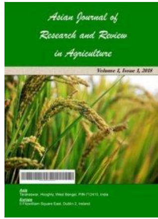

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Three SRAP Molecular Markers Linked to Yield Component Traits in Wheat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

In order to better understand the relationship genotype-phenotype, marker-trait associations were studied in eight wheat genotypes using a set of 95 DNA-based SRAP molecular markers. SRAP analysis showed that the average of the percentage of polymorphism (P%) was 62.59%, as well as the average of polymorphic information content (PIC) was 0.23. Moreover, the means of marker index (MI) was 1.49. The ME1F-EM5R and ME9F-EM3R primers combination showed higher levels of polymorphism of 88.89% and 90.91%, respectively. Single-marker analysis (SMA) indicated that ME-7F-EM-6R1250bp was probably as a candidate marker which linked to 1000-grain weight. In addition, ME-7F-EM-5R900bp and ME-4F-EM-6R490bp SRAP markers were identified for weight of grains/spike and No. of grains/spike, respectively. The cluster analysis based on SRAP and means of morphological data revealed similarity coefficient values ranged from 57.40% to 80.40% and from 90.61% to 98.81%, respectively. Similarity matrices generated by SRAP markers and means of morphological traits showed a positive but non-significant correlation (r = 0.03, $P > 0.05$).

Keywords: Wheat; mean performance; correlation; polymorphism; similarity.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the first important and strategic cereal crop for the majority of world's population. It is important stable food (edible grain) of about two billion people (36% of the world population), provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally [1]. Most of the currently cultivated wheat varieties belongs to hexaploids, which is known as common bread wheat and valued for bread making. Through the last 20 years, the global wheat average varied between 207 and 227 million hectares [2]. Egypt total wheat production of grain reached about 9 million tons resulted from 3.4 million faddens with 2.65 ton/faddan, while the consumption of wheat grains is about 15 million tons [3]. In spite of the high increase in production of wheat in Egypt that reached 8.8 million ton produced from

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1.34 million ha [4] this gab still there between production and consumption.

Yield and its component traits are controlled by polygene, whose expression is greatly affected by environments [5]. Hence, the development of high yielding wheat cultivars by crossing the lines with good general combining ability and selecting desirable genotypes within its segregating population consider one of the most tasks for wheat breeding program [6,7].

The assessment of the genetic variation in crop plants has been conducted on basis of phenotypic characters, which frequently resolving power needed to identify individual genotypes [8]. Molecular markers provide an excellent tool for obtaining genetic information and their use in the assessment of genetic diversity in wheat [9,10,11]. Because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development, SRAP is based on the amplification of open reading frames (ORFs) by targeting the exonic regions, intronic regions, and regions with promoters [12]. SRAP marker continue to be the main marker type for quantitative trait loci (QTL) studies in wheat, either alone or in combination with other types of markers, and they cover all 21 wheat chromosomes [12,13,14,15,16]. Jinwang et al. [17] utilized recombined inbred $F_{2:3}$ population to locate the molecular markers associated with sugar content. The results showed that SRAP marker (M3E7-S248) linked to the sugar content gene on chromosome 7. Moreover, Galal et al. [18] evaluated the level of genetic diversity in ten grain sorghum genotypes by selected SRAP primer combinations. The results showed that Single-marker analysis (SMA) indicated four of the SRAP markers were significant association with six morphological traits. Therefore, the objective of this investigation were to estimate the performance of wheat genotypes, level of polymorphism and marker traits association using morphological traits and SRAP technique.

2. MATERIALS AND METHODS

2.1 Genetic Materials

The genetic materials in this study involved eight different bread wheat parental genotypes: Misr-1 (P_1), Sids-12 (P_2), Sahel-1 (P_3), Katela (P_4), Sakha-94 (P_5), Deibera (P_6), Weiber (P_7) and Canada-462 (P_8), which presented wide range of variability in their yield component traits. The present study was carried out at the El-Kawther Experimental Research Farm of Faculty of Agriculture, Sohag University, Sohag, Egypt.

2.2 Field Experimental Design

The seeds of genotypes were sown on winter season 2018/2019 in a randomized complete block design (RCBD) with three replications. Each plot consisted of 3 rows with long 3 m. and 30 cm. wide. Plants were spaced by 10 cm. within row. The soil at the experimental site was sandy to loamy sand.

