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Morphological and sequence-related amplified polymorphism-based molecular diversity of local and exotic wheat genotypes

S.M. Abdelkhalik[1], A.K.M. Salem[2], A.R. Abdelaziz[1] and M.H. Ammar[2]

[1]Botany and Microbiology Department, College of Science,
King Saud University, Saudi Arabia
[2]Plant Production Department, Faculty of Food and Agricultural Sciences,
King Saud University, Riyadh, Saudi Arabia

Corresponding author: M.H. Ammar
E-mail: ammarrice@gmail.com

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ABSTRACT. Assessing genetic diversity is a prerequisite for the genetic improvement of wheat. Molecular markers offer accurate and reproducible means for assessing genetic diversity. Field performance and sequence-related amplified polymorphism (SRAP)-based assessment of molecular diversity was carried out on a set of 10 local and introduced bread wheat (*Triticum aestivum* L.) genotypes grown in the middle arid region of Saudi Arabia. The results revealed highly significant differences among the studied phenological traits and revealed a significant amount of genetic diversity across the tested genotypes. The overall performance revealed the superiority of KSU 102 in terms of yield and its components, with a yield potential of 8.7 tons/ha. Highly significant and positive correlations were observed among grain yield and biological yield, and also, spike length and spike weight. Thirteen SRAP primer combinations successfully amplified 954 fragments. The total number of genetic loci analyzed was 312. The overall polymorphism ratio was 99.67%, ranging from 98 to 100%. The

polymorphic information content values ranged from 0.67 for ME11 x EM5 to 0.97 for ME9 x EM4 and ME11 x EM6, respectively. The wheat genotypes were clustered based on their genetic constitution and origin. The results demonstrate the power of SRAP primers for detecting molecular diversity and for varietal discrimination. The results show that high levels of genetic diversity exist, and suggest the potential of the tested materials for wheat crop improvement in the arid central region of Saudi Arabia.

Key words: Bread wheat; Field performance; SRAP; Molecular diversity

INTRODUCTION

Wheat is considered the world's first cereal crop, and presently, it is the main staple food for nearly half of the world's populations, providing 20% of the calories and protein required for a healthy human diet (Gupta et al., 2008). Most wheat produced worldwide is used for human food, and is a main source of protein and dietary fiber for people across the globe. Wheat grains, along with other cereals, are strongly recommended for infants and children for better health performance, and they represent the first food that is introduced into the diet of infants. Studies on human nutrition suggest that diets should obtain most of their calories from complex carbohydrates, such as cereal starch. A balanced diet should contain approximately 20-30 g/day of dietary fiber, which can be obtained through cereal grains, particularly wheat (Dendy and Dobraszczyk, 2001). With global population increase, significant increases in wheat yield are needed to avoid famine and ensure food security. Under arid and semi-arid environments, water scarcity and elevated temperatures during grain filling periods are among the main production constraints for wheat (Sio-Se Mardeh et al., 2006). In 1990, the crop cultivated area of Saudi Arabia was about 744,422 ha (i.e., 55% of the total cultivated area), and the total production was 3.5 million tons (Al-Hazmi, 1997). This area has significantly reduced in recent years owing to water scarcity for 100,000 ha in the 2013/2014 growing season, during which the production was approximately 600,000 tons (Mousa, 2014). This significant decline in the cultivated area has pushed wheat breeders towards developing new lines with the potential for higher yields under such arid conditions.

Successful breeding programs require significant levels of crop genetic diversity, which are essential for crop manipulation, optimum germplasm utilization, the selection of elite lines, and varietal fingerprinting (Engles et al., 2002). Molecular marker technology provides efficient and accurate tools that can be used to estimate genetic diversity and varietal discrimination at the molecular level. Numerous polymerase chain reaction (PCR)-based molecular marker techniques have been developed and efficiently used in plant breeding programs. These include random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), sequence tagged site (STS), and sequence characterized amplification region (SCAR). These marker systems are now routinely employed for genetic assessments and better selection through marker-assisted breeding (Jones et al., 2009).

Among the various molecular marker systems used, sequence related amplified polymorphisms (SRAP) is thought to be an efficient marker system, which generates simple and reproducible results. It has better throughput and reproducibility compared with RAPD

markers. In terms of simplicity, SRAP methods are easier to use than AFLP methods. This marker system is considered an open reading frame (ORF) trap, since it targets ORFs (Li and Quiros, 2001). These advantages of SRAP have made it the marker of choice for investigating molecular diversity, particularly when combined with a sensitive electrophoresis system. SRAP markers have been used to assess diversity among durum wheat landraces (Zaefizadeh and Goliev, 2009), CIMMYT bread wheat lines (Filiz, 2012), for mapping the stripe rust resistance gene (Chen et al., 2012), and for mapping physiological traits of wheat (El Shafei et al., 2013).

The objective of this study was to assess variations in the field performance of 10 local and exotic bread wheat genotypes grown under the arid environments of the central region of Saudi Arabia and to assess molecular diversity among these genotypes using the SRAP marker system.

MATERIAL AND METHODS

Ten wheat genotypes were selected for use in this study, including Yecoro Rojo, Pavon76, Veery and Lang as exotic introduced genotypes, and a set of advanced breeding lines with high adaptability to the local environment, developed at the wheat breeding program, Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Saudi Arabia. The advanced breeding lines were KSU102, KSU 105, KSU106, L11-19, L11-21, and L11-23. The list, origin, and basic features of the tested genotypes are shown in Table 1. The 10 wheat genotypes were grown at Dirab Agricultural Research Station, Riyadh (South Riyadh (24°43'34"N, 46°37'15"E, altitude 600 m) in a randomized complete block design RCBD layout with three replications in the 2011-12 growing season (December 20th), for field evaluations. The experimental soil was loamy sandy soil, with very low organic matter (0.03%) and nutrients, field capacity 14%, pH 7.5. Each plot (4 m^2), consisted of eight rows, 2 m long, and plant spacing of 20 cm. All cultural practices were applied as recommended for the central region of Saudi Arabia. Data on yield and yield components were collected and analyzed using *MSTAT C* software package (Freed et al., 1991). Seeds of each genotypes were grown in a laboratory for 15 days prior to DNA isolation. Leave samples from 3-week-old wheat seedlings were collected, directly immersed in liquid N_2, and then stored at -80°C. DNA was isolated from leaf samples using a modified SDS method, as described by Hoelzel (1998) and Alghamdi et al. (2014). Following initial screening with 21 primer combinations (three forward and seven reverse primers), 13 SRAP primer pairs were selected based on their reproducibility and ability to amplify loci from all tested genotypes. Those 13 primer combinations were used to estimate molecular diversity across the 10 genotypes. SRAP primer pairs used in this study are shown in Table 2. The amplification reactions were carried out in 20-μL volumes, which contained the following: 1X *GoTaq* Green Master Mix (Cat. No. M7123, Promega Corporation, Madison, USA), 0.25 μM forward and reverse primers, 50 ng DNA template and volume were completed using nuclease-free water. The three forward primers were labeled with FAM dye at the 5' end. Amplifications were carried out using a TC-5000 thermal cycler [Bibby Scientific - UK]. The SRAP profile used for amplification was as follows: initial denaturation at 94°C for 5 min followed by five cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, and elongation at 72°C for 1 min. The remaining 30 cycles were performed with an annealing temperature of 50°C for 1 min. A final extension step was carried out at 72°C for 7 min to ensure amplification was complete. Amplified PCR products were separated using a 36-cm 16-capillary based system from Applied Biosystems (ABI) 3130xl Genetic Analyzer. Fragment analysis of PCR products

was performed with GeneMapper software v3.7 (ABI). The allele calling threshold was set at 200 relative florescence units (rfu) according to the method described by Wooten and Tolley-Jordan (2009). Generated data for the presence (1) and absence (0) of alleles were analyzed using Jaccard similarity coefficient (Jaccard, 1908). Based on SRAP data, a dendrogram was constructed to explain the genetic relationships among tested genotypes using the Jaccard similarity coefficient and unweighted pair group method with arithmetic average (UPGMA) employing the SAHN (sequential, agglomerative, hierarchical, and nested clustering) from the NTSYSpc (ver.2.10) program (Rohlf, 2005).

