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Marker assisted screening of wheat (*Triticum aestivum* L.) cultivars for drought tolerance and yield improvement

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Abstract

Wheat being cultivated in diverse types of environmental conditions is facing different physiological stresses like drought that result in low yield, food scarcity and economic loss. The problem can be minimized by careful selection and identification of drought resistant varieties and their further breeding. The present study was conducted having fifty two wheat varieties based on ten parameters of morphology along with molecular analysis using nine SSR markers to analyze their potential against drought. Variation in qualitative and quantitative traits including number of Peduncle length, Spike length, Plant height, Days to 50% heading, Biological yield, Flag leaf area, Yield per plant, 1000 grains weight, Number of spikelet's per spike, and HI were recorded. Morphological data were subjected to analysis of variance (ANOVA), correlation and cluster analysis. ANOVA showed that all the parameters were significant. There was also significant correlation among the parameters. The highest values of mentioned traits were observed in genotype 010810(42cm), 010797(13cm), 010791(118cm), 010808(158), Sahar(19.7gm), ZAM(70.3), Sahar(12.7gm), Sahar(59gm), 010810(67) and 010817 (84.94), respectively. Dendrogram clustered all the genotypes into six groups. Nine molecular markers used for screening of drought tolerant genotypes, amplified maximum number of drought tolerant genes in genotypes 010817, 010771, 010803, 010726, 010810, Sahar, 010772, 010732, PS-2004, 010727, 010718, 010725, Siran-2010, 010786 and ARE-10. Sahar, 010810, 011786 and 010817 genotypes showed best performance both in morphological and molecular screening and are therefore, recommended for utilization in future wheat breeding programs for rain fed areas of Pakistan.

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Introduction

Wheat is the most widely cultivated cereal crop, and is used as staple food for more than one third of the world's population (Asif *et al.,* 2005). It is one of the largest cereals with annual global production at more than 651.4 million metric tons (Anonymous, 2012). Water stress changes the plant growth and development. Depressed water potential effect cell division, growth of organ, photosynthesis and change hormonal balances of major plant tissues (Gusta and Chen, 1987). Wheat (*Triticum aestivum* L.) is commonly grown in drought-prone environments where grain yields are limited by low seasonal rainfall. Drought stress remains an ever-growing problem that severely limits crop production worldwide and causes important agricultural losses particularly in arid and semi-arid areas (Boyer, 1982).

The percentage of drought affected land areas has been doubled from the 1970s to the early 2000s in the world (Isendahl and Schmidt, 2006). Drought affects more than 99 million hectare area across the developing nations and more than 60 million hectares across the developed parts of the world (Rajaram, 2000). About 15 million hectares of land In Pakistan is affected by the stress caused by the short supply of water (Mujtaba and Alam, 2002). It has been proved that the crop losses due to water shortage are greater than any other type of biotic or abiotic stress globally (Kramer, 1983).

Wheat yield can be increased through the development of improved cultivars with wider genetic base capable of producing better yield under various agro-climatic conditions and stresses (Zhu *et al.,* 2000). The genetic variability can be either studied with morphological markers or isozyme markers but due to some limitations these markers are not used now a day due to their limitations (Andersen and Lubberstedt, 2003; Brown, 1979). An invaluable alternative offered DNA-based markers, such as restriction fragment length polymorphism (RFLPs) (Glaubitz *et al.,* 2000), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995), simple sequence repeats (SSRs) (Zietkiewicz *et al*., 1994), single nucleotide polymorphisms (SNPs) (Rafalski, 2002). These basic biotechnology tools can be effectively employed to manage stress tolerance and hence improving yield stability, whereas different genetic markers are identified as linked to different traits of interest (Iqbal *et al.,* 2012). This study was aimed at exploitation of SSR markers linked with drought tolerance as genetic markers are important determinants for the study of polymorphism. Our main focus was on DNA fingerprinting of different wheat genotypes cultivated in Pakistan on the basis of polymorphism. SSR analyses were complemented with morphological analysis linked drought stress as well. This research will improve wheat crop production to meet with the shortage situation of this valuable crop of Pakistan.

Materials and Methods

Plant sample

The present research work was carried out under field conditions of Mansehra during 2013-2014. Fifty two genotypes comprising of germplasm and varieties of common wheat (*Triticum aestivum* L) collected from PGRI and NARC Islamabad for morphological and molecular study.

Morphological studies

Morphological studies were carried out in the Hazara University (Garden campus) research field. Fifty two wheat germplasm were planted in Randomized Complete Block Design (RCBD) with three replications. The data were collected on 03 randomly selected plants in each row for different morphological parameters *viz*., Peduncle length, Spike length, Plant height, Days to 50% Heading, Biological yield, Flag leaf area, Yield per plant, 1000 grain weight, No of spikelets per spike and Harvest index (HI).

DNA isolation and PCR amplification

DNA was isolated from selected plant materials using Weining and Langridge (1992) protocol with some minor modifications. Polymerase Chain Reaction (PCR) was carried out using the protocols of Roder *et al.* (1998) with a little modification. The thermocyclic

conditions for PCR reaction were as follows: Initial denaturation step of 94C° for 4min followed by 35 cycles of 94C° for 1min for denaturation, 50 to 61C° for 1min for annealing and 72C° for 2 min extension and final extension of 72C° for 10 min was given. All DNA samples were amplified using 9 SSR markers viz. Wmc-27, Wmc-94, Wmc-104, Wmc-105, Wmc-147, Wmc-154, Wmc-169, Wmc-173 and Wmc-213. The PCR product was run in 2% agarose gel and visualized under UV light in gel documentation system for checking the required DNA fragment.

Statistical analysis

Data obtained for morphological analysis were subjected to analysis of variance (ANOVA) and correlation analysis using statistical software SPSS ver. 16. Bivariate data were subjected to cluster analysis for computing a dendrogram.

Results

Comparative performance for morphological traits

In the present study the comparative performance of wheat (*Triticum aestivum* L.) morphological traits was done.

Table 1a. Comparative Performance of Ten Superior Genotypes on the Basis of Morphological Characterization.

Varieties	Peduncle Length (cm)	Varieties	Spike Length (cm)	Varieties	Plant Height (cm)	Varieties	50% Heading (Days)	Varieties	Biological Yield (gm)
10810	42	10771	13	10791	118	10808	158	Sahar	19.7
10786	42	10797	13	10772	109	10791	158	PS-05	19.1
10808	41	10727	12	10810	106	10797	157	11786	18.9
ZAM	41	10772	11	10797	105	10771	156	10791	18.2
10791	41	10718	11	10808	101	10772	156	10755	18.1
10772	39	10801	11	10805	99	10810	156	10743	17.6
10727	38	10808	11	10786	97	10803	155	10740	16.3
10799	37	10755	11	10799	95	10801	155	10797	15.4
10771	36	ARE-10	11	10804	92	10804	155	10727	15.2
Hashim	35	10817	10	Gomal	90	10719	152	10732	15.1

Peduncle Length

The analysis of variance at (P≤0.01) level confirmed that peduncle length was highly significant (Table 2). The correlation analysis showed that peduncle length was positively correlated to flag leaf area, plant height, spike length, 50% heading, No of spikelets per spike, 1000 grain weight and harvest index (HI) while was found negatively correlated to biological yield and yield per plant as shown in (Table 3).

Spike length

The analysis of variance (P≤0.01) showed that spike length was highly significant (Table 2).

Table 2. Analysis of Variance for Yield Associated Parameters of Wheat (*Triticum aestivum* L.*)*.

ANOVA						
		Sum of Squares	Df	Mean Square	F	Sig.
Replication	Between Groups	0.000	51	0.000	0.000	1.000
Peduncle Length	Between Groups	1462.077	51	28.668	15.972	.000
Spike Length	Between Groups	230.769	51	4.525	4.525	.000
Total Plant Height	Between Groups	31592.481	51	619.460	619.460	.000
fifty percent Heading	Between Groups	3704.019	51	72.628	72.628	.000
Biological Yield	Between Groups	1312.876	51	25.743	25.743	.000
Leaf Flag Area	Between Groups	13780.029	51	270.197	270.197	.000
No of Grains per Plant	Between Groups	15269.308	51	299.398	299.398	.000
thousand Grain Weight	Between Groups	6990.173	51	137.062	137.062	.000
No of Spikelets per Spike	Between Groups	1088.308	51	21.339	21.339	.000
Harvest Index	Between Groups	3841.335	51	75.320	75.320	.000

The correlation analysis also showed that spike length was positively correlated to peduncle length, flag leaf area, plant height, spike length, Days to 50% heading, No of spikelets per plant, 1000 grain weight, biological yield, yield per plant and harvest index (HI) and was not found negatively correlated to any one of the observed morphological traits (Table 3).

Table 3. Statistical Analysis on the Base of Correlation of Ten Yield Associated Morphological Traits.

Correlations										
	Peduncle Length	Spike Length	Plant Height	Days to 50% Heading	Biological Yield	Flag Leaf Area	Yield per Plant	1000 Grain Weight	No of Spikelets per Spike	Harvest Index
Peduncle Length	1									
Spike Length	.291*	1								
Plant Height	.583**	.176	1							
Days to 50% Heading	.407**	.383**	.497**	1						
Biological Yield	-.013	.199	.168	.027	1					
Flag leaf Area	.071	.081	-.053	-.230	.359**	1				
Yield per Plant	-.121	.146	-.100	-.025	.495**	.247	1			
1000 Grain Weight	.072	.193	.036	-.223	.366**	.162	.119	1		
No of Spikelets per Spike	.116	.178	-.002	.236	.144	-.099	.303*	.249	1	
Harvest Index	.130	.133	.281*	.074	.630**	.117	-.346*	.326*	-.066	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Plant height

The Analysis of variance (P≤0.01) showed that plant height was highly significant (Table 2).The correlation analysis was also showed that plant height was positively correlated to peduncle length, spike length, days to 50% heading, biological yield, 1000 grain weight and harvest index (HI) and was found negatively correlated to flag leaf area, No of spikelets per spike and yield per plant (Table 3).

Days to 50% heading

The Analysis of variance (P≤0.01) showed that Days to 50% heading was highly significant (Table 2).The correlation analysis showed that days to 50% heading was found positively correlated to peduncle length, spike length, plant height, biological yield, No of spikelets per spike and harvest index (HI) and was found negatively correlated to flag leaf area, yield per plant and 1000 grain weight (Table 3).

Biological yield

The Analysis of variance (P≤0.01) showed that biological yield was highly significant (Table 2).The correlation analysis also showed that biological yield was found positively correlated to peduncle length, spike length, plant height, 50% heading, flag leaf area, yield per plant, 1000 grain weight, no of spikelets per spike and harvest index and was not found negatively correlated to any one of the morphological trait (Table 3).

Flag leaf area

The Analysis of variance (P≤0.01) showed that Flag leaf area was highly significant (Table 2).The correlation analysis also suggest that flag leaf area was found positively correlated to peduncle length, spike length, biological yield, yield per plant, 1000 grain weight, No of spike lets per spike and harvest index (HI) while was found negatively correlated to plant height and days to 50% heading (Table 3).

Yield per plant

The Analysis of variance (P≤0.01) showed that Yield per plant was highly significant (Table 2).The correlation analysis also showed that yield per plant was found positively correlated to spike per plant, biological yield, flag leaf area, 1000 grain weight and spikelets per plant and was found negatively correlated to peduncle length, plant height, days to 50% heading and harvest index (HI) (Table 3).

Thousand grain weight

The Analysis of variance (P≤0.01) showed that Thousand grain weight was highly significant (Table 2).The correlation analysis also showed that Thousand grain weight was found positively correlated to peduncle length, spike length, plant height, biological yield, flag leaf area, yield per plant, No of spikelets per spike and harvest index (HI) while was found negatively correlated to days to 50% heading (Table 3).

Table 4. Molecular Marker Banding Pattern (+ for Presences and – for Absence of Band**).**

S.NO	Genotypes	WMC	27	WMC 105	WMC 105	WMC 147	WMC 154	WMC 169	WMC 173	WMC 213	WMC 94
1	010817	+	+	+	+	+	+	+	+	+	
2	010771	+	+	+	+	+	+	+	+	+	
3	010803	+	+	+	+	+	+	+	+	+	
4	010726	+	+	+	+	+	+	+	+	+	
5	010772	+	+	+	+	+	+	+	+	+	
6	010727	+	-	+	+	+	+	+	+	+	
7	010718	+	+	+	+	+	+	-	+	+	
8	010732	+	+	+	+	+	+	+	+	+	
9	010801	+	+	-	-	-	-	+	+	+	
10	010805	+	-	+	+	+	-	+	+	-	
11	010808	+	+	+	+	+	-	+	+	-	
12	010809	+	+	+	+	+	-	+	+	-	
13	011863	+	+	-	+	+	-	+	+	+	
14	010717	-	+	-	-	-	-	-	-	-	
15	010735	+	-	+	-	+	-	+	+	+	
16	010810	+	-	+	+	+	+	+	+	-	
17	010759	+	+	-	+	-	+	-	+	+	
18	010725	+	+	+	+	+	+	+	+	-	
19	010755	+	+	-	+	-	+	-	+	+	
20	010733	-	+	-	-	-	-	-	-	+	
21	010719	+	-	-	+	-	-	-	+	+	
22	010780	+	-	+	+	+	-	+	+	-	
23	010740	-	-	+	-	-	-	-	+	+	
24	010743	+	+	+	+	-	-	-	+	+	
25	Shafaq-2006	+	-	-	-	-	-	-	+	+	
26	PS-2004	+	+	+	+	+	+	+	+	+	
27	Siran-2010	+	+	+	+	+	+	-	+	+	
28	010786	+	+	+	+	-	+	+	+	+	
29	Khyber-87	+	-	-	+	+	+	-	+	-	
30	010800	+	-	-	+	+	+	-	+	+	
31	Hashim	-	-	-	+	-	-	-	-	+	
32	PS-08	+	-	-	+	+	+	+	+	+	
33	ZAM	+	-	-	+	-	-	-	+	+	
34	010802	-	-	-	+	-	-	-	-	+	
35	PS-05	+	-	+	+	+	+	+	+	-	
36	010798	-	-	-	+	-	-	-	+	+	
37	Haider-2000	+	-	+	+	-	+	+	+	+	
38	ARE-10	+	-	+	+	+	+	+	+	+	
39	010799	+	-	+	+	+	-	+	+	+	
40	Faisalabad-08	+	-	+	-	+	+	+	+	-	
41	011786	+	-	-	+	-	+	+	+	-	
42	Gomal	+	-	-	+	-	+	+	+	+	
43	010797	+	-	-	+	+	+	-	+	+	
44	NARC -2009	+	-	-	+	+	+	-	+	+	
45	Sahar	+	+	-	+	+	+	-	+	+	
46	Dera-98	+	-	-	+	-	-	-	+	+	
47	Atta- habib	+	-	-	+	-	-	-	-	-	
48	KT-2000	+	-	-	+	+	+	-	+	+	
49	010791	-	-	-	+	-	-	-	-	+	
50	010804	+	-	-	+	+	-	-	+	+	
51	PS-85	+	-	-	+	-	-	-	+	+	
52	Tatara	+	-	-	+	-	-	-	+	+	

No of spikelet per spike

The analysis of variance showed that No of spikelets per spike was highly significant at level (P≤0.01) (Table 2).the correlation analysis also suggest that No of spikelets per spike was found positively correlated to peduncle length, spike length, days to 50%heading, biological yield, yield per plant and 1000 grain weight and was found negatively correlated to plant height,

flag leaf area and harvest index (HI) as shown in (Table 3).

Harvest index

The analysis of variance (P≤0.01) showed that No of harvest Index (HI) was highly significant (Table 2).The correlation analysis also showed that harvest Index (HI) was found positively correlated to peduncle length, spike length, plant height, days to 50% heading, biological yield, flag leaf area and 1000 grain weight and was found negatively correlated to yield per plant and no of spikelets per plant (Table 3).

Cluster analysis on the bases of morphological traits

Dendrogram was constructed for cluster analysis on the base of morphological traits of tested wheat varieties. The dendrogram was consisted of six groups as A, B, C, D, E and F.

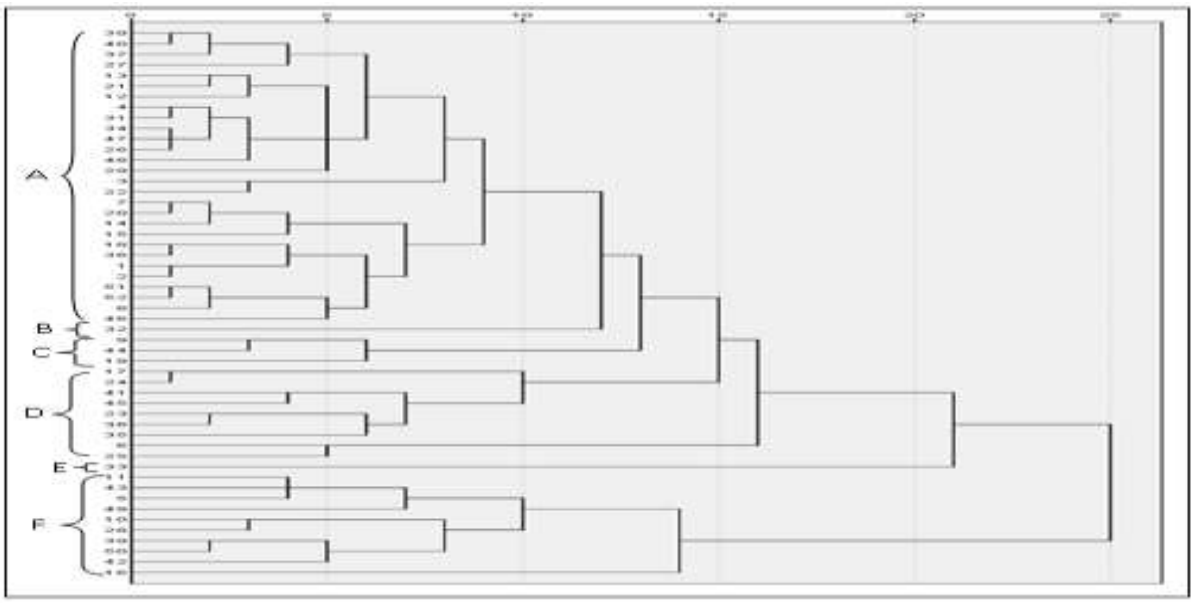

Fig. 1. Morphological Dendrogram Representing Different Clusters.

The germplasm of different clusters shows the dissimilarity among genotypes and the genotypes present in the same cluster show the similarity level. Group A was sorted into 28 genotypes containing germplasm 30 (10800), 40 (Faisalabad-08), 37 (Haider-2000), 27 (Siran-2010), 13 (11863), 21 (10719), 12 (10809), 04 (10726), 31 (Hashim), 34 (10802), 47 (Atta habib), 26 (PS-2004), 48 (KT-2000), 29 (Khyber-87), 03 (10803), 22 (10780), 07 (10718), 20 (10733), 14 (10717), 15 (10735), 18 (10725), 36 (10798), 01 (10817), 02 (10771), 51 (PS-85), 52 (Tatara), 08 (10732) and 46 (Dera-98). Group B was sorted into one genotype as 32 (PS-08). Group C consist of three genotypes as 09 (10801), 44 (NARC-2009) and 19 (10755). Group D consist of nine genotypes as 17 (10759), 24 (10743), 41 (11786), 45 (Sahar), 23 (10740), 38 (ARE-10), 35 (PS-05), 06 (10727) and 25 (Shafaq-2006). Group E consist of only one genotype as 33 (ZAM). Group F consist of ten genotypes as 11 (10808), 43 (10797), 05 (10772), 49 (10791), 10 (10805), 28 (10786), 39 (10799), 50 (10804), 42 (Gomal) and 06 (10727).

Molecular marker screening for drought tolerance

All the 52 genotypes were screened out for drought tolerance on the base of molecular markers. Total of nine drought markers were selected from Wheat microsatellite consortium.

Discussion

Pakistan's climatic conditions are well suited for the development of agriculture but still the present indigenous production of wheat is not sufficient to meet the needs of the growing population. Therefore, the only visible way to increase wheat production is the development of improved wheat varieties having better yield associated traits and disease resistance (Ejaz-ul-hasan, 2008).

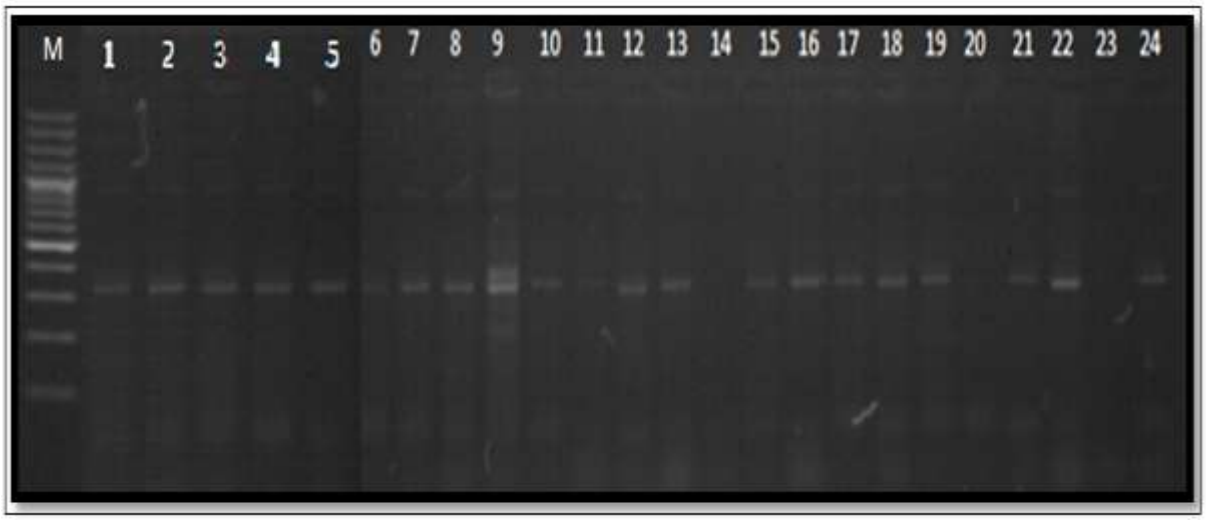

Fig. 2a. PCR amplification profile of 24 wheat genotypes using SSR marker WMC 27. M(marker). Sample No. 1-24 represent wheat genotypes enlisted in table 4.

In present study fifty two wheat varieties were studied for ten parameters of morphology along with molecular analysis through drought specific markers. Based on quantitative traits the analysis of variance (ANOVA) showed that all the parameters were significant. With respect to morphological quantitative traits the superior genotypes included 010810, 010786, 010797, 010771, 010791, 010772, 010808, Sahar, PS-05, ZAM, 011786, 010717 and 010817. The dendrogram based on morphological traits confirmed that the genotype PS-08 is placed in the separate group as considered being quite different from the remaining groups.

Fig. 2b. PCR amplification profile of 28 wheat genotypes using SSR marker WMC 27. M(marker). Sample No. 25-52 represent wheat genotypes enlisted in table 4.

The varieties 010759, 010743, 011786, Sahar, 010740, ARE-10, PS-05, 010727 and Shafaq-2006 were grouped in closely related clusters that reflected the association among these varieties both morphologically and genetically. The genotypes 010808, 010797, 010772, 010791, 010805, 010786, 010799, 010804, Gomal and 010727 were placed in the same group and showed closed morphological results. The genotypes 010801, NARC-2009 and 010755 were placed in the same group. The genotype ZAM was placed in separate group while all the remaining genotypes showed similarity and came

under the same group. Cluster analysis showed uniformity with quantitative and molecular characteristics of studied genotypes. This study is in close agreement with the studies of Ashraf, (2010); Thoday, (1991): Nachit *et al.* (1993) who demonstrated that different molecular markers, currently available for tagging of different traits are useful for Marker-assisted breeding technique in wheat in stress conditions and are intensively used to create stress-tolerant lines in different crops. Some markers in wheat are linked to grain yield and morpho physiological characteristics for drought tolerance (Nachit *et al.,* 1993). These results suggest the usefulness of morphological studies complemented with molecular markers to enhance drought tolerance in wheat in drought condition.

The molecular markers screening for drought resistance also confirmed that 13 genotypes including 010817, 010771, 010803, 010726, 010772, 010732, PS-2004, 010727, 010718, 010725, Siran-2010, 010786 and ARE-10 have amplified maximum number of drought resistance genes and therefore, these genotypes were considered the most resistant among the studied genotypes. Current study is in close agreement with previous results of Kirigwi *et al.* (2007) who found association of molecular markers with some quantitative traits in wheat genotypes. Based on combined performance with respect to morphology and SSR markers analyses vigorous genotypes were selected as Sahar, 010810, 010817 and 011786. Thus these genotypes can better be adopted in drought habitats for high yield. All these genotypes can also be used in breeding programs to produce high yielding varieties in drought stress environment.

Conclusion

The present research concluded that the following genotypes including 010810, 010786, 010797, 010771, 010791, 010772, 010808, Sahar, PS-05, ZAM, 011786, 010717 and 010817 showed best performance based on morphological analysis.

In SSR analysis maximum number of drought tolerant genes were amplified in genotypes 010817, 010771, 010803, 010726, 010810, Sahar, 010772, 010732, PS-2004, 010727, 010718, 010725, Siran-2010, 010786, 011786 and ARE-10.

Thus genotypes revealing the best performance both on morphological and molecular base were Sahar, 010810, 010817 and 011786 and are recommended for utilization in future wheat breeding programs for rain fed areas of Pakistan. Marker assisted selection (MAS) is cost effective and more reliable technique and could be successfully utilized in modern research.

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