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Morphogenetic screening of Pakistani spring wheat germplasm for drought tolerance

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Abstract

Wheat (*Triticum aestivum L.*) is the most vital cereal crop and ranks first in world crop production. In Pakistan drought is the main environmental constraint that reduce 20% yield annually. The present study was carried out to screen fifty wheat genotypes for drought stress on the basis of morphological and molecular techniques. All the genotypes were sown in the field of Hazara University Mansehra Pakistan for three consecutive years (2013-2015). The highest Peduncle length, Spike length, Plant height, Days to 50% heading, Biological yield, Flag leaf area, Yield per plant, Spikelets per spike, 1000 grain weight and HI were observed in LYP-73(50 cm), Wardak-85(35 cm), 010724(105.6 cm), 010724(155 days), Sonalika(23 gm), soghat-90(65.4 cm/sq), 010737(13.1), Shahkar-95(72), 010748(68) and 010737(109.16) respectively. The analysis of variance (ANOVA) revealed that all parameters were found to be highly significant at ($P \leq 0.01$) level. Seven molecular markers were used for screening of drought tolerant genotypes concluded that Wafaq-2008, C-273, SA-2002 and Punjab-96 showed more resistant genes and recommended for rain fed areas of Pakistan. Furthermore, these genotypes could be used for breeding purposes to improve the crop yield against drought stress.

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Introduction

Wheat (*Triticum aestivum* L.) is a vital and essential cereal crop around the world. Its annual production was 713 million metric tons (Reynolds, 2014). Wheat ranks first due to its area and production. Wheat also maintains its first rank among cereals due to high gluten content (Palmer and John, 2012). It is also used for fermentation, biofuel and other alcoholic beverages (Neill, 2002).

Drought is the most important abiotic stress in arid and semi-arid regions of the world (Rajala *et al.,* 2009). Wheat production is 20% lesser in drought areas as compared to irrigated areas. (Delmer, 2005; Farooq *et al.,* 2009). The crucial way to protect the crop against unfavorable effects of drought is the drought tolerant wheat varieties (Bernardo, 2008).

Larger genetic variability can be explored in wheat from its centers of origin and diversity in germplasms (Dvorak *et al.* 2011). To improve drought tolerance among cultivars can be achieved through genetic variation or selection for adaptive mechanisms; including drought escape and dehydration tolerance (Blum, 2010). Drought is quantitative trait controlled by several genes and greatly influenced by ecological conditions. Yield improvement is difficult in water-limited environments and depends on the drought system i.e. time of occurrence, intensity, and drought duration (Blum, 2011). Through marker assisted breeding (MAB) it is now possible to study the efficacy of thousands of genomic regions of a crop germplasm under drought region, which was not possible previously (Ashraf, 2010). Therefore, the present research was conducted to screen out wheat genotypes for drought tolerance using molecular markers.

Materials and methods

Plant materials

The present research work was carried out under field conditions of Mansehra during 2013-2014. Fifty genotypes of common wheat (*Triticum aestivum* L.) collected from PGRI and NARC Islamabad were evaluated under rainfed condition for yield performance and possible marker association with drought potential. The data was analyzed using statistical software SPSS version 22.

Fifty wheat germplasm are 010724, Wardak-85, Potohar-90, Potohar-70, Faisalabad-85, C-273, 010737, SA-2002, LYP-73, Barani-70, C-591, C-250, Saleem-2000, Chenab-79, Uqaab-2000, NIAB-83, Wafaq 2008, MH-97, Momal-2002, AUP-2008, PUNJAB-96, Iqbal-2000, SUSSI, Mehran-89, Marwat-01, LR-230, Bakhtawar-94, Punjab-88, 010742, Sonalika, Maxipak, 010748, Pak-81, Fakhr-e-sarhad, Dirk, Zarlashta-90, Local white, Shahkaar-95, Wadanak-85, Khyber-79, Chenab-70, Manther, 010760, Khyber-83, Soghat-90, Bahawalpur-79, Nori-70, Blue silver, 010776 and Haider-2002.

Morphological studies

Fifty wheat germplasms were planted in Randomized Complete Block Design (RCBD) with three replications. The data was collected on 3 randomly selected plants in each row for different morphological parameters *viz.*, peduncle length, spike length, plant height, 50 % heading, biological yield, flag leaf area, yield per plant, 1000-grain weight, and number of spikelets per spike and harvest index.

