50 to 659 bp in size as shown in **Figure (3) and Table (7)**. S₁₃ and S₁₂ genotypes scored the highest positive unique marker (7 and 6), while, five genotypes presented only one positive unique marker (S₁, S₃, S₇, S₈ and S₁₀), respectively. S₉, S₁₂ and S₁₃ genotypes counted the highest three molecular sizes 659, 579 and 567 bp, respectively.

While, S11 recorded the three highest positive unique marker (539,448 and 411bp), respectively. However, S1, S14, S13, S12, S3 and S2 genotypes exhibited the lowest molecular size ranged

from 50 to 95 bp, respectively. Whereas, three genotypes were failed to record any positive unique marker (S4, S5 and S6), respectively.

Table 6. Summary of positive unique F-AFLP markers via fourteen sunflower genotypes across the C2 and C5- Green primers combination

Genotype	Unique positive markers						Total grand markers	
	Combinations name	Amplicon size (bp)	Total number of markers	Combinations name	Amplicon size (bp)	Total number of markers		
S1	C2-Green	220	1	C5-Green	431,385,154,93	4	5	
S2		334,185,138,137	4		267,98	2	6	
S3		216	1		-	-	1	
S4		-	-		-	-	-	
S5		-	-		-	-	-	
S6		-	-		-	-	-	
S7		-	-		-	382,314,227,226,176,105	6	6
S8		-	-		-	263,201,89	3	3
S9		116	1		78	1	2	
S10		339	1		377	1	2	
S11		328,319,56	3		294,290,125,104,80	5	8	
S12		120,117,56	3		163	1	4	
S13		218,83	2		-	-	2	
S14		400,285,95	3		149	1	4	
Total			19			23	43	

Table 7. Summary of positive unique F-AFLP markers *via* fourteen sunflower genotypes across the C3 and C6- yellow primers combination

Genotype	Unique positive markers						Total grand markers
	Combinations name	Amplicon size (bp)	Total number of markers	Combinations name	Amplicon size+ (bp)	Total number of markers	
S1		50	1		281,252,140	3	4
S2		301, 273, 95	3		628,598	2	5
S3		92	1		-	-	1
S4		-	-		-	-	-
S5		-	-		563,502	2	2
S6		-	-		595,484	2	2
S7		297	1		119,98	2	3
S8		388	1		128,89,57	3	4
S9		659,189	2		565,540,214	3	5
S10		156	1		594,483,468	3	4
S11	C3-yellow	539,448, 411,305	4	C6-yellow	596,589,574, 534,477,350, 260,211,126	9	13
S12		579,182,120, 102,99,56	6		641,569,453, 352,246,162, 127	7	13
S13		567,356,264, 246,134,83,66	7		133	1	8
S14		479,224,95,53	4		267,249,232, 143,119,84	6	10
Total			31			43	74

For the last combination (C6- Yellow), a total of 43 positive unique marker were observed ranging in size from 57 to 641 bp. S₁₁, S₁₂ and S₁₄ genotypes recorded the highest positive unique marker (9, 7 and 6), respectively, while, S₁₃ showed only one positive unique marker. S₂, S₅, S₆, S₉, S₁₀, S₁₁ and S₁₂ genotypes exhibited the highest three molecular size ranging from (641 to 453 bp), in contrast, S₆ and S₁₄ genotypes recorded the lowest molecular size being 57 and 84 bp. Two genotypes, S₃ and S₄ did not show any positive unique marker. In summary, the total positive unique marker for each two pairs of primer combination C₁ & C₄ –Blue, C₂& C₅ green and C₃ & C₆ yellow exhibits (61), (43) and (74) markers, respectively. The results of F-AFLP in sunflower agreed with Gonthier et al (2013) who constructed the high-density genetic maps between two plants, K28

(male-sterile) and K59 (pollen-fertile), at the S-locus. Bulks and parental plants K28 and K59 were screened using AFLP analysis, with a complete set of 256 primer combinations of EcoRI-ANN and MseI-CNN. Thirteen AFLP markers were identified close to the NMS1-locus and six in the vicinity of the S-locus. The results suggested that a total of 31,000 fragments were generated, of which 2,350 showed polymorphism between K59 and K28. Additionally, Reif et al (2013) evaluated 133 intra-group and 104 intergroup hybrids with their parental lines in sunflower. The results showed that there was a total of 572 AFLP markers across the parental lines. Moreover, Zhang et al (2014) studied two sunflower varieties using SSR and AFLP. The results indicated that a total of 84 and 108 polymorphic bands were recorded for SSR and AFLP, respectively.