2.3 Traits Definitions

The following agronomic traits were recorded as the mean of ten plants/genotype chosen randomly in each plot for No. of spikes/plant, spike length, No. of grains/spike, weight of grains/spike, 1000-grain weight and grain yield/plant traits.

2.4 Analysis of Variance

Data were subjected to the analysis of variance to test the significance of the differences among the eight genotypes according to Cochran and Cox [19].

2.5 DNA Extraction and PCR Procedure

Genomic DNA was extracted from fresh young leaf pieces using cetyltrimethyl ammonium bromide (CTAB) protocol as described by Poresbski et al. [20] at the Laboratory of Biotechnology, Genetics Department, Faculty of Agricultural, Sohag University. For each genotype, 0.2 g of ground leaf tissue was suspended in 2 ml of extraction bufffer (20 mM of EDTA, 0.1 M of Tris-HCl, 1.4 of NaCl, 2% CTAB, 1% of PVP). The suspension was mixed well with 50 µl of Betamercapoethanol, incubated at 65°C for 30 min, followed by chlorophorm-isoamyl alcohol (24:1) extraction and precipitation with 2/3 of the volume of cold isopropanol and then kept at -20°C for one hour. The genomic DNA pellet formed after centrifugation at 5000 rpm for 5 min and washed with 1 ml of 75% ethanol. The DNA pellet was then suspended in 100 µl of TE buffer. The quality of genomic DNA was measured in spectrophotometer Genova (UK) and in a 0.8%

Table 1. Sequence and TM of the SRAP Primers used in this study

Primers name	Primer sequence 5`............3`	TM
ME-1(F)	TGAGTCCAAACCGGATA	49.00
ME-2(F)	TGAGTCCAAACCGGAGC	54.00
ME-4(F)	TGAGTCCAAACCGGACC	56.00
ME-7(F)	TGAGTCCAAACCGGACG	54.00
ME-9(F)	TGAGTCCAAACCGGTGC	56.00
ME-5(F)	TGAGTCCAAACCGGAAG	52.00
EM-3 (R)	GACTGCGTACGAATTGAC	50.00
EM-5(R)	GACTGCGTACGAATTAAC	47.00
EM-6(R)	GACTGCGTACGAATTGCA	53.00
EM-2(R)	GACTGCGTA CGAATTTGA	49.90
Em-10(R)	GACTGGGTACGAATTCCA	53.00
EM-1(R)	GACTGGGTACGAATTAAT	50.00
EM-10(R)	GACTGGGTACGAATTCCA	54.00

gel agarose stained with 0.2 µl ethidiumbromide. Genomic DNA was diluted 10-fold in water prior to 35 cycles of PCR amplification.

The PCR assays were performed in a 25 µl volume containing 12.5 µl of Go Taq® Green Master Mix (Promega, Madison, USA), 3.5 µl of primer 8 pmol, 7 µl of free nuclease water and 2 µl of 100 ng genomic DNA templates. The Thermal Cycler 96-labmet (USA) was programmed as: 1 cycle (an initial denaturing step) of 5 min at 95°C, 35 cycles of 30 sec at 95°C (denaturation step), 30 sec at 47°C to 56°C (annealing step, optimized for each primer combination), 2 min 30 sec at 72°C (elongation step) and 5 min at 72°C (final extension), then stored at 8°C. The amplified products were electrophoresed in a 1.0% agarose gel stained with 0.2 µl ethidiumbromide. The amplified fragments were visualized and photographed using UVP Bio Doc-It imaging system (USA) [21]. SRAP technique was conducted using 12 primers combinations (forward and reverse) Table 1.