Molecular studies

Simple sequence repeats markers were used for screening of drought resistance genes. DNA was isolated using protocol of Weining and Langridge (1992). DNA quality and quantity was checked on 1% agarose gel. PCR was carried out using the protocols of (Mago *et al.*, 2002; Stepien *et al.*, 2003) with a little modification. Components of PCR reaction were genomic DNA used as template, dNTPs, specific Primers, Taq polymerase buffer, $MgCl_2$, Taq polymerase and distilled water. The primers used in the present research work are *wmc*-97, *wmc*-104, *wmc*-105, *wmc*-147, *wmc*-166, *wmc*-175 and *wmc*-216**.** There sequences, fragment size and sources are given in the table 1.

Results and discussion

Fifty wheat varieties were studied for morphological traits. Seven drought specific molecular SSR markers were also run for tagging drought genes in selected genotypes. The highest peduncle length was found in LYP-73(50) and Soghat-90(44.6). The highest spike length was found in Wardak-85(15) and Soghat-90(12.3). The highest plant height was found in 010724(105.6).

Table 1. Primer sequences of *wmc*-97, *wmc*-104, *wmc*-105, *wmc*-147, *wmc*-166, *wmc*-175 and *wmc*-216.

SSR marker	Primer sequence	Fragment size	source
wmc-97	Forward GTCCATATATGCAAGGAGTC Reverse GTACTCTATCGCAAAACACA	184bp	Roder *et al.* 1998
wmc-104	Forward TCTCCCTCATTAGAGTTGTCCA Reverse ATGCAAGTTTAGAGCAACACCA	140bp	Erum *et al., 2013*
wmc-105	Forward AATGTCATGCGTGTAGTAGCCA Reverse AAGCGCACTTAACAGAAGAGGG	192bp	Erum *et al., 2013*
wmc-147	Forward AGAACGAAAGAAGCGCGCTGAG Reverse ATGTGTTTCTTATCCTGCGGGC	152 bp	Somers *et al.*, 2004
wmc-166	Forward ATAAAGCTGTCTCTTTAGTTCG. Reverse GTTTTAACACATATGCATACCT	305bp	Matthews *et al.*, 2003
wmc-175	Forward GCTCAGTCAAACCGCTACTTCT Reverse CACTACTCCAATCTATCGCCGT	253bp	Somers *et al.*, 2004
wmc-216	Forward ACGTATCCAGACACTGTGGTAA Reverse TAATGGTGGATCCATGATAGCC	123bp	Somers *et al.*, 2004

Table 2. Comparative analysis of ten morphological traits on the base of analysis of variance.

		Sum of Squares	Df	Mean Square	F	Sig.
Peduncle Length	Between Groups	7895.033	49	161.123	161.123	.000
Spike Length	Between Groups	2071.428	49	42.274	42.274	.000
Plant Height	Between Groups	38068.503	49	776.908	776.908	.000
50% Heading	Between Groups	637.740	49	13.015	13.015	.000
Biological Yeild	Between Groups	1772.212	49	36.168	36.168	.000
Flag Leaf Area	Between Groups	43066.080	49	878.900	878.900	.000
yeild per Plant	Between Groups	156629.040	49	3196.511	3196.511	.000
1000 Grain Weight	Between Groups	10343.040	49	211.082	211.082	.000
No of Spikelets per Spike	Between Groups	18102.000	49	369.429	369.429	.000
Harvest Index	Between Groups	11390.943	49	232.468	232.468	.000

The maximum days for 50% heading was counted in 010724(155) and Barani-70(148). The highest biological yield was weighted in Sonalika(23) and 010742(22). Soghat-90(65.4) is the variety having largest leaf area. Among the studied varieties 010737(13.1) and wadanak-85(10.45) are the highest yielding varieties. The highest 1000 grain weight was found in Shahkar-95(72) and Wadanak-85(67). The highest number of spikelets per spike was found in 010748(68) and 010742(64). Among the studied varieties 010737(109.16) and Shahkar-95(82.83) recorded the highest harvest index.

The analysis of variance (ANOVA) showed that all the parameters are significant at ($P \leq 0.01$) as shown in Table 2. The dendrogram based on morphological traits confirmed that the genotype C-273 and Khyber-83 is placed in separate group as considered being quite different from the remaining groups. The varieties 010742, 010748, Chenab-79, soghat-90, SA-2002, NIAB-83 and wardak-85 are grouped in closely related clusters therefore showed similarity to greater extent while all the remaining genotypes show similarity and are come under the same group as shown in Fig. 1.

All the 50 genotypes were screened for drought tolerance on the base of molecular markers. Total of seven SSR drought markers were selected from online grain gene 2 database. The visible and reliable bands were included in scoring sheet. The required fragment size was compared with 100 bp molecular ladder. The presence of required band denoted by (+) and absent by (-) as shown in table 3.

Table 3. Molecular markers banding pattern (+ for presence and – is absence of band).

S. No.	Genotypes	*Wmc-97*	*Wmc-104*	*Wmc-105*	*Wmc-147*	*Wmc-166*	*Wmc-175*	*Wmc-216*
1	010724	+	-	-	+	-	-	+
2	Wardak-85	-	-	-	+	-	-	-
3	Potohar-90	-	-	-	+	-	-	-
4	Potohar-70	-	+	-	+	-	-	-
5	Faisalabad-85	+	-	-	+	-	-	-
6	C-273	+	+	-	+	-	+	+
7	010737	+	-	-	+	-	+	-
8	SA-2002	+	+	-	+	+	+	-
9	LYP-73	+	-	-	-	-	-	-
10	Barani-70	+	-	-	+	-	-	-
11	C-591	+	-	-	+	-	-	-
12	C-250	+	-	-	+	-	-	-
13	Saleem-2000	+	+	+	+	-	-	-
14	Chenab-79	+	+	-	+	-	+	-
15	Uqaab-2000	+	+	-	+	-	+	-
16	NIAB-83	+	+	+	+	-	-	-
17	Wafaq 2008	+	+	-	+	+	+	+
18	MH-97	+	+	-	+	+	-	-
19	Momal-2002	+	+	-	+	-	+	-
20	AUP-2008	+	+	-	-	-	-	-
21	PUNJAB-96	+	+	-	+	-	+	+
22	Iqbal-2000	+	+	+	+	-	-	+
23	Sussi	+	-	+	+	-	-	+
24	Mehran-89	+	+	-	+	+	+	-
25	Marwat-01	+	+	-	+	-	-	-
26	LR-230	+	+	-	+	-	+	-
27	Bakhtawar-94	-	+	-	-	-	-	-
28	Punjab-88	+	-	-	+	-	-	-
29	010742	-	+	-	-	+	-	+
30	Sonalika	+	-	-	+	-	-	-
31	Maxipak	+	+	-	+	-	-	-
32	010748	+	+	-	+	+	+	-
33	Pak-81	-	-	-	+	-	-	-
34	Fakhr e sarhad	+	-	+	+	+	-	-
35	Dirk	+	-	-	+	+	-	+
36	Zarlashta-90	+	+	-	+	-	-	-
37	Localwhite	+	+	-	+	+	-	-
38	Shahkaar-95	+	-	-	-	+	-	-
39	Wadanak-85	-	-	-	-	-	-	+
40	Khyber-79	+	-	-	-	-	+	-
41	Chenab-70	+	-	-	+	-	-	-
42	Manther	+	-	-	+	-	-	+
43	010760	+	-	+	+	-	-	+
44	Khyber-83	+	+	-	-	-	-	+
45	Soghat-90	+	+	-	-	-	-	+
46	Bahawalpur-79	+	+	-	-	-	+	+
47	Nori-70	+	+	-	-	-	-	+
48	Blue silver	+	-	-	+	-	+	-
49	010776	+	-	-	-	-	-	-
50	Haider-2002	+	+	-	-	-	-	+

The molecular markers screening for drought resistance also confirmed that the genotypes Wafaq-2008, C-273, SA-2002, Punjab-96, Iqbal-2000, Mehran-89 and 010748 have amplified maximum number of drought resistance genes as 6, 5, 5, 5, 5, 5 and 5 respectively. Therefore, these genotypes can better be adopted in drought habitats for high yield. The minimum number of drought tolerant genes was found in Wardak-85, Potohar-90, LYP-73, Bakhtawar-94, Pak-81, Wadanak-85 and 010776 and therefore are considered to be drought susceptible.

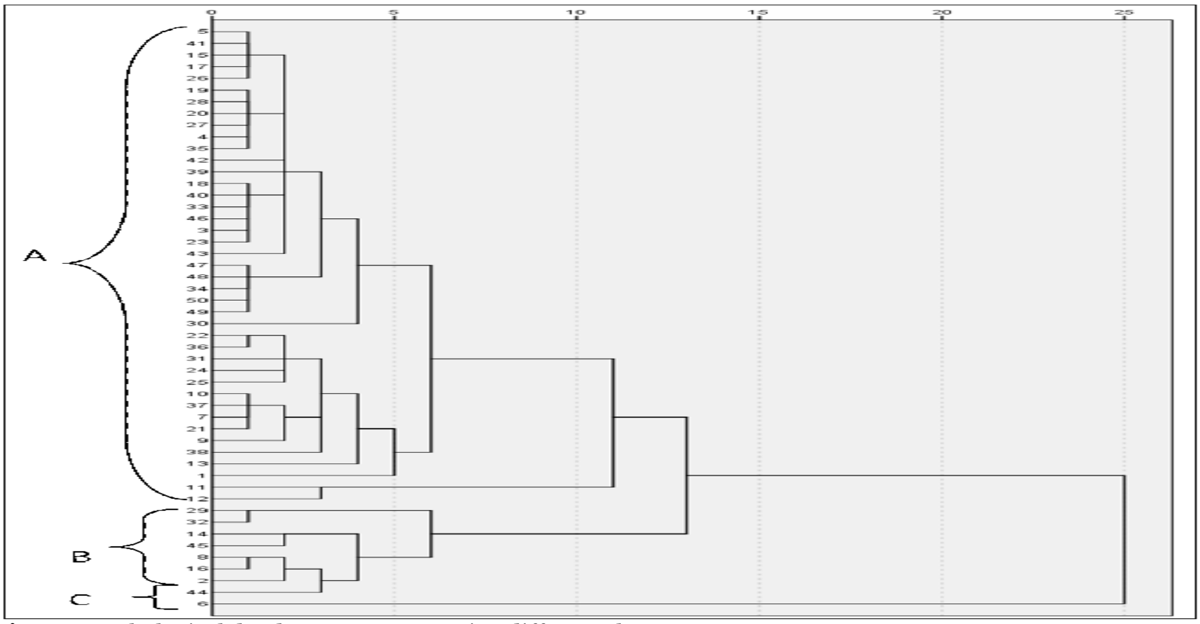

Fig. 1. Morphological dendrogram representing different clusters.

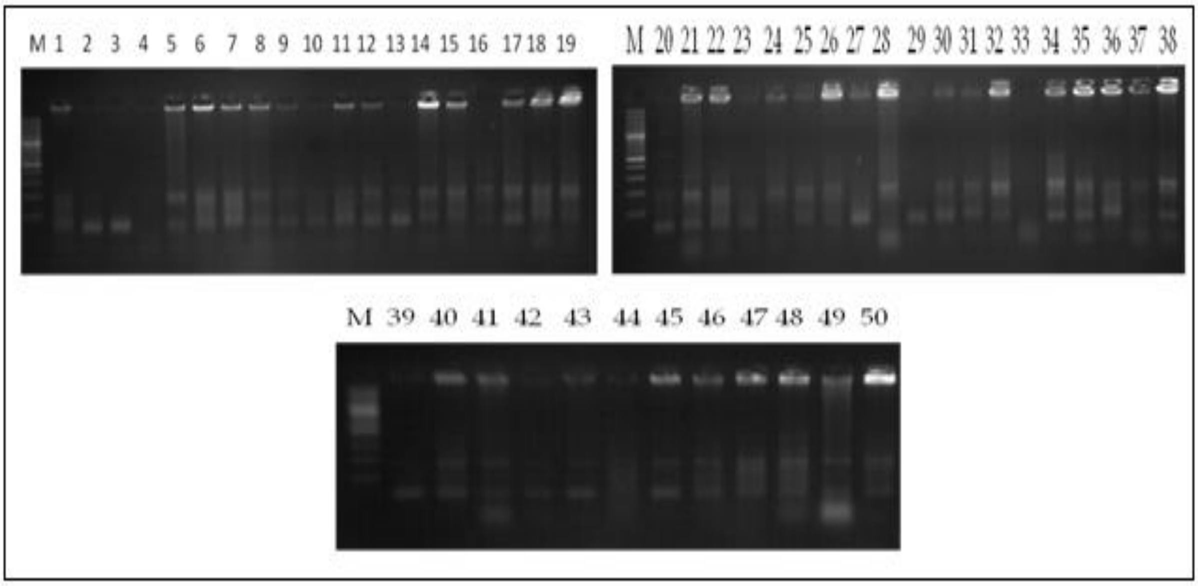

Fig. 2. Representative gel pictures of *wmc 97 marker*.

Conclusion

The present research concluded that Wafaq-2008, C-273, SA-2002 and Punjab-96 have showed more resistant genes and are recommended for rain fed areas of Pakistan. All these genotypes can also be used in breeding programs to produce high yielding varieties for drought stress environment. The morphological parameters could be used for screening of wheat germplasm for drought. Marker assisted selection (MAS) are cost effective, more reliable technique and advance technique for screening of drought tolerance and could be utilized in modern research.

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