Discrimination capacity of F-AFLP

For the estimation of the discrimination capacity of F-AFLP, several parameters were evaluated as shown in **Table (8)**. The percentages of polymorphic amplicons were 97.42 % with a total of 1007 amplicons. While the total number of polymorphic amplicons (PA) was 981 amplicons ranged from 119 within the combination C5-green

to 208 amplicons in C3-yellow combination. Combination C3-yellow showed the highest number of amplicons (222), whereas combination C5-green showed the lowest number of amplicons (120). The highest percentage of polymorphic was obtained within the combination C1-blue with 100%, while the lowest PA% was revealed within the combination C3- yellow being 93.69%.

Table 8. Summary of the polymorphic, monomorphic amplicons, % of polymorphic and positive unique marker monomorphic amplicons by six F-AFLP combinations

Combination name	Monomorphic amplicons (MA)	Polymorphic amplicons (PA)	Total number of amplicons	Percentage of monomorphic amplicons	Percentage of polymorphism	Total of positive unique marker
C6-yellow	1	207	208	0.48%	99.52%	43
C3-yellow	14	208	222	6.31%	93.69%	31
C2-Green	8	155	163	4.91%	95.09%	19
C4-blue	2	166	168	1.19%	98.81%	27
C1-Blue	0	126	126	0.00%	100.00%	34
C5-green	1	119	120	0.83%	99.17%	23
Total	26	981	1007	2.58%	97.42%	178

In this regards, our results were in partial agreement with those of **Gonthier et al (2013)** who used AFLP analysis with a complete set of 256 primer combinations. A total of 31,000 fragments were generated, of which 2,350 showed polymorphism. Moreover, **Zhang et al (2014)** reported that a total 1460 bands were detected by AFLP analysis using 48 of AFLP primer combinations among some sunflower genotypes. The total number of PA detected by AFLP analysis was 1119 bands (60.48%), while 557 bands were polymorphic with the percentage of PA 39.52%.

Marker traits association in sunflower genotypes

With regard to the total positive unique marker which were counted 73 positive markers ranged from 52 to 682 bp in size, while the P-value thresholds for the association between F-AFLP markers and the different measured sunflower traits ranged from 2 to 3.66 under ($p \leq 0.01$), and their densitometric analysis as shown in **Table (9)**.

Table 9. Summary of positive unique markers for eight traits of sunflower genotypes across six combination of F-AFLP

Traits Combination	Positive unique markers with traits										Total
	Seeds weight	Stem diameter	Plant height	Number of seeds /head	Number of leaves	Head weight	Head diameter	1000 Seeds weight			
C1-Blue	-	161	-	-	81	-	81,161	125	5		
'-log10 value	-	3.09	-	-	2.89	-	3.66,2.24	3.36	-		
C4-Blue	-	244	-	-	102	-	-	-	2		
'-log10 value	-	2.52	-	-	2.03	-	-	-	-		
C2-Green	-	187	52,116,126,187	-	135	-	133,345	79,89,112,115,226,2	18		
'-log10 value	-	2.37	2.06,2.06,2.06,290	-	2.93	-	2.40,2.40	54,266,382,434,446 2.59,2.41,2.34,2.13, 2.59,3.58,2.38,2.13, 3.58,2.01	-		
C5-Green	-	-	78	86	-	-	-	-	2		
'-log10 value	-	-	2.06	2.30	-	-	-	-	-		
C3- yellow	146,522	-	58,59,121,127,130, 269,659	79, 332	163,180,189,262,2	-	163,226,262	93,181,268,522	24		
'-log10 value	2.12,2.25	-	2.06,2.06,2.06,2.06, 2.06,2.06,2.06	2.53,2.85	3.51,2.01,2.76,2.1	-	3.10,2.37,2.11	2.40,2.32,2.52,2.31	-		
C6- yellow	161, 682	173, 502,563	60,173,214,243,430, 540,565	75	60,284	139,682	60,173,502, 563	139	22		
'-log10 value	2.48, 3.06	2.47,3.09, 3.09	2.74,2.74,2.06,2.02, 2.11,2.06,2.06	2.41	2.52,2.30	2.26,3.40	2.35,2.22,2.24, 2.24	2.22	-		
Total	4	6	19	4	11	2	11	16	73		

The highest number of positively associated markers traits were observed within four traits, *i.e.* plant height, 1000 seeds weight, head diameter and number of leaves being 19, 16, 11 and 11 positive unique marker, respectively. With respect to the lowest marker associated traits, head weight, number seeds per head and seed weight recorded the lowest value of positive unique marker: 2, 4 and 4, respectively.