Table 1. List and basic features of the studied genotypes.

Genotype	Origin	Pedigree	
YecoraRojo	USA	Ciano 67/Sonora 64/Klein Rendidor/3/II-8156	Hard red spring wheat, semi dwarf
Pavon76	China	-	Spring bread wheat
Verry	CYMMET	Kavkaz x Buho	Spring bread wheat
Lang	Australia	QT3765 x Sunco	Hard grain spring wheat
KSU102	KSU	Sama X YecoraRojo	Spring bread wheat
KSU105	KSU	HD2172 x RI474	Spring bread wheat
KSU106	KSU	Parok x RI474	Spring bread wheat
L11-19	KSU	Sama\Yecora Rojo-11-19	Spring bread wheat
L11-21	KSU	Sama\Yecora Rojo-11-21	Spring bread wheat
L11-23	KSU	Sama\Yecora Rojo-11-23	Spring bread wheat

Table 2. Forward and reverse sequence-related amplified polymorphism (SRAP) primers used to assess molecular diversity among the wheat genotypes.

Primer name	Forward 5'-3'	Primer name	Reverse 5'-3'
ME9	5-TGAGTCCAAACCGGAAG-3	EM3	5-GACTGCGTACGAATTACA
ME11	5-TGAGTCCAAACCGGTAA-3	EM4	5-GACTGCGTACGAATTACG
ME12	5-TGAGTCCAAACCGGTCC-3	EM5	5-GACTGCGTACGAATTACT
		EM6	5-GACTGCGTACGAATTAGC
		EM7	5-GACTGCGTACGAATTATG
		EM9	5-GACTGCGTACGAATTCAA
		EM10	5-GACTGCGTACGAATTCAC

RESULTS

Field performance

The mean square estimates for yield performance parameters of the 10 studied genotypes are presented in Table 3. The results show that there are highly significant differences among genotypes for all studied traits. In addition, they highlight the presence of substantial genetic diversity among genotypes and indicate their potential utilization in wheat breeding programs for yield improvement. Table 4 shows the mean performance of exotic and locally developed wheat materials under field conditions. L11-19 showed semi-dwarf stature at 89.7 cm high, while the Australian variety Lang was the tallest at 103.7 cm high, and was also relatively late maturing (135.7 days) as compared to L11-19, which required 129.3 days to complete its life cycle. For spike length, the two newly developed lines, L11-21 and L11-23, were 17.2 and 17.9 cm long, respectively. Conversely, the Chinese variety Pavon76 and the Australian variety Lang had the shortest spikes, at 9.8 and 9.4 cm, respectively. KSU-developed lines also had the heaviest spikes as compared with those of the exotic varieties.

The 1000-grain weight ranged from 29.3 g for Lang to 43.3 g for Verry. KSU 102 recorded the highest number of spikes/m^2, with 610.7 spikes, as compared with 371.7 for the L11-23 line. The grain yield/ha ranged from 8.7 to 5.9 tons for KSU102 and L11-23, respectively. KSU 102 showed superiority for both grain and biological yield. The results showed clear superiority of the locally developed lines KSU 102, KSU 105, and KSU 106 over the introduced, widely grown Yecora Rojo, indicative of the successful impact of the wheat breeding program. The overall performance revealed the superiority of KSU 102 in terms of yield and its components compared with other tested genotypes. The correlation coefficient among yield components is presented in Table 5. The results revealed a highly significant and positive correlation between grain yield and biological yield (r = 0.83). The same was true for spike length and spike weight (r = 0.93) and between plant height and days to maturity (r = 0.64).