2.6 Data of Molecular Markers Analysis

The DNA banding patterns generated by SRAP markers was analyzed by computer programme Gene Profiler (version 4.03). The presence (1) or absence (0) of each band was recorded for each genotype for all studied primers. Genetic distance was estimated according to Jaccard [22]. To measure the informativeness of the SRAP technique in differentiating among genotypes, the polymorphic information content (PIC) was calculated according to the formula of Ghislain et al. [23] as PIC= $1- [(p)^2 + (q)^2]$, where p is the frequency of allele band present and q is frequency of allele band absent across the tested genotypes. The marker index (MI) was also calculated for SRAP primer as MI= PIC x ηβ, where PIC is the mean PIC value, η the number of bands, and β is the proportion of polymorphism. Analysis of variance (ANOVA) was conducted using the 0-1 data. The association analysis was conducted using simple linear regression. For this, data on individual phenotypic traits were regressed on whole 0-1 binary marker data for each individual marker using Excel programme. The coefficient of determination $(R)^2$ was calculated as $R^2 = 1 - (SSE / SST)$, where SSE is the sum of squares of error and SST is the total sum of squares.

2.7 Dendogram construction

The genetic similarities among the parental genotypes were computed and UPGMA-dendrogram was performed according to Jaccard's coefficient of similarity using NTSYS-pc version 2.20 (Applied Biostatics Inc.) [24]. Mantel test [25] was performed to estimate the correlation between the distances matrices conducted based on phenotypic data using Euclidean's coefficient [26] and SRAP markers according to Jaccards coefficient.

3. RESULTS AND DISCUSSION

3.1 Mean Performance of Linked Morphological Traits with SRAP Marker

Mean performance of linked morphological traits with SRAP marker are presented in Table 2. Concerning of No. of grains/spike, the results cleared that the best genotype was P_3 with mean value of 62.69. Furthermore, the genotype P_2 recorded the highest weight of grains/spike (2.77

gm.). Moreover, the genotype P_5 recorded the highest mean value (50.07 gm) for 1000-grain weight trait.

3.2 Level of Polymorphism based on SRAP

In this study, 14 pairs of SRAP primers were screened among the eight wheat parental genotypes, and 12 pairs of them (forward and reverse) were polymorphic. A total of 95 bands were amplified, of which 64 bands (62.59 %) were found polymorphic (Table 3). The total number of bands varied from 2 (ME7F-EM3R and ME7F-EM1R) to 12 (ME4F-EM6R). Also, the polymorphic bands number ranged from 1 (ME7F-EM6R and ME7F-EM63R) to 10 bands (ME4F-EM6R and ME9F-EM3R). The percentage of polymorphism (P%) ranged from 16.67% to 90.91% which was detected by ME7F-EM6R and ME9F-EM3R primers combination, respectively, with an average of 62.59% (Table 3). The mean number of total bands and polymorphic bands were 7.92 and 5.33 per primer, respectively. The ME1F-EM5R and ME9F-EM3R primers combination showed higher levels of polymorphism of 88.89% and 90.91%, respectively. The size of polymorphic bands ranged from 200 bp to 1750 bp, generated by ME1F-EM6R as well as ME7F-EM5R primers combination, respectively (Table 3). Al-Doss et al. [27] used nineteen SRAP primers for detecting the polymorphism among six wheat durum genotypes. The results showed that 128 amplified fragments including 35% polymorphic exposed to heat stress. These findings imply that SRAP markers are useful and efficient to estimate genetic diversity level in wheat genotypes. Moreover, Filiz [28] screened 30 wheat genotypes collected from International Maize and Wheat Improvement Centre (CIMMYT) using SRAP markers. His results exhibited that 23 SRAP primers combination were amplified 686 DNA bands with 90% polymorphism. Khaled and Hamam [29] studied 36 bread wheat for genetic variability by SRAP. Their results showed that, the percent of polymorphism ranged from 20% to 100%. Furthermore, thirteen SRAP primers combination successfully amplified 954 fragments in bread wheat genotypes, and the overall polymorphism ratio was 99.67%, ranging from 98 to 100% [30].

The polymorphism information content (PIC) index has been used extensively in many genetic diversity studies [31,32]. Moreover, the PIC values indicate the usefulness of DNA markers for gene mapping, molecular breeding and germplasm evaluation [33]. In this study the PIC values for SRAP primers combination varied from 0.06 (ME7F-EM6R) to 0.38 (ME9F-EM3R) with an average of 0.23 (Table 3). The SRAP primers combinations showed that the marker index (MI) values ranged from 0.06 (ME7F-EM6R) to 3.80 (ME9F-EM3R) with an average of 1.49 (Table 3). [29] found among 36 bread wheat genotypes that the average of polymorphic information content was about 0.15 and 0.16, respectively, also the marker index was 1.03 reflecting that SRAP marker more efficient in genetic diversity assessment. Moreover, Abdelkhalik et al. [30] demonstrated in studied bread wheat that the polymorphic information content values ranged from 0.67 for ME-11 x EM-5 to 0.97 for ME-9 x EM-4 and ME-11 x EM-6, respectively. Furthermore, Jahnvi et al. [34] assessed genetic diversity by SRAP marker among 16 cumin genotypes including five released varieties. They found that, out of 65 amplified bands, a total of 60 polymorphic bands were detected which demonstrated with polymorphism information content (PIC) value of 0.34 with a range from 0.14 to 0.51. Also, the distinctive value of MI (marker index) for studied markers was 2.43. In addition, Samah et al. [35] used SRAP markers to determine the genetic diversity and relationship among 19 Egyptian

Table 2. Mean performance of the genotypes

Genotypes		No. of grains/spike	weight of grains/spike	1000-grain weight
Misr-1	P_1	40.96	2.3	46.98
Sids-12	P_2	58.19	2.77	49.97
Sahel-1	P_3	62.69	2.5	48.5
Katela	P_4	49.57	1.99	47.97
Sakha-94	P_5	60.25	2.48	50.07
Diebera	P_6	55.75	2.1	49.77
Weiber	P_7	50.3	1.19	44.17
Canada-462	P_8	43.6	1.08	46.2

Table 3. Primers used for SRAP markers, total number of fragment detected by each pair of primers, %P, PIC, MI and fragments sizes for eight wheat genotypes

Primer combinations	Amplified bands		P %	PIC	MI	Fragments size (bp)	
	Bands number	Polymorphic bands				Largest	Smallest
ME-7F-EM-6R	6	1	16.67	0.06	0.06	1250	490
ME-7F-EM-5R	7	4	57.14	0.24	0.96	1750	420
ME-7F-EM-3R	4	1	25	0.13	0.13	100	500
ME-2F-EM-6R	11	7	63.64	0.2	1.4	1250	300
ME-1F-EM-5R	9	8	88.89	0.34	2.72	1200	460
ME-1F-EM-2R	7	3	42.86	0.15	0.45	1200	480
ME-5F-EM-2R	5	3	60	0.24	0.72	1480	445
ME-7F-EM-10 R	8	6	75	0.29	1.74	1400	525
ME-1F-EM-6R	11	8	72.73	0.28	2.24	1420	200
ME-4F-EM-6R	12	10	83.33	0.32	3.2	1500	370
ME-9F-EM-3R	11	10	90.91	0.38	3.8	1500	490
ME-7F-EM-1R	4	3	75	0.16	0.48	1000	560
Means	7.92	5.33	62.59	0.23	1.49		
Total	95	64					

P %: Percentage of polymorphism; PIC: Polymorphic Information Content; MI: Marker Index

Table 4. Details of variance (ANOVA) involving simple linear regression (R^2) for traits using 64 SRAP polymorphic bands

Marker	Traits	S.V	d.f	SS	MS	R^2	P- value
ME-7F-EM-$6R_{1250bp}$	1000-grain weight	Genotypes	1	15.09	15.09*	49.51	0.052
		Error	6	15.39	2.57		
		Total	**7**	**30.48**			
ME-7F-EM-$5R_{900bp}$	weight of grains/spike	Genotypes	1	1.70	1.70*	64.29	0.017
		Error	6	0.95	0.16		
		Total	**7**	**2.66**			
ME-4F-EM-$6R_{490bp}$	No. of grains/spike	Genotypes	1	228.50	228.50*	52.84	0.041
		Error	6	203.93	33.99		
		Total	**7**	**432.43**			

S.V: Source of variance, d.f: Degrees of freedom, S.S: Sum of squares, M.S: Mean squares, R^2: Coefficient of determination

barely cultivars for water tolerance. The results exhibited that primer combination “me5+em1” gave the highest polymorphism (100%) and the highest polymorphic information content PIC was (0.97). In the same direction, Khaled et al. [36] studied the relationship among SRAP marker and morphological traits in seven sorghum genotypes. They showed the polymorphism percentage was 51.37%, as well as the average of PIC was 0.17. Moreover, the mean of marker index (MI) was 0.68.

3.3 Single Marker Analysis

The present study involved a set of eight wheat genotypes, exhibiting moderate to high genetic variability for the eight phenotypic traits included in this work. Using simple linear regression method, a total of 64 polymorphic molecular markers were identified; three of them were significantly associated with some studied traits (Table 4 and Figs. 1, 2). The results in Table 4 showed that the SRAP marker ME-7F-EM-$6R_{1250bp}$ was probably as a candidate marker which linked to 1000-grain weight. In addition, ME-7F-EM-$5R_{900bp}$ and ME-4F-EM-$6R_{490bp}$ molecular markers were identified for weight of grains/spike and No. of grains/spike, respectively. These results revealed significant regression (49.51, *P= 0.052*), (64.29, *P= 0.017*) and (52.84, *P= 0.04*) on 1000-grain weight, weight of grains/spike and No. of grains/spike,

respectively. Similarly, Khaled and Hamam [29] reported that the SRAP marker ME-7F – EM-$6R_{420bp}$ was regarded as a candidate marker linked to No. of grains/spike in bread wheat. In addition, Khaled et al. [36] showed four SRAP markers linked to 1000-grain weight and grain yield/plant in sorghum genotypes.

3.4 Cluster Analysis

A cluster analysis realized using Jaccard's coefficient for the data of SRAPs molecular marker, revealed similarity values ranging from 57.4% (between Sahel-1 and Sakha-94) to 80.4% (between Misr-1 and Canada-462) with an average of 68.9% (Table 5).

The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis based on SRAP markers separated the studied wheat parental genotypes into four significantly different clusters (Fig. 3). Genotype Sakha-94 was in the third cluster, branched at 64.50% percent of similarity with genotype Wieber which belonged to the fourth cluster. The first cluster subdivided into two groups at 72.2%, the first group contains Misr-1 and Canada-462 but the second group contains Sahel-1 and Diebera.

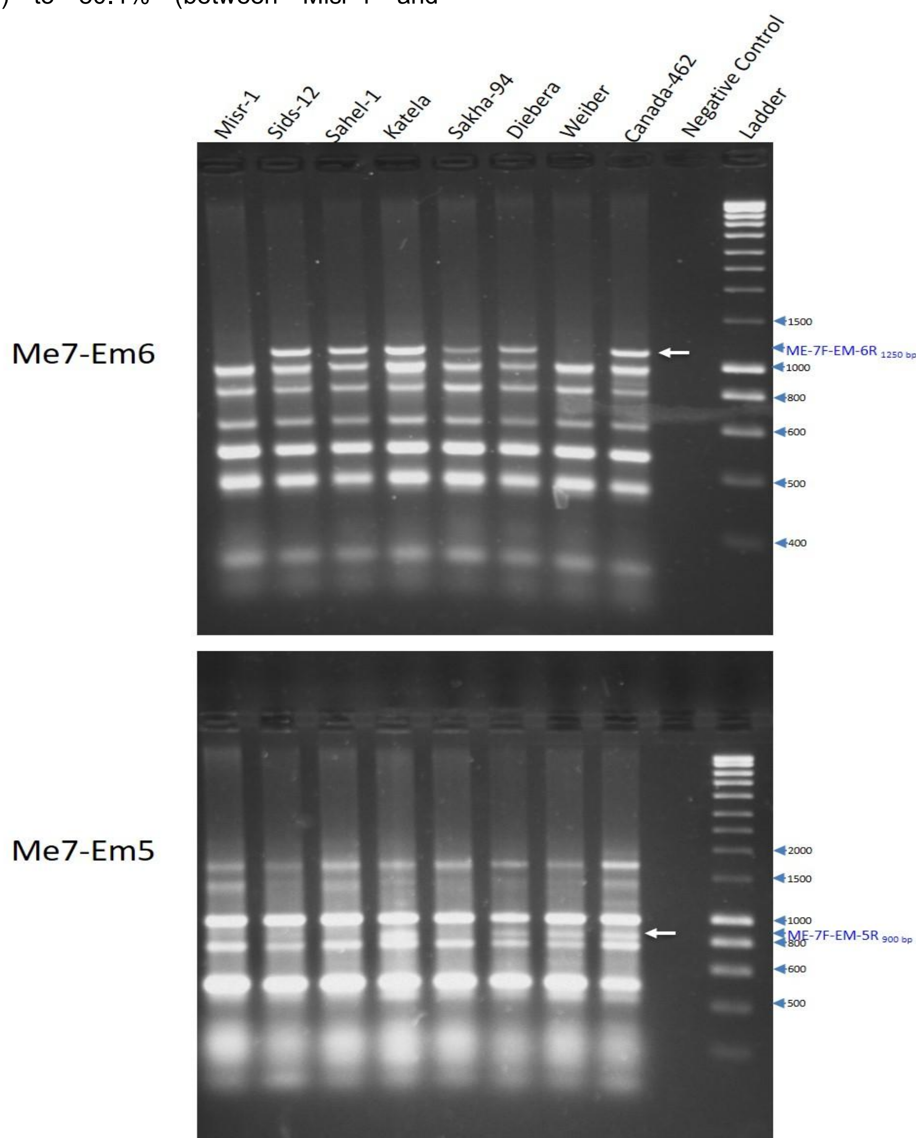

Fig. 1. SRAP profiles obtained from eight wheat genotypes amplified by primers (ME-7/EM-6 and ME-7/EM-5). M= 100_{bp} ladder size marker

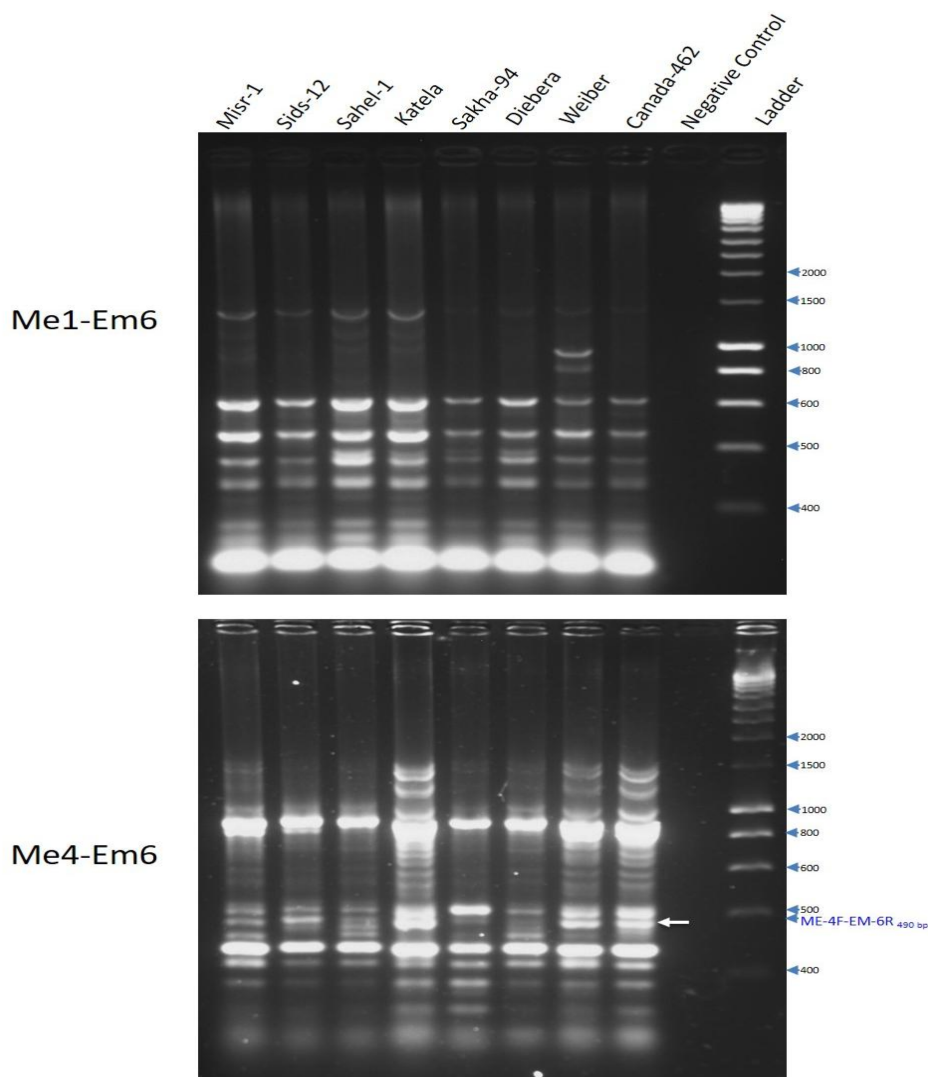

Fig. 2. SRAP profiles obtained from eight wheat genotypes amplified by primers (ME-1/EM-6 and ME-4/EM-6). M= 100_{bp} ladder size marker

Table 5. Similarity matrix for the eight wheat genotypes according to Jaccard's coefficient obtained from 95 SRAP fragments

Genotypes	Miser-1	Sids-12	Sahel-1	Katela	sakha-94	Diebera	Weiber	Canada-462
Miser-1	100.00							
Sids-12	70.00	100.00						
Sahel-1	76.60	66.70	100.00					
Katela	67.30	71.20	67.30	100.00				
Sakha-94	66.70	64.20	57.40	70.90	100.00			
Diebera	72.00	66.00	80.00	69.80	66.00	100.00		
Weiber	70.00	57.70	60.00	61.80	61.10	69.40	100.00	
Canada-462	80.40	64.80	70.60	74.50	70.90	69.80	71.20	100.00

Table 6. Genetic distance among tested eight wheat genotypes using eight agronomic traits based on percent of similarity

Genotypes	Misr-1	Sids-12	Sahel-1	Katela	akha-94	Diebera	Weiber	Canada-462
Misr-1	100.00							
Sids-12	90.61	100.00						
Sahel-1	94.19	95.09	100.00					
Katela	94.93	93.20	95.72	100.00				
Sakha-94	93.20	93.89	96.87	95.69	100.00			
Diebera	93.69	93.29	96.13	95.97	98.81	100.00		
Weiber	93.96	91.71	94.32	97.54	95.63	95.94	100.00	
Canada-462	97.43	90.81	93.29	93.42	92.48	93.08	93.64	100.00

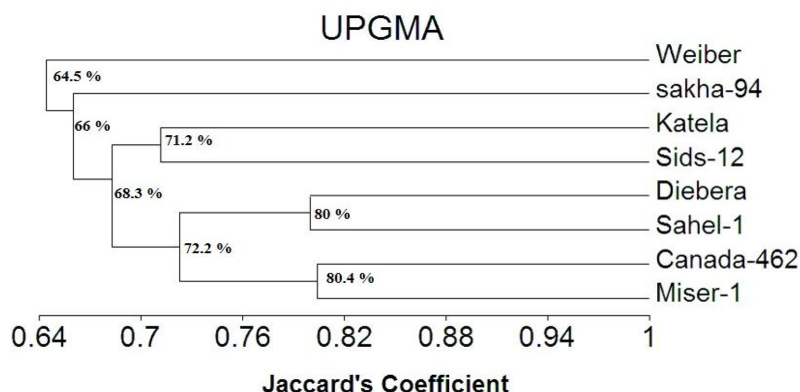

Fig. 3. Phylogenetic tree of the eight wheat genotypes obtained using 95 SRAP bands

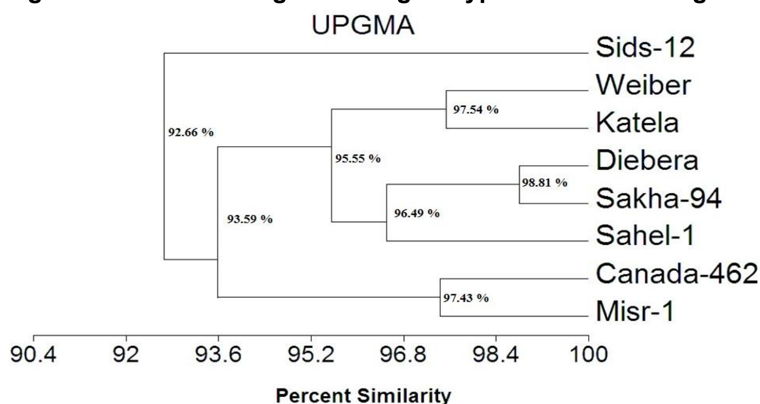

Fig. 4. Phylogenetic tree of genetic distance among tested wheat genotypes using eight agronomic traits based on percent similarity

The cluster analysis based on the means of morphological and agronomical traits revealed similarity coefficient values ranging from 90.61% (between Misr-1 and Sids-12) to 98.81% (between Sakha-94 and Diebera) (Table 6).

The dendogram based on means of traits divided the wheat parental genotypes into three different clusters (Fig. 4). The second cluster contains Canada-462 and Misr-1 which clustered at 93.59% percent of similarity with the big first cluster. At percent of similarity of 92.66% branched genotypes Sids-12 in the last cluster with the first big cluster. Similar results were found by Galal et al. [18], Khaled and Hamam [29], Jahnvi et al. [34], Samah et al. [35],

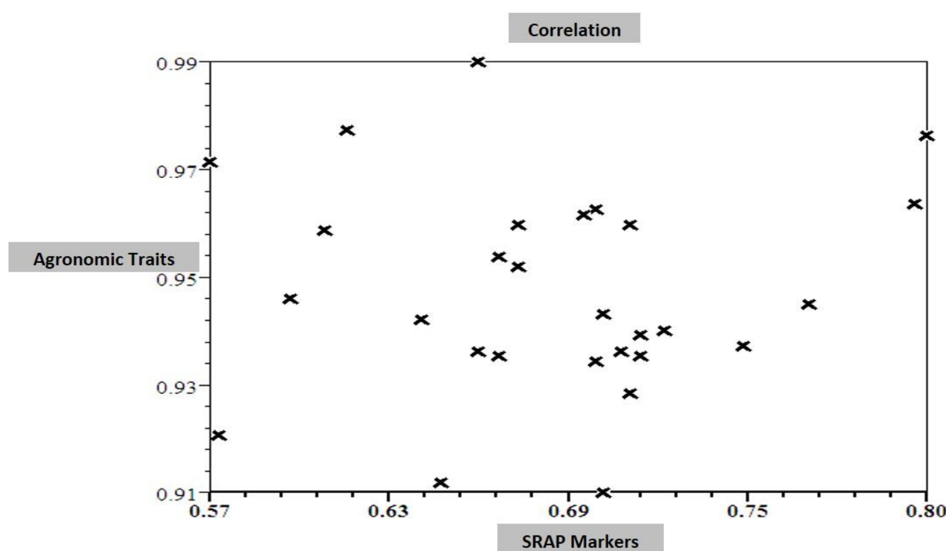

Fig. 5. Correlation between similarities percent obtained from SRAP markers and studied agronomic traits for the eight wheat genotypes

Said et al. [37] They showed that each group in the UPGMA cluster analysis includes the most relative genotypes according to their response to drought stress.

3.5 Combined Molecular Markers and Morphological Markers

The correlation (r) and the Mantel test statistic (Z) were calculated to measure the degree of relationship between the similarity matrices obtained with SRAP and agronomic traits data (Fig. 5).

The Mantel test revealed that there was a positive and non-significant correlation between the genetic similarities based on phenotypic data and SRAP marker (r = 0.03).

Fahmi et al. [38] obtained a negative correlation of -0.20 between RAPD markers and agronomic characterization in wheat. In addition, Khaled and Hamam [29] found a positive correlation (0.63) among 36 bread wheat genotypes generated by SRAPs. Moreover, Khaled et al. [36] showed positive correlation (0.21) among sorghum genotypes generated by SRAPs.

4. CONCLUSION

The optimal strategies of the breeding system require extensive knowledge of the breeding materials employed. Results presented here will be useful to understand the current status of genetic diversity between wheat genotypes. Genetic markers like SRAPs may accurately assay the degree of genetic change between two genomes, but they may not necessarily reflect the divergence in terms of changes in traits of agronomic importance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard was written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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