

ORIGINAL ARTICLE

Investigation of grain yield and drought resistance in selected wheat lines based on molecular markers

D. Khan¹, I. Muhammad¹, M. Shuaib^{2*}, F. Hussain³, M. Romman⁴, N. Azam⁵, S. Abidullah⁶, A. Zeb⁶, A. Rauf⁶, S. Bahadur⁷

¹Laboratory of Plant Metabolic Engineering, Faculty of Life Science and Technology, Kunming University of Science and Technology P. R. China

²School of Ecology and Environmental Science, Yunnan University, Kunming, China

³Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan

⁴Department of Botany, University of Chitral, KPK-Pakistan

⁵Cenetre of Plant Biodiversity, University of Peshawar, Pakistan

⁶Department of Botany, Abdul Wali Khan University Madan, Pakistan

⁷Institute of Tropical Agriculture and Forestry, Hainan University, Haikou, China

*Corresponding author E-mail: zeyadz44@yahoo.com

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The current work aims to performed and know the fifty-one wheat genotypes and evaluate for drought stress. The analysis of variance (ANOVA) was performed, which shows that all the parameters are significant. Variations in quantitative traits include Peduncle length, Spike length, Plant height, Days to 50% heading, Biological yield, Flag leaf area, Yield per plant, 1000 grains weight, Number of spikelets per spike Harvest Index were recorded. 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 10831 (46.33), 10843 (14), 10821 (116.33), 10845 (157), 10826 (18.4), 10874 (58.46), 10854 (2.944gm), 10841(26), 11881 (52) and 10738 (24.571) respectively. Seven molecular markers were used for screening drought-tolerant genotypes. The genotypes 11861, 11860, 10850, 10815, 10819, 11866, and 10825 have amplified a maximum number of drought resistance genes as 7, 7, 7, 6, 6, 6, and 6, respectively.

Keywords: ANOVA, drought, quantitative traits, wheat.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop for most of the world's populations. It is the essential staple food of about two billion people (36% of the world population). Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally. Wheat-breeding programs worldwide are operational to improve grain yield with better quality, agronomic performance, and disease resistance (Zhang *et al.* 2011; Naeem *et al.*, 2020). The problems of climate change affecting several crops, including wheat (increase in temperature and drought), are being addressed globally (Plaut *et al.* 2004, Passioura 2007, Reynolds *et al.* 2001, Najafian 2003 and Rajki 1982). It has been reported that about 20% of irrigated land has been affected by drought and soil salinization, and crop yield has been reduced by 20-30% throughout the world. In general, breeding for tolerance to drought involves combining good yield potential in the absence of stress and selecting traits that provide drought stress tolerance (Blum 1988). The present study tested 51 wheat varieties and detected drought-resistant varieties based on molecular markers and high grain yielding varieties based on morphological parameters.

Materials and Methods

Plant collection

The present research work was carried out under field conditions of Mansehra during 2013-2014. Fifty-one genotypes comprising germplasm and common wheat varieties (*Triticum aestivum* L.) collected from PGRI, and NARC Islamabad were evaluated at the morphological and molecular levels.

Primers design

Using NCBI to design the seven different primers with their base pairs, temperature, times for the 51 different wheat varieties shown in Table 1.

S No	Primer Name	Primer Sequence	Fragment Size	Tm
01	WMC-97	Forward GTCCATATATGCAAGGAGTC Reverse GTA CTCTATCGCAAAACACA	184 bp	51°C
02	WMC-105	Forward AATGTCATGCGTGTAGTAGCCA Reverse AAGCGCACTTAACAGAGAGGG	192 bp	59°C
03	WMC-147	Forward AGAACGAAAGAAGCGCGCTGAG Reverse ATGTGTTTCTTATCCTGCGGGC	152 bp	61°C
04	WMC-154	Forward ATGCTCGTCAGTGTCATGTTTG Reverse AAACGGAACCTACCTCACTCTT	147 bp	61°C
05	WMC-169	Forward TACCCGAATCTGGAAAATCAAT Reverse TGGAAGCTTGCTAACTTTGGAG	167 bp	61°C
06	WMC-182	Forward GTATCTCACGAGCATAACACAA Reverse GAAAGTGTATGGATCATTAGGC	159 bp	56°C
07	WMC-219	Forward TGCTAGTTTGCATCCGGGCGA Reverse CAATCCCGTTCTACAAGTTCCA	180 bp	61°C

Table 1. Details of the primers used in the present study.

Research design, treatment, and DNA extraction

Morphological studies were undertaken in the Hazara University (garden campus) research field. Fifty-one wheat germplasm was planted in Randomly Complete Block Design (RCBD) with three replications (Table 1). DNA was isolated from selected plant materials using (Weining and Langridge 1992) protocol. To check the quality and quantity, the extracted genomic DNA was electrophoresed on 1% agarose gel. After that, the gel was observed under UV light using a Uvtech system (model BTS-20.M).

PCR

Polymerase Chain Reaction (PCR) was carried out using the protocols of (Stepien *et al.* 2003 and Mago *et al.* 2002) with a little modification. The PCR was conducted for seven primers viz., wmc97, wmc105, wmc147, wmc154, wmc169, wmc182 and wmc219. The Thermo cyclic conditions were kept as initial denaturation at 94°C for 4 min followed by 37 cycles of 94°C for 1 min, 51 to 61°C for 1 min, 72°C for 2 min a final extension of 72°C for 10 min (Fig. 1).

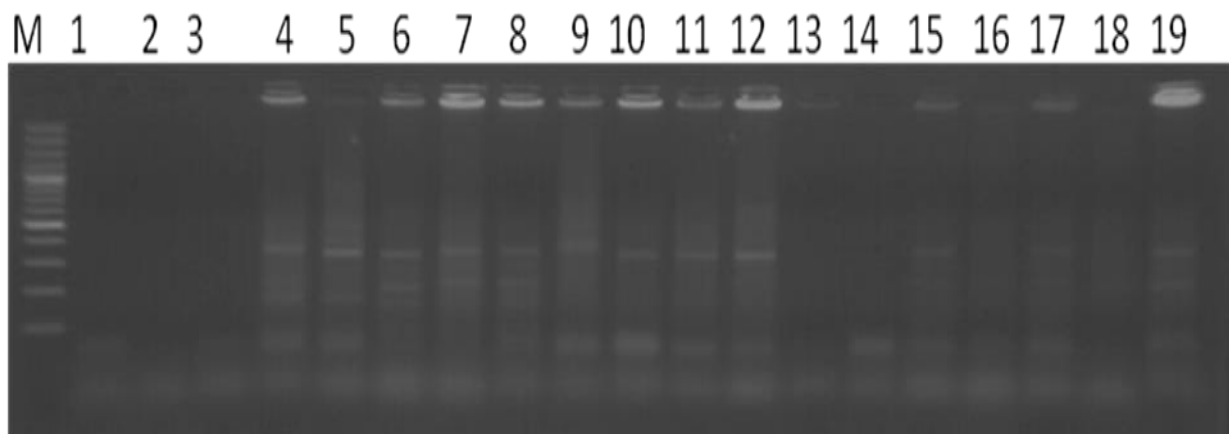


Fig. 1. PCR amplification profile of the genotypes WMC 105.

Data analysis

One-way ANOVA was used to analyze the data. Fifty-one wheat germplasm was planted in Randomly Complete Block Design (RCBD) with three replications shown in (Table 1) in detail.

Results and Discussion

Fifty-one wheat varieties were studied for different morphological characters. Seven droughts specific SSR markers were also run to identify drought resistance genes.

The highest peduncle length was counted for 10831 (46.33 cm) and 10814 (45.66 cm) among morphological parameters. The highest spike length was recorded in 10843 (14 cm) and 10841 (13 cm). The highest flag leaf area was counted for 10874 (58.46 cm) and 10848 (55.06 cm). The maximum number of days to 50% heading was counted in 10845 and 10842, 157 days each. The highest plant height was counted for 10821 (116.33 cm) and 10819 (155.66 cm). The highest 1000 grain weight was counted in 11881 (52 gm) and 10874 (48 gm). Biological yield counted in 10826 and 11881, 18.4 gm each was found highest among other varieties. Among the studied varieties, 10854 (2.94) and 10814 (2.82) have the highest yield per plant. The highest number of spikelets per spike was counted in 10841 (26) and 11873 (25), while the highest Harvest Index was counted for 10738 (24.57) and 10813 (19.86). Among the fifty-one germplasm, the superior genotypes were observed based on repetition in morphological traits are 10845 (7times), 10842 and 10843 (5times), 10841 (4times), while the remaining varieties are repeated at least one time. The analysis of variance showed that all the parameters are highly significant, as shown in Table 2.

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig
Replication	Between Groups	0.000	50	0.000	0.000	1.000
Peduncle Length	Between Groups	8929.517	50	178.590	178.590	.000
Spike Length	Between Groups	331.888	50	6.638	6.638	.000
Total Plant Height	Between Groups	57658.261	50	1153.165	1141.860	.000
Fifty percent Heading	Between Groups	4206.471	50	84.129	84.129	.000
Biological Yeild	Between Groups	1294.289	50	25.886	25.886	.000
Flag Leaf Area	Between Groups	6984.562	50	139.691	139.691	.000
Yield per Plant	Between Groups	15171.529	50	303.431	303.431	.000

Thousand Grain Weight	Between Groups	4670.549	50	93.411	1.647	.017
No of Spikelets per Spike	Between Groups	1074.118	50	21.482	21.482	.000
Harvest Index	Between Groups	3807.497	50	76.150	76.150	.000

Table 2. Analysis of Variance for yield and yield associated traits of wheat.

The morphological dendrogram was constructed for wheat (*Triticum aestivum* L.) germplasms. The fifty-one wheat germplasms were sorted into seven groups as A, B, C, D, E, F, and G. The germplasm of different clusters shows the dissimilarity among genotypes and the genotypes present in the same clusters shows the similarity level. Group A was further sorted into five clusters containing 21 genotypes, group B containing three genotypes; group C has consisted of 8 genotypes, group D consisted of 3 genotypes, group E consisted of only one genotype, group F containing two genotypes. In contