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Assessment of Genetic Diversity in Saudi Wheat Genotypes under Heat Stress Using Molecular Markers and Agronomic Traits

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ABSTRACT

The objectives of this study were to compare the application and utility of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) marker techniques for analysis of genetic diversity among Saudi wheat genotypes under heat stress, to compare genetic diversity estimated using molecular markers with agronomic performance under heat stress and to establish the degree of association between these techniques and develop heat tolerance-associated DNA markers. Twelve wheat genotypes were used in this study. They were evaluated phenotypically for heat tolerance and were planted on two sowing dates (20th December and 20th January) over two seasons to expose genotypes to different levels of heat stress during the grain-filling period. The UPGMA dendrogram generated from standardized agronomic data separated the 12 wheat genotypes into three main groups. RAPD and ISSR markers were assayed to determine the genetic diversity of the 12 wheat genotypes. In RAPD analysis, 336 out of 343 bands (98%) were polymorphic while in ISSR analysis, 199 out of 254 bands (75.98%) were polymorphic. The dendrogram based on RAPD markers differed from that based on ISSR markers. The combined dendrogram agreed better with the groups of wheat genotypes based on pedigree analysis. The correlation coefficient between RAPD and ISSR matrix was highly significant (0.53**, $p > 0.001$). Specific RAPD and ISSR markers were developed successfully to identify heat-tolerant 'Ksu106' from heat-sensitive 'Yecora Rojo'. Thus, the markers identified in this study should be applicable for marker-assisted selection for heat tolerance in wheat breeding programs.

Keywords: genetic diversity, heat tolerance, ISSR markers, RAPD markers, *Triticum aestivum* L.

INTRODUCTION

Heat stress due to increased temperature is an agricultural problem in many areas in the world (Wahid *et al.* 2007). Post-anthesis high temperature stress in wheat is a major cause of yield reduction in some regions in Saudi Arabia as well as in many wheat-growing regions of the world. Some attempts to develop heat-tolerant genotypes via conventional plant breeding protocols have been successful (Ehlers and Hall 1998; Camejo *et al.* 2005). Molecular breeding and genetic engineering have provided additional tools to develop crops with improved heat tolerance.

In a breeding program, knowledge of the degree of genetic diversity among parental materials for key selection traits will facilitate the development of high-yielding, stress-tolerant wheat cultivars. Thus, the correct choice of parents employed in the development of a basic population can influence the final result of artificial selection and promote better allocation of financial resources during the whole process of adjusting genotypes to a given environment (Bohn *et al.* 1999). However, to confirm such expectations, it is necessary that the parents combine high means with an increase in variability for the characters under selection.

Molecular and morphological analyses are among the most used tools for the estimation of genetic distances within a group of genotypes. Molecular markers provide an excellent tool for obtaining genetic information and their use in the assessment of genetic diversity in wheat (*Triticum aestivum* L.) has increased over the last few years (Manifesto *et al.* 2001; Corbellini *et al.* 2002; Almanza-Pizón *et al.* 2003; Máric *et al.* 2004; Roy *et al.* 2004; Bolaric *et al.* 2005; Shoaib and Arabi 2006; Chao *et al.* 2007; Jin *et al.* 2008; Wicker *et al.* 2009). Molecular markers are useful complements to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects, and allow cultivar identification early in plant development. Molecular characterization of cultivars is also useful to evaluate potential genetic erosion, defined as a reduction of genetic diversity in time (Manifesto *et al.* 2001). Better understanding of the genetic basis of phenotypic variability will improve the efficiency of wheat improvement to heat tolerance. The objectives of the present study were to: (1) compare the application and utility of RAPD and ISSR marker techniques for analysis of genetic diversity among Saudi wheat genotypes under heat stress, (2) compare the estimated genetic diversity using molecular markers with agronomic performance under heat stress to establish the degree of association between these techniques and (3) develop a heat tolerance-associated DNA marker.

MATERIALS AND METHODS

Field trials and traits evaluation

Twelve wheat genotypes were used in this study. These included the two recommended cultivars ('Yecora Rojo' and 'West Bred 911') as well as 10 advanced lines (F_8) (**Table 1**) selected from the wheat breeding program at the Plant Production Department, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia. They were evaluated phenotypically for heat tolerance using two sowing dates (20th December and 20th January) over two seasons (2006/2007 and 2007/2008) to expose genotypes

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Table 1 Name and origin of the 12 genotypes used in the study.

Genotypes	Pedigree	Origin
Ksu122	Er4/WB-24-1-2	Plant production
Ksu124	YR/E2-94-5-30-16-4	Plant production
Ksu102	YR/E2-94-2-5-30-19-5	Plant production
Ksu103	L9/Er2-31-15-6	Plant production
Ksu128	YR/WB-8	Plant production
Ksu104	L9/Sak69-9	Plant production
Ksu130	YR/E2-94-2-6-43-10-10	Plant production
Ksu107	YR/E2-94-2-6-60-20-11	Plant production
Ksu105	L9/RI474-1-3-161-10-10	Plant production
Yecora Rojo		USA
West Bread		USA
Ksu106	Barouk/RI474-75-3-53-3-3	Plant production

to different levels of heat stress during the grain-filling period. These dates represent heat and stress conditions in Saudi Arabia.

A split-plot design with four replicates was used. The main-plot was planting date and the sub-plot was genotype. Six agronomic traits were scored for the 12 bread wheat genotypes. These were grain yield (GY), biological yield (BY), spike number/m^2 (SN), kernels per spike and 1000-kernels weight (KW). Grain and biological yields were determined from the central rows and converted into grain yield/ha. Harvest index (HI) was calculated as GY/BY.

Molecular analysis

1. DNA extraction

Different young leaves from different plants of each genotype were used for DNA extraction. Frozen leaves (500 mg) were ground to a powder in a mortar with liquid nitrogen. The powder was poured into tubes containing 9.0 ml of warm (65°C) CTAB extraction buffer (Sagahi-Maroof *et al.* 1984). The tubes were incubated at 65°C for 60-90 min. 4.5 ml chloroform: octanol (24: 1) was added and tubes were shaken for 10 min, and centrifuged for 10 min at 3200 rpm. The supernatants were pipetted off into new tubes and 6 ml isopropanol was added. After 60 min, the tubes were centrifuged for 10 min and the pellets obtained were put in sterile Eppendorf tubes, containing 400 µl of TE buffer of a pH 8.0 (10 mM Tris-HCl, pH 8.0 + 1.0 mM EDTA, pH 8.0). The DNA was stored at -20°C until use. The reagents were obtained from Pharmacia Biotech (Amersham Pharmacia Biotech Ltd., UK).

2. RAPD and ISSR analysis

A total of 37 10-mer oligonucleotides with arbitrary sequences were used in RAPD analysis (**Table 2**) and 27 primers based on di-, tri- and tetranucleotide repeats were used in ISSR analysis (**Table 3**), all purchased from Pharmacia Biotech.

The PCR reaction mixture consisted of 20-50 ng genomic DNA, 1× PCR buffer, 2.0 mM $MgCl_2$, 100 µM of each dNTP, 0.1 µM primer and 1 U *Taq* polymerase in a 25 µL volume. The amplification protocol was 94°C for 4 min to pre-denature, followed by 45 cycles of 94°C for 1 min, 36°C (for RAPD analysis) or 50°C (for ISSR analysis) for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplification products were fractionated on a 1% (for RAPD analysis) or 2% (for ISSR analysis) agarose (Agarose 25, BDH Laboratory Supplies, UK) gel.

Statistical analysis

Analysis of variance was performed for all measured traits (agronomic traits) in order to test the significance of variance among genotypes (Steel and Torrie 1980). Data from agronomic traits were standardized and used to estimate the distance matrix, according to Jaccard's coefficient (Jaccard 1908).

RAPD and ISSR data were scored for the presence (1), absence (0) or as a missing observation (Fernandez *et al*. 2002), and each band was regarded as a locus. Two matrices, one for each marker, were generated. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes, was constructed using the algorithm UPGMA (Unweighed Pair Group Method with Arithmetic Average) (Sokal and Michene 1958) through the software NTSYS.pc (Numercial Taxonomy and Multivariate Analysis System, version 1.80 (Applied Biostatics Program; Rohlf 1993).

Comparison between the Jaccard distance matrix based on agronomic traits and genetic distance matrix obtained with molecular markers was performed for the wheat genotypes for which both agronomic traits and molecular markers data were available by calculating the correlation between the two data sets using the Mantel test (1967) in NTSYS-pc.

RESULTS AND DISCUSSION

Morphological analysis

Previously, 'Ksu105' and 'Ksu106' had significantly out-yielded 'Yecora Rojo' on the 20th December (Al-Doss *et al*. 2004). That study also reported 'KSU106' to have the highest yield under heat stress condition (20th January) yielding 5.10 ton/ha over the two seasons and out-yielding 'Yecora Rojo' (3.84 ton/ha) by 33%. Those authors suggested that 'Ksu106' be recognized as a heat-tolerant genotype.

A dendrogram generated from standardized agronomic data is presented in **Fig. 1**. The UPGMA dendrogram separated the 12 wheat genotypes into three main groups, which diverged at a similarity index of 0.28.

The average genetic similarity among the 12 wheat genotypes was 0.36, with values ranging from 0.15 to 0.56. 'Ksu104' and 'West Bred 911' showed a very high degree of similarity (0.56) indicating that these two genotypes had similar agronomic traits under heat stress. On the other hand, 'Yecora Rojo' and 'Ksu103' showed a very low degree of similarity (0.15) indicating that these are not closely related genotypes and had different agronomic traits under heat stress. Although 'Ksu107' and 'Ksu124' are closely related genotypes (both originated from the cross YR X E2; **Table 1**), they had the lowest similarity (0.15).

Molecular markers analysis

1. Identification and evaluation of RAPD and ISSR markers for diversity estimates

Thirty seven RAPD primers were screened for their ability to amplify the genomic DNA from 12 wheat genotypes. The number of amplified DNA fragments ranged from 0.0 to 20.0 depending on the primer and the DNA sample with a mean value of 9.3 bands per primer (**Table 2**). These values are rather high for RAPD amplification, compared to the average numbers of amplified bands recorded in other crops; namely, three fragments in *Triticum turgidum* L. (Joshi and Nguyen 1993), 4.3 fragments in *Solanum tuberosum* L. (Masuelli *et al*. 1995), 6.7 in *Zea mays* L. (Heun and Helentjaris 1993) and 4.4 in barley (Hou *et al*. 2005). In the present investigation, the size of fragments ranged from 150 to 1500 bp. A total of 343 fragments were produced by the 37 primers. Of these 343 amplified fragments, 2.0% were not polymorphic while 98.0% were polymorphic among the 12 genotypes. Primer OPJ10 generated the greatest polymorphism (100%), while the lowest level of polymorphism (11.1%) was obtained by primer OPH 13. Out of the 37 primers, 24 revealed more than 80% polymorphism (**Table 2**). **Fig. 2** shows the amplification profiles, generated by primer OPC15 (5′-GACGGATCAG-3′) across the 12 wheat genotypes, all of which had distinguishable banding patterns. Polymorphism between genotypes can arise through nucleotide changes that prevent amplification by introducing a mismatch at one priming site; deletion of a priming site; insertions that render priming sites too distant to support amplification; and insertions or deletions that change the size of the amplified product (Williams *et al*. 1990). RAPD markers have been used to detect the genetic diversity of some South Tunisian barley races (Guasmi *et al.* 2009).

Table 2 Number of amplifications and polymorphic products of 37 RAPD primers.

Primer	Nucleotide sequence (5'→3')	No. of amplification products	No. of polymorphic products	Polymorphism (%)
Pr_1	CAGGCCCTTC	9	5	55.6
Pr_2	TGCCGAGCTG	7	0	00.0
Pr_3	AGTCAGCCAC	7	7	100.0
Pr_4	AATCGGGCTG	7	4	57.1
Pr_5	AGGGGTCTTG	20	18	90.0
Pr_6	GGTCCCTGAC	5	4	80.0
Pr_7	GAAACGGGTG	11	8	72.7
Pr_8	GTGACGTAGG	12	11	91.7
Pr_9	GGGTAACGCC	10	7	70.0
Pr_{10}	GTGATCGCAG	12	11	91.7
Pr_{11}	CAATCGCCGT	12	10	83.3
Pr_{12}	TCGGCGATAG	12	10	83.3
Pr_{13}	CAGCACCCAC	8	3	37.5
Pr_{14}	TCTGTGCTGG	8	8	100 .0
Pr_{15}	TTCCGAACCC	11	9	81.8
Pr_{16}	AGCCAGCGAA	18	17	94.4
Pr_{17}	GACCGCTTGT	9	9	100.0
Pr_{18}	AGGTGACCGT	5	1	20.0
Pr_{19}	CAAACGTCGG	10	5	50.0
Pr_{20}	GTTGCGATCC	12	12	100.0
UBC321	ATCTAGGGAC	0	0	00.0
UBC475	CCAGCGTATT	0	0	00.0
UBC532	TTGAGACAGC	0	0	00.0
OPA02	TGCCGAGCTG	8	7	87.5
OPA06	GGTCCCTGAC	0	0	00.0
OPA07	GAAACGGGTG	8	7	87.5
OPB09	TGGGGGACTC	6	6	100.0
OPB13	TTCCCCCGCT	7	6	85.7
OPC04	CCGCATCTAC	20	18	90.0
OPC15	GACGGATCAG	14	14	100.0
OPE20	AACGGTGACC	8	7	87.5
OPF15	CCAGTACTCC	11	11	100 .0
OPH13	GACGCCACAC	9	1	11.1
OPJ04	CCGAACACGG	9	9	100.0
OPJ10	AAGCCCGAGG	14	14	100.0
OPU06	ACCTTTGCGG	16	14	87.5
OPZ03	CAGCACCGCA	8	8	100.0

Table 3 Number of amplifications and polymorphic products of 27 ISSR primers.

Primer	Nucleotide sequence (5'→3')	No. of amplification products	No. of polymorphic products	Polymorphism (%)
AD1	(GA)9C	8	6	75.0
AD2	(AGC)6G	7	4	57.1
AD3	(ACC)6G	8	8	100
AD5	(CA)10C	6	5	83.3
AD6	GT(CAC)7	12	7	58.3
AD9	(AC)9G	6	5	83.3
M-1	(AC)8CG	11	10	91.0
M-2	(AC)8CC-T	7	2	28.5
M-6	(CAC)5	6	4	66.6
M-7	(CAG)5	5	3	60.0
M-8	(GTG)5	7	6	85.7
M-11	(CA)6A/G	6	4	66.6
M-12	(CA)6RY	5	5	100
M-13	(AGC)3Y	9	5	55.5
ISSR-1	(GA)8T	13	9	69.2
ISSR-3	(CT)8A	12	9	75.0
ISSR-4	(CT)8G	11	9	81.8
M-11	(CA)6A/G	6	4	66.6
ISSR-5	(TC)8A	8	5	62.5
ISSR-8	(GA)8YT	11	9	81.8
ISSR-11	(GGGGT)3G	15	11	73.3
ISSR-808	A(GA)7GC	7	7	100
ISSR-811	G(AG)7AC	15	12	80.0
ISSR-816	C(AC)7AT	17	16	94.1
ISSR-817	C(AC)7AA	17	13	76.4
ISSR-821	G(TG)7TT	11	9	81.8
ISSR-827	A(CA)7CG	8	7	87.5

Forty-five ISSR primers were used to amplify DNA segments from 12 wheat genotypes. The number of amplified bands per primer varied between 0 and 17. 27 primers out of 45 were selected for further analysis based on the intensity, size and number of amplified products (**Table 3**). A total of 254 bands were observed, with 9.41 bands per

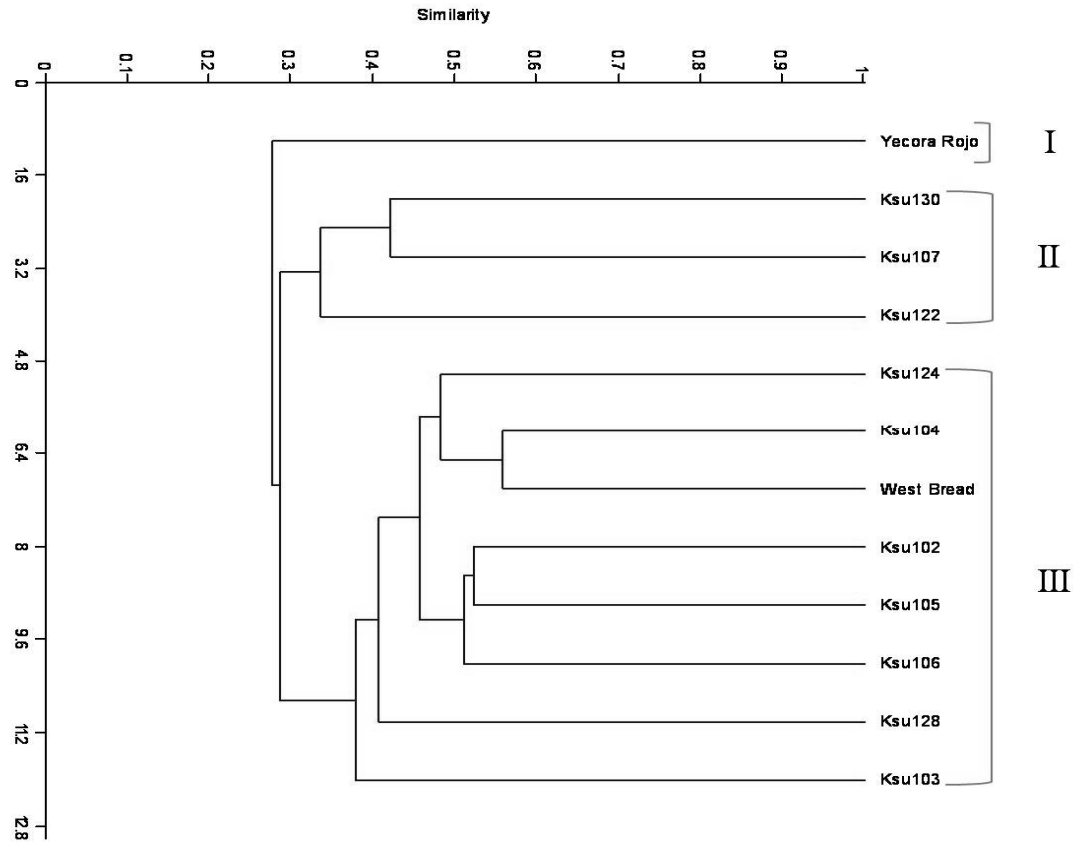

Fig. 1 Dendrogram based on Jaccard Similarity Coefficient of 12 wheat genotypes, generated by six agronomic traits over tow seasons under heat stress condition.

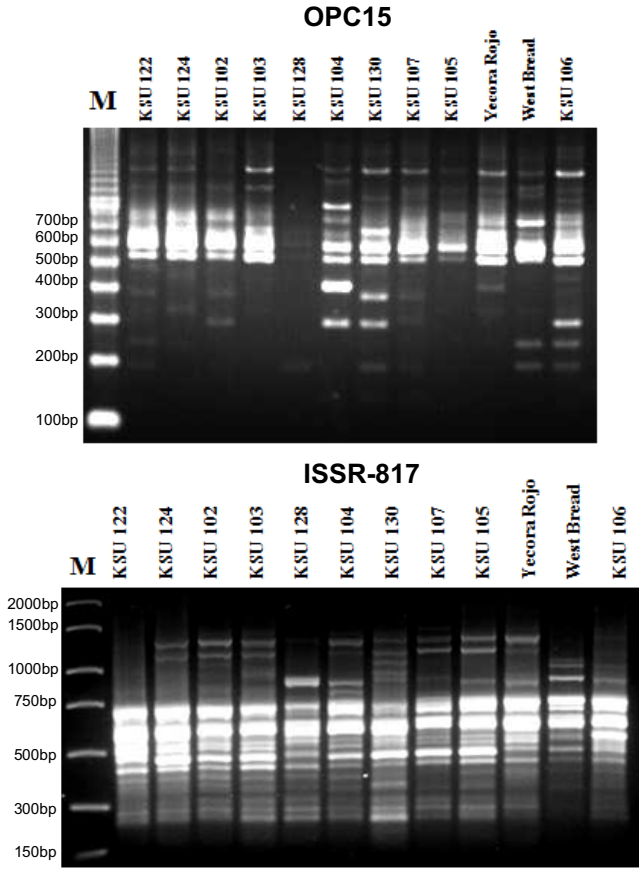

Fig. 2 Polymorphism revealed using RAPD primer OPC15 (5'-GACGGATCAG-3') and ISSR primer ISSR-817 (5'-C (AC) 7AA-3') to amplify genomic DNA purified from wheat genotypes. M: Molecular weight, followed by wheat genotypes.

primer (**Table 3**). 199 out of 254 bands (75.98%) were polymorphic. The number of amplification bands per primer varied between 5 and 17. An example of polymorphism is shown in **Fig. 2**. The dinucleotide repeats $(AC)_n$ primer had more average number of bands than $(GA)_n$ $(CA)_n$ $(CA)_n$ and $(CT)_n$ primers and trinucleotide repeats primers (**Table 3**). The dinucleotide repeats primer generated a maximum of bands (12) with 6 polymorphic ISSR markers in wheat (El Maati *et al*. 2004). On the other hand, tetranucleotide repeats did not amplify DNA of wheat lines. This might indicate that di- and trinucleotide-based ISSR-PCR markers could provide potential markers for wheat genome mapping.

2. Genetic diversity of molecular markers

The relationships among wheat genotypes were estimated by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters obtained using RAPD markers alone (**Fig. 3**), ISSR markers alone (**Fig. 4**), and using both RAPD and ISSR markers together (**Fig. 5**) revealed similar groupings in some cases.

Cluster analysis using RAPD data grouped the 12 wheat genotypes into three main clusters with Jaccard's similarity coefficient ranging from 0.45 to 0.75 (**Fig. 3**). The highest similarity was found between 'Ksu122' and 'Ksu124' (0.75) and the lowest was between 'Ksu130' and 'Ksu122' (0.45). Although 'Ksu124' and 'Ksu130' were more closely related with each other and derived from the cross YR X E2 (**Table 1**), they were different at the DNA level. Comparison of the agronomic relationships of wheat genotypes with RAPD clustering showed that the heat-tolerant genotypes 'Ksu105' and 'Ksu106' in **Fig. 1** were located in the same cluster in the RAPD dendrogram in **Fig. 3**.

The dendrogram generated from ISSR data clearly indicated three main clusters (**Fig. 4**). The Jaccard similarity coefficient ranged from 0.54 to 0.76. Maximum similarity was found between 'Ksu105' and 'Ksu107'. The first cluster included 'Ksu 106', which is heat tolerant while the second cluster included the commercial cultivar 'Yecora Rojo',

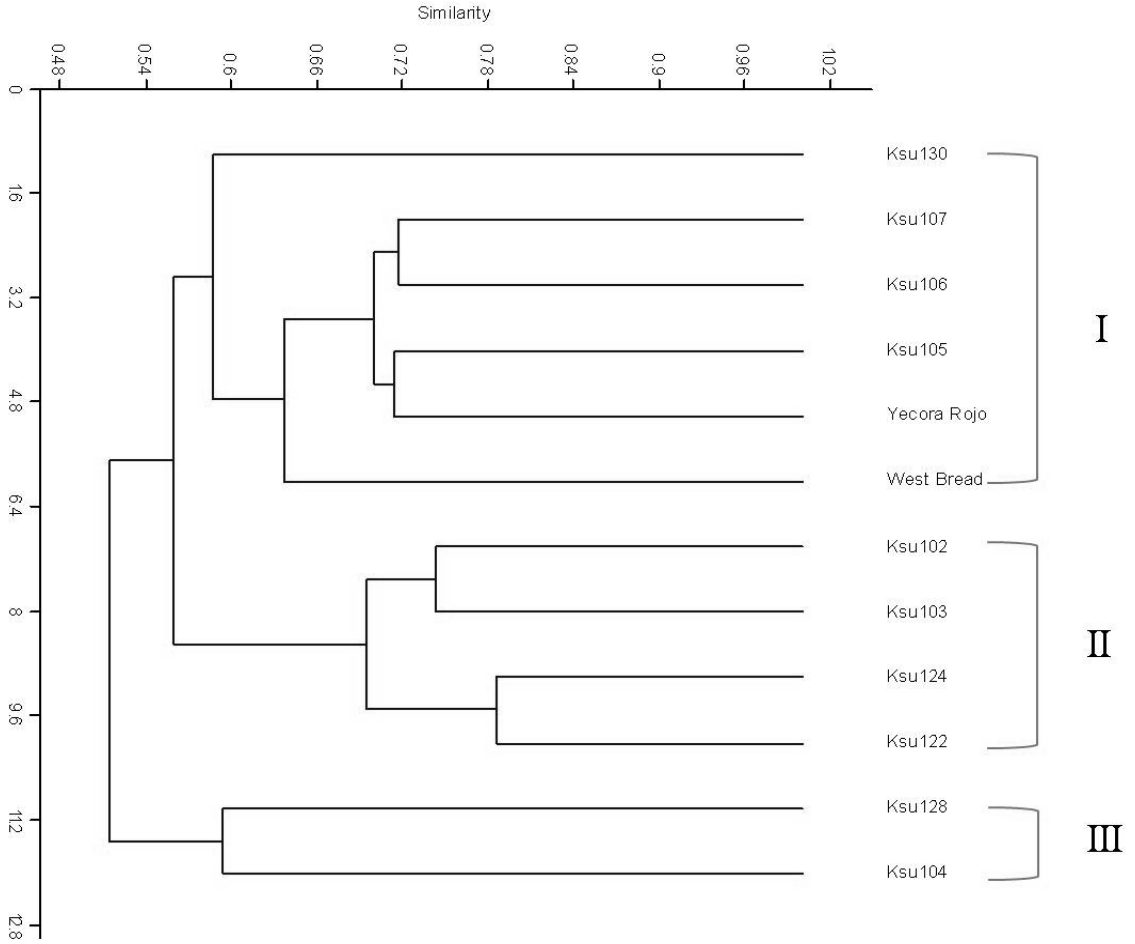

Fig. 3 Dendrogram based on Jaccard's Similarity Coefficient of 12 wheat genotypes generated using RAPD markers.

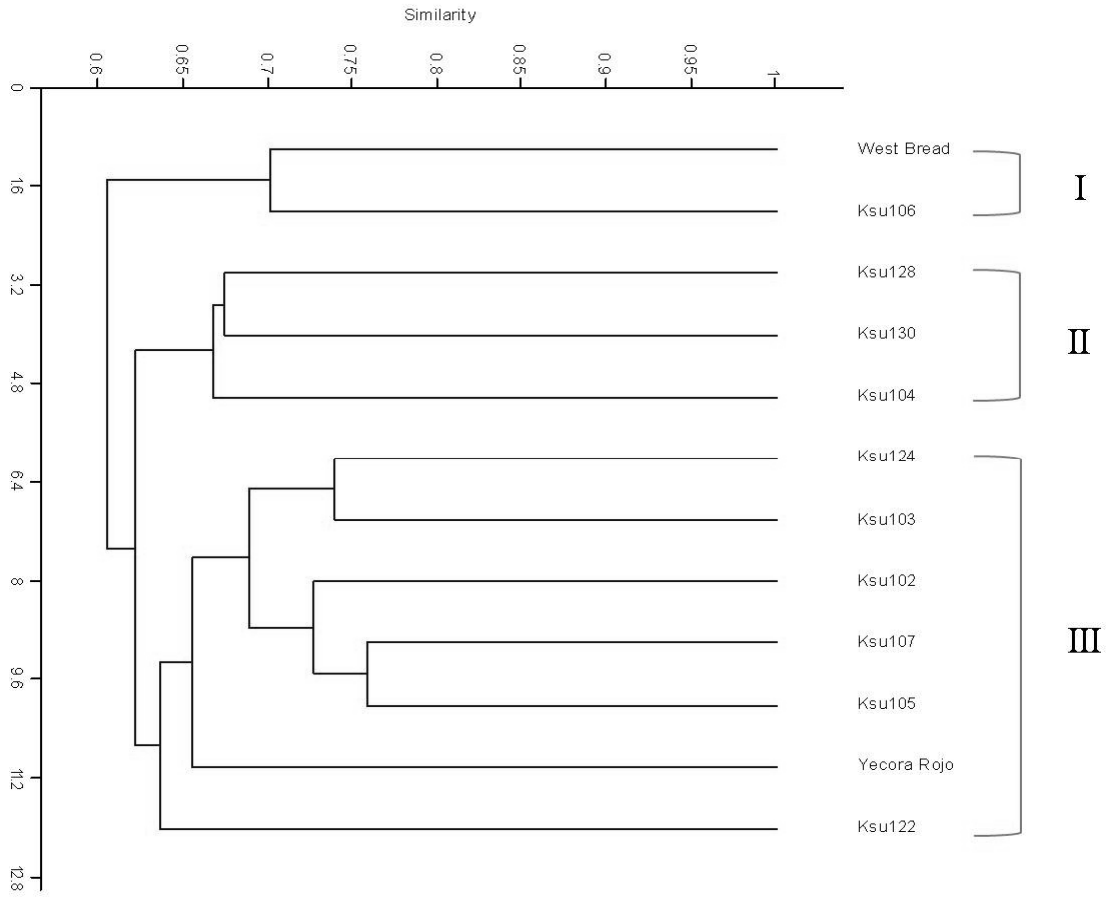

Fig. 4 Dendrogram based on Jaccard's Similarity Coefficient of 12 wheat genotypes generated using ISSR markers.

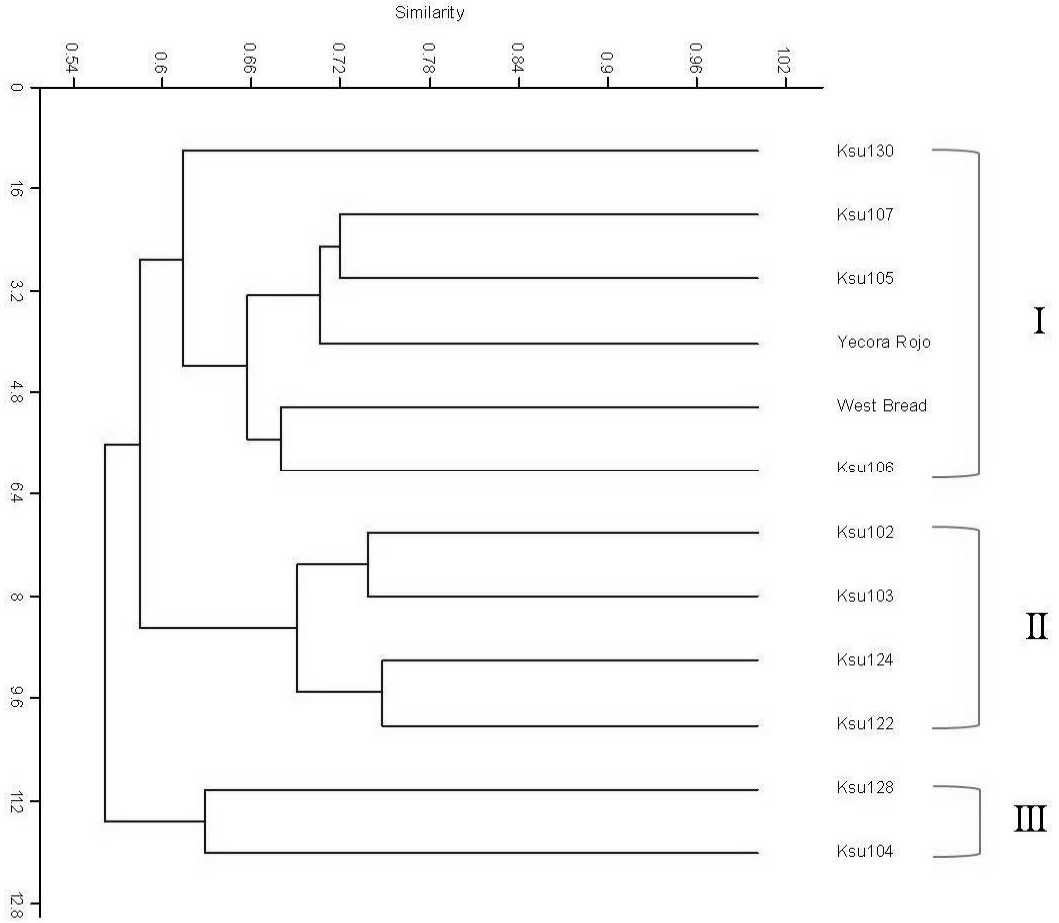

Fig. 5 Dendrogram constructed from similarity coefficients showing the clustering of 12 wheat genotypes using RAPD and ISSR markers.

which is heat sensitive. Previously, ISSR was successfully used to estimate the genetic diversity in wheat (Ben El Maati *et al.* 2004; Motawei *et al*. 2007), rice (Joshi *et al.* 2000; Qian *et al.* 2001), barley (Fernandez *et al.* 2002; Hou *et al.* 2005) and maize (Ye *et al*. 2005).

RAPD and ISSR data were combined to produce a dendrogram, according to the Mantel test (1967). The similarity coefficient among the wheat genotypes varied from 0.51 to 0.75 with the highest being between 'Ksu124' and 'Ksu122' and the lowest being between 'Ksu130' and 'Ksu122', also as revealed by RAPD analysis only. Cluster analysis revealed three main clusters (**Fig. 5**). 'Ksu105' and 'Ksu106' were the most heat tolerant in the first cluster (Al-Doss *et al.* 2004). These wheat genotypes had one parent in common (IR474) and they also clustered together. The combined dendrogram agrees better with the groupings of the wheat genotypes depending on their pedigree and the dendrogram generated by RAPD data alone than the dendrogram generated by ISSR data alone. RAPD markers have shown to be associated with various traits contributing to kernel hardness in bread wheat (Galande *et al.* 2001). RAPD and ISSR markers have proved to be the most popular polymorphic markers in wheat and hence are highly useful markers for various applications in wheat (Nagaoka *et al.* 1997).

3. Correlation between agronomic traits and molecular markers

In order to compare the extent of agreement among dendrograms, derived from morphological and molecular markers, a distance matrix was constructed for each assay and compared, using the Mantel matrix correspondence test. Comparison of matrices of either RAPD or ISSR and morphological data showed a low correlation among dendrograms (r = 0.14 and 0.05, respectively). Despite this low correlation between morphological and molecular analysis, there were similar genotypes formed in the respective dendrograms. Three clusters were consistently formed in the three analyses, although some discrepancies between the molecular and morphological dendrograms could be found. For example, 'Yecora Rojo' was clearly clustered separately into one group alone in the morphological analysis, while, in RAPD analysis, the same genotype was clustered into a larger cluster. Another discrepancy concerned 'Weast Bred 911', which fell into the third cluster in the morphological dendrogram, but, in the ISSR analysis, it was clustered into the first cluster. Several reasons might be responsible for these discrepancies among results, based on morphology and molecular markers. Although some RAPD markers might be associated with functionally important loci (Penner 1996), most might be amplified from non-coding regions of the genome (Williams *et al.* 1990). Morphological traits are, generally, believed to be under the pressure of natural selection, and their expression is partially under the influence of environmental factors. In contrast to morphological traits, molecular variation is directly based on DNA sequence variation. A single nucleotide change can result in a change of the molecular phenotype. Beside, these differences of morphological and molecular markers, different combinations of alleles might result in morphological similarities or differences that are not proportional to the underlying genetic differences. Semagn (2002) suggested two reasons for the low correlation between DNA and morphological markers: (a) DNA markers cover a larger proportion of the genome, including coding and non-coding regions, than the morphological markers, and (b) DNA markers are less subjected to artificial selection compared with morphological markers.

A low correlation between RAPD and morphological traits had been reported in other studies in European barley varieties (Schut *et al.* 1997), in rye grass varieties (Roldan-Ruiz *et al.* 2001) and synthetic hexaploid wheat and their parents (Lage *et al.* 2003). Schut *et al.* (1997) reported no significant correlation between molecular markers and 25

morphological traits in barley. Working with 16 rye grass varieties, Roldan-Ruiz *et al.* (2001) reported a correlation value of r = -0.06 between molecular markers and 15 morphological characters. Similar results were found in wheat by Lage *et al.* (2003), who detected differences between dendrograms generated from morphological traits and AFLP markers and they suggested that morphological and molecular differences were apparently independent, due to different selection and evolutionary factors. Although AFLP markers can cover a high proportion of the genome because of the high number of bands scored in each analysis, due to its neutral origin, there is no guarantee that such bands fall in coding regions of the genome involved in morphological traits (Roldan-Ruiz *et al.* 2001).

The correlation coefficient between the RAPD and ISSR matrix was highly significant (0.53**, p > 0.001). In addition, both RAPD and ISSR matrices showed a significantly positive correlation (r = 0.94** and r = 0.78**, respectively) with the RAPD+ISSR matrix.

Development of heat tolerance-associated DNA markers

In the present investigation, RAPD and ISSR analysis were undertaken with the aim of developing a heat tolerance-associated DNA marker. For the RAPD analysis presented here some wheat genotypes reported to be heat tolerant/sensitive (on the basis of field performance) were used. **Fig. 6** and **Table 4** indicated that DNA bands at about 1300 and 310 bp were present in heat-tolerant 'Ksu106' but not in heat-sensitive 'Yecora Rojo' when primer OPE20 was used. On the other hand, specific DNA bands at 1400 and 1200 bp were present in 'Yecora Rojo' but not in 'Ksu106' when the same primer was used. Moreover, specific DNA bands generated from RAPD primers (**Table 4**) could be used to characterize between 'Ksu106' and 'Yecora Rojo'. In the ISSR analysis, polymorphic DNA fragments of 690, 500, 470 and 360 bp were identified in 'Ksu106' but were absent in 'Yecora Rojo' when primer ISSR-811 was used (**Fig. 6; Table 4**). These fragments appeared to be linked to heat tolerance genes. Previously, specific DNA markers associated with drought tolerance genes in tea plants had been reported (Mishra *et al.* 2004). Recently, specific markers linked to heat tolerance alleles in wheat have been detected by RAPD and ISSR analysis (Motawei *et al.* 2007). On the other hand, specific DNA bands at 1100 and 290 bp were present in 'Yecora Rojo' but not in 'Ksu106'. In addition, specific DNA bands generated from ISSR primers (**Table 4**) could be used to characterize between 'Ksu106' and 'Yecora Rojo'.

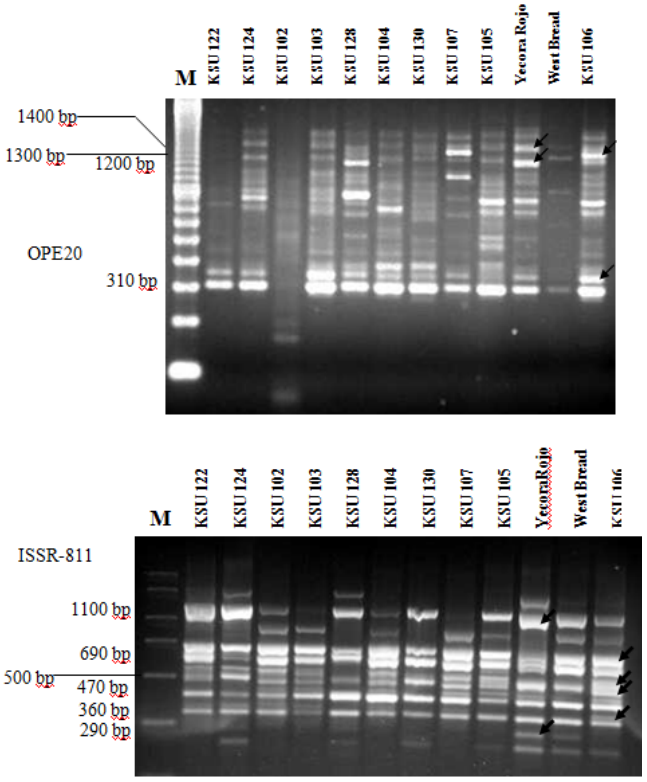

Fig. 6 Polymorphic DNA fragments linked to heat tolerance genes, generated by RAPD primer OPE20 (5'-AACGGTGACC-3') and generated by ISSR primer ISSR-811 (5'-G (AG) 7AC-3'). M: Molecular weight, followed by wheat genotypes.

Molecular marker technology has allowed the identification and genetic characterization of quantitative trait loci (QTLs) with significant effects on stress tolerance during different stages of plant development and facilitated determination of genetic relationships among tolerance to different stresses (Foolad 2005). Comparatively, however, limited research has been conducted to identify genetic markers associated with heat tolerance in different plant species.

CONCLUSIONS

The 12 characterized wheat genotypes were mainly classified according to agronomic traits under heat stress condi-

Table 4 Specific DNA fragments generated from RAPD and ISSR analysis to develop heat tolerance–associated DNA markers between Yecora Rojo and Ksu 106.

RAPD				ISSR			
Primer	**Marker (bp)**	**Yocora Rojo**	**KSU106**	**Primer**	**Marker (bp)**	**Yocora Rojo**	**KSU106**
Pr-7	550	+	-	AD6	2000	+	-
	490	+	-	ISSR-1	840	+	-
	330	+	-		700	+	-
Pr-29	650	-	+	ISSR-3	740	+	-
OPC15	650	+	-	ISSR-811	1100	+	-
	290	-	+		690	-	+
	230	-	+		500	-	+
	190	-	+		470	-	+
OPE20	1400	+	-		360	-	+
	1300	-	+		290	+	-
	1200	+	-	ISSR-816	1400	+	-
	310	-	+		950	-	+
OPF15	1150	+	-		750	-	+
	600	-	+		600	+	-
	400	-	+	ISSR-817	1350	+	-
Opu06	900	+	-		570	-	+
OPZ03	1450	+	-				
	510	-	+				
	410	-	+				

tions which are complex and multigenic characters. Such characters are environmentally affected and, therefore, liable to subjective evaluation. In this sense, molecular characterization was more efficient in generating an unbiased picture of diversity than an agronomic approach. However, agronomic characterization is still important in wheat germplasm management, and determination of molecular diversity should not be seen as replacing traditional characterization, but rather as a complement to it.

In conclusion, these results indicate that both morphological analysis and molecular markers showed a high degree of variation among the 12 analyzed wheat genotypes. These genotypes represent an important source of genetic diversity in Saudi wheat, and could be used in future breeding programs. Although the correlation between morphological and molecular data was low, both techniques could be complementary used in wheat characterization. These distinct genotypes can be crossed to produce mapping population for detection of QTLs against various important agronomical traits. A future study will be conducted to develop wheat cultivars that are highly tolerant to heat stress in addition to other desirable wheat characters. Production of these wheat lines can minimize yield losses due to growing heat-sensitive wheat cultivars under stress condition. This consequently can lead to maximize farm income for wheat growers.

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