In this context, sampling strategies for yield components and morphological traits, along with marker trait association was analysed and exploitation in sunflower, by Luevanos- Escareño et al (2010) who estimated the hybridization between the cultivated sunflower *Helianthus annuus* and the wild species with ornamental potential *Tithonia rotundifolia*. The results of AFLP-based on DNA fingerprints were analyzed in a sample of ten familial trios, with an average of 28 polymorphisms per trio. The DNA fingerprinting analysis of the plants complemented the morphological study which allowed confirmation of the hybrid nature of the offspring. Moreno et al (2013) studied the genetic characterization of sunflower breeding resources in Argentina via marker trait association analysis for seed oil content. The results indicated that this information could take into account the diversity and selection of appropriate parental genotypes for successful hybridization. Purwati and Herwati (2016) analyzed marker-trait associations between TE-AFLP markers and oil content. The results showed that, two markers namely E_{ac} x P_{a04} and E_{ac} x P_{a39} were found to be associated oil content with high statistical significance ($P = 0.005$). These results indicated that on the basis of their qualitative morphological traits, sunflower accessions possessed high diversity. The seed size and seed thickness showed significant positive correlation with 100 seeds weight. These two traits might be used as selection criteria in sunflower breeding programs for appropriate screening of parental genotypes included in hybridizing process aiming at the increase of plant productivity.

CONCLUSION

F-AFLP markers facilitate the simultaneous selection of several characters and can thus improve the efficacy of selection based on markers associated traits in the plant-breeding activities.

REFERENCES

- Aboki M.A., Mohammed M., Musa S.H., Zuru B.S., Aliyu H.M., Gero M., Alibe I.M. and Inuwa B. 2012. Physicochemical and antimicrobial properties of Sunflower (*Helianthus annuus* L.) seed oil. *Int. J. Sci. Tech.*, **2(4)**, 151-154.
- Black W.C., Baer C.F., Antolin M.F. and DuTeau N.M. 2001. Population genomics: genome-wide sampling of insect populations. *Annual Review of Entomology*, **46**, 441-469.
- Bonin A., Ehrich D. and Manel S. 2007. Statistical analysis of amplified fragment length polymorphism data: A toolbox for molecular ecologists and evolutionists. *Mol. Ecol.*, **16(18)**, 3737-3758.
- Bukhsh M.A.H.A., Iqbal J., Kaleem S., Wasaya A. and Ishaque M. 2011. Qualitative analysis of spring planted sunflower hybrids as influenced by varying nutritional area. *Pakistan J. of Nutrition*, **10(3)**, 291-295.
- Chambó E.D., Escocard de Oliveira N.T., Garcia R.C., Ruvolo-Takasusuki M.C.C. and de Toledo V.D.A.A. 2017. Phenotypic correlation and path analysis in sunflower genotypes and pollination influence on estimates. *Open Biological Sci. J.*, **3(3)**, 9-15.
- Duncan D.B. 1955. Multiple ranges and multiple F test. *Biometrics*, **11(1)**, 1-42.
- Ei-Wakil D.A. 2014. Seed-Borne Fungi of Sunflower (*Helianthus annuus* L.) and their Impact on Oil Quality. *IOSR J. of Agric. and Veterinary Sci.*, **6(6)**, 38-44.
- Gonthier L., Blassiau C., Mörchen M., Cadalen T., Poiret M., Hendriks T. and Quillet M.C. 2013. High-density genetic maps for loci involved in nuclear male sterility (NMS1) and sporophytic self-incompatibility (S-locus) in chicory (*Cichorium intybus* L., Asteraceae). *Theor Appl Genet* **126(8)**, 2103-2121.
- Kandil A.A., Sharief A.E. and Amira A.M. 2016. Seedling parameters of some sunflower cultivars as affected by seed priming and salinity stress. *Int. J. Agro. Agri. R.* **9(2)**, 117-136.
- Luevanos-Escareño, M.P., Reyes-Valdes M.H., Villarreal-Quintanilla J.A. and Rodriguez R. 2010. Obtención de híbridos intergenéricos *Helianthus annuus* x *Tithonia rotundifolia* y su análisis morfológico y molecular. *Acta Botánica Mexicana*. **90**, 105-118.

- Luikart G., England P.R., Tallmon D., Jordan S. and Taberlet P. 2003.** The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**, 981–994.
- Magdy M., Werner O., McDaniel S.F., Goffinet B. and Ros R.M. 2016.** Genomic scanning using AFLP to detect loci under selection in the moss *Funaria hygrometrica* along a climate gradient in the Sierra Nevada Mountains, Spain. *Plant Biology*, **18(2)**, 280-288.
- Mahmoud A. and Abdel-Fatah B.E. 2012.** Analysis of Genetic Diversity among Sunflower Genotypes using Agro-morphological Traits and Molecular Markers. *Australian J. of Basic and Applied Sci.*, **6(13)** 419-432.
- Moreno M.V., Nishinakamasu V., Loray M.A., Alvarez D., Gieco J., Vicario A. and Lia V.V. 2013.** Genetic characterization of sunflower breeding resources from Argentina: assessing diversity in key open-pollinated and composite populations. *Plant Genetic Resources*, **11(3)**, 238-249.
- Purwati R. and Herwati A. 2016.** Evaluation of quantitative and qualitative morphological characters of sunflower (*Helianthus annuus* L.) germplasm. *Bio Diversitas* **17(2)**, 462-465.
- Razzaq H., Tahir H.N. and Sadaqat A. 2014.** Genetic variability in sunflower (*Helianthus annuus* L.) for achene yield and morphological characters. *Inter. J. Sci. Nat*, **5(4)**, 669-676.
- Reif J.C., Zhao Y., Würschum T., Gowda M., and Hahn V. 2013.** Genomic prediction of sunflower hybrid performance. *Plant Breeding*, **132(1)**, 107-114.
- Riaz A., Tahir M., Rizwan M., Nazir M.F. and Riaz B. 2017.** Combining Ability Analysis for Achene Yield and Related Components in Sunflower (*Helianthus annuus* L.). *Helia*, **40(67)**, 177-188.
- Storz J.F. 2005.** Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, **14**, 671–688.
- Suresha P.G., Kulkarni V.V., Supriya S.M., Darshan S. and Patil C.B. 2017.** Genetic Diversity Analysis in Sunflower (*Helianthus annuus* L.) parental Lines Using SSR and RAPD Markers. *Int. J. Curr. Microbiol. App. Sci.*, **6(7)**, 2069-2076.
- Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Friters A., Pot J., Paleman J., Kuiper M. and Zabeau M. 1995.** AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407-4414.
- Zhang, Z., van Parijs, F.R.D. and Xiao, B. 2014.** The status of AFLP in the genomics era and a pipeline. *Mol Breeding*, **34**, 1245–1260.



المسح الجينومي باستخدام فلوروسنت AFLP اربعة عشر تركيب وراثي لزهرة الشمس

[183]

مختار سعيد رزق^{1,2*} - فتحى محمد عبد التواب¹ - أحمد أبو دومة¹ -

محمد حمدي عمار² - محمود مجدى¹

1- قسم الوراثة - كلية الزراعة- جامعة عين شمس- ص.ب. 68 - حدائق شبرا 11241 - القاهرة - مصر

2- قسم الاصول الوراثة - مركز بحوث الصحراء - ص.ب. 1 شارع متحف المطرية 11753- المطرية - القاهرة -

مصر

*Corresponding author: drmokhtarsaid@yahoo.com

Received 6 June, 2018

Accepted 2 July, 2018

الموجز

وجود ارتباط إيجابي عالي بين معظم الصفات. وقد نجحت ستة بادئات من F-AFLP فى استنباط 178 من الواسمات الجزيئية من اجمالى 1007 حزمة متعددة الاشكال المظهرية بنسبة مئوية 97.42%. بالنسبة للواسمات الجزيئية المرتبطة بالصفات، لوحظ وجود 73 من الواسمات مرتبطة بالصفات المورفولوجية ومكونات المحصول.

الكلمات الدالة: زهرة الشمس، فلوروسنت AFLP، الواسمات المساعدة لانتخاب الصفات

ينتمى زهرة الشمس الى العائلة النجمية. استخدمت الدراسة الحالية تقنية الفلوروسنت AFLP لتقييم التنوع الوراثي لاربعة عشر تركيباً وراثياً من عباد الشمس متضمنة دراسة ثمانية صفات مورفولوجية واخرى مرتبطة بالصفات المحصولية. اظهرت النتائج اختلافات عالية المعنوية بين التراكيب الوراثة لجميع الصفات المدروسة عند مستوى معنوية 0.01. كما أظهرت نتائج الارتباط بين الصفات المدروسة عند مستوى معنوية 0.05