Table 3. Mean square estimates for yield and its component traits.

S O V	d.f.	Plant height	Days to maturity	Spike length (cm)	Spike weight (g)	1000-grain weight (g)	Number of spikes (m^2)	Grain yield (t/ha)	Biological yield (t/ha)
Replication	2	1.6	2.5	0.4	0.04	2.0	802.9*	0.05	0.6
Genotype	9	50.7**	14.3**	29.0**	1.05**	63.4**	20,060.5**	3.64**	61.0**
Error	18	2.8	3.3	0.1	0.014	1.2	192.5	0.03	1.3

S.O.V = source of variation; d.f. = degrees of freedom.

Table 4. Mean performance of the wheat genotypes under field evaluation, Dirab Research station, 2012.

Genotype	Plant height	Days to maturity	Spike length cm	Spike weight g	1000 - grain weight g	Number of spikes m^2	Grain yield - ton/ha	Biological yield t/ha
YECORA ROJO	98.7	133.7	10.3	2.4	33.9	500.3	5.4	16.1
PAVON76	102.7	134.7	9.8	2.1	42.5	524.0	7.6	26.7
VERRY	101.3	131.7	11.3	2.5	43.3	432.0	8.3	26.0
LANG	103.7	135.7	9.4	1.7	29.3	552.0	7.1	26.8
KSU102	95.0	130.0	11.2	2.5	42.2	610.7	8.7	30.0
KSU105	100.7	130.7	14.4	2.5	40.5	543.3	8.6	29.1
KSU106	100.7	130.7	11.1	2.3	37.9	529.3	8.4	27.9
L11-19	89.7	129.3	15.1	3.2	42.2	564.0	7.5	20.3
L11-21	98.0	134.3	17.2	3.6	42.0	370.7	7.2	20.9
L11-23	100.3	132.0	17.9	3.4	42.0	371.7	5.9	22.0
Mean	99.1	132.3	12.8	2.6	39.6	499.8	7.5	24.6

Table 5. Correlation coefficients among the eight studied traits.

	Plant height	Days to maturity	Spike length (cm)	Spike weight (g)	1000-grain weight (g)	Number of spikes (m^2)	Grain yield (t/ha)	Biological yield (t/ha)
Plant height	1.00							
Days to maturity	0.64*	1.00						
Spike length cm	-0.37	-0.27	1.00					
Spike weight g	-0.55	0.-31	0.93**	1.00				
1000-grain weight g	-0.37	-0.51	0.50	0.60	1.00			
Number of spikes, m^2	-0.26	-0.32	-0.60	-0.61	-0.26	1.00		
Grain yield, ton/ha	-0.06	-0.48	-0.19	-0.21	0.39	0.45	1.00	
Biological yield t/ha	0.33	-0.22	-0.32	-0.48	0.12	0.45	0.83**	1.00

Molecular analysis

Results of the SRAP analysis are summarized in Table 6. The 13 primer combinations successfully amplified 954 fragments across the 10 genotypes. The total number of genetic loci analyzed was 312. The number of amplified loci per primer pair combination ranged from

7 for ME9 x EM8 and ME11 x EM5 to 48 for ME9 x EM4. The overall polymorphism rate was 99.67% and ranged from 98% in primer combination ME9 x EM4 to 100% in the remaining primer combinations. The polymorphic information content PIC values ranged from 0.67 for ME11 x EM5 to 0.97 for ME9 x EM4 and ME11 x EM6, respectively. The overall PIC value for the SRAP primers was relatively high at 0.89.

Table 6. Summary of data using SRAP primer combinations on the ten tested genotypes.

Primer combination	Total No. of fragments	Total No. of loci	Polymorphic loci	% Polymorphism	PIC* value
ME9 x EM3	119	38	38	100	0.96
ME9 x EM4	187	48	47	98	0.97
ME9 x EM5	77	26	26	100	0.93
ME9 x EM6	22	5	5	100	0.75
ME9 x EM7	32	12	12	100	0.87
ME9 x EM8	26	7	7	100	0.84
ME11 x EM5	13	7	7	100	0.67
ME11 x EM6	106	45	45	100	0.97
ME11 x EM7	88	30	30	100	0.90
ME11 x EM9	110	40	40	100	0.96
ME12 x EM4	108	28	28	100	0.96
ME12 x EM6	31	11	11	100	0.89
ME12 x EM10	35	15	15	100	0.90
Total	954	312	311		
Average	73.38	24	23.92	99.67	0.89

*PIC = polymorphic information content.

Based on the SRAP banding patterns, a similarity matrix was calculated based on the Jaccard coefficient (Table 7). The closest pair of genotypes was L11-21 and L11-23, with 63% similarity, while the most diverse pair of genotypes was Lang and KSU105. Based on the similarity matrix, a dendrogram, explaining the genetic relationships among the tested genotypes, was constructed (Figure 1). The 10 tested genotypes were clustered into two main groups, A and B, with group A comprising eight genotypes and group B containing two exotic genotypes, Verry and Lang. The A group contained the six Saudi genotypes, along with Yecora Rojo and Pavon76. At 30% similarity level, Saudi genotypes were sub-clustered in a single group. Results showed that wheat genotypes clustering were largely based on genetic background and origin since four out of the six Saudi genotypes share Yecora Rojo as common parent and were all clustered together in group A. KSU102 and KSU106 showed some divergence from other Saudi lines and formed a subgroup at 49% similarity. At 64% similarity, all tested genotypes were in separate subgroups.

Table 7. Similarity coefficients among the genotypes as revealed by SRAP data.

	YR	PAV.76	VERRY	LANG	KSU102	KSU105	KSU106	L11-19	L11-21	L11-23
YR	1.00									
PAV.76	0.29	1.00								
VERRY	0.12	0.11	1.00							
LANG	0.05	0.04	0.20	1.00						
KSU102	0.22	0.35	0.09	0.04	1.00					
KSU105	0.19	0.37	0.08	0.03	0.36	1.00				
KSU106	0.26	0.29	0.11	0.06	0.45	0.37	1.00			
L11-19	0.17	0.34	0.07	0.02	0.37	0.34	0.27	1.00		
L11-21	0.20	0.35	0.08	0.03	0.42	0.40	0.38	0.38	1.00	
L11-23	0.15	0.30	0.04	0.01	0.34	0.39	0.23	0.41	0.63	1.00

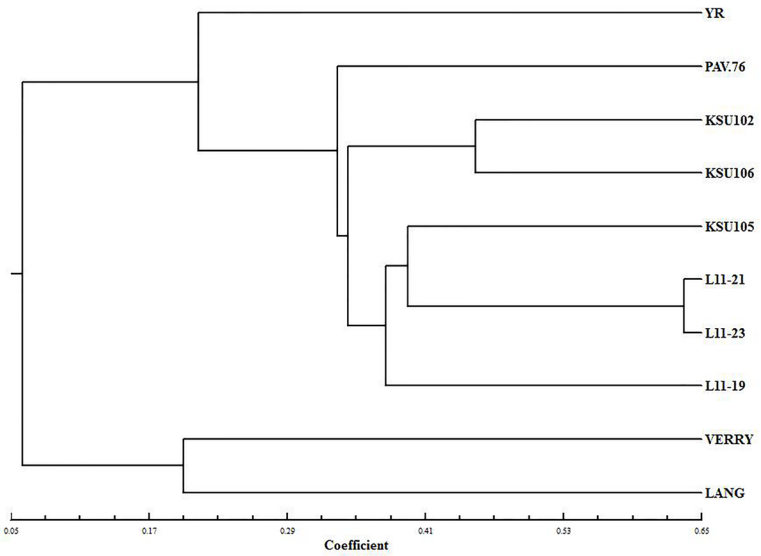

Figure 1. Dendrogram explaining the genetic relationships among 10 wheat genotypes using the un-weighted pair group method with arithmetic mean (UPGMA) method.

DISCUSSION

The field performance of the 10 tested genotypes revealed the presence of significant variations in all studied parameters. The results obtained were consistent with those reported by Houshmandfar and Asli (2011), who observed significant variations among different bread wheat genotypes in field and pot conditions. They concluded that wheat grain yield was driven by yield components, i.e., number of tillers, number of grains per spike, and grain weight. Those yield components together comprise the total build-up of grain yield. KSU 102 proved to have high yield potential for both seed and biological yield (Table 4) compared with the other tested genotypes, including the widely grown Yecora Rojo. This highlights its potential for use as a recommended variety for large scale production in the central region of Saudi Arabia. Yield gaps between demonstration, adjacent, and farmer's fields are large in many spots worldwide (Houshmandfar and Moraghebi, 2010). These yield gap need to be filled in order to enhance the national average and achieve maximum yield potential (Houshmandfar and Asli, 2011). Large scale production of developed lines like KSU 102 would indeed enhance yield potential in the central region of Saudi Arabia. Our results were consistent with those of Yavas and Ünay (2011). Doğan (2010) studied the agronomical performance of CIMMYT/ICARDA genotypes along with standard Turkish genotypes, and also revealed significant variations among the tested genotypes for agronomic and biochemical characteristic. Theses variations were influenced by the genotypes and the growth environment.

The molecular diversity of wheat has been investigated by many researchers. Ejaz et al. (2014) studied two groups of sterile and maintainer male wheat genotypes using AFLP markers, and reported the presence of molecular diversity among studied accessions. They highlighted their usefulness for wheat improvement and hybridization. Furthermore, Ejaz et al. (2015) used the same method to study diversity among 10 cytoplasmic male sterile CMS

lines and their restorers and found similar results, and emphasized the power of molecular markers for detecting genetic variations. Similarly, the results of the present study were somewhat consistent with those reported by Filiz (2012) who screened 30 Russian wheat genotypes collected from CIMMYT (International Maize and Wheat Improvement Center) using SRAP markers. In that study, 23 SRAP primer combinations were used to successfully amplify 686 DNA bands with 90% polymorphism. The author concluded that SRAP markers can be utilized for assessing genetic diversity and have potential use in linkage mapping studies. The implementation of a SRAP marker system may provide accurate, sensitive, and cost effective genetic tool for use in wheat. The SRAP technique can generate co-dominant or dominant markers, which can be used for molecular characterization and marker assisted selection (MAS) in wheat breeding programs. The higher number of genetic loci detected and PIC values for SRAP markers in our study compared to previously published studies resulted from the higher resolution power of capillary based band separation using an ABI system compared with other gel-based conventional electrophoretic methods. An important advantage of the SRAP marker system is the targeting of ORFs (Li and Quiros, 2001), which makes these the markers of choice to trap functional gene linked sequences.

The studied wheat genotypes exhibited significant levels of genetic variation and hence, are of high potential for the development of elite genotypes that are suitable for growth in the central region of Saudi Arabia with better yield potential under arid environments. Furthermore, KSU 102 showed superiority in yield and its attributes compared with those of other genotypes, and may be a potential candidate for large-scale production in the central region of Saudi Arabia. The SRAP marker system proved to have a high discrimination power, particularly when combined with capillary based electrophoresis. These results show that SRAP is an efficient tool for the detection of genetic diversity at the molecular level and for the establishment of MAS in wheat breeding programs.

Conflicts of interest

The authors declare no conflict of interest.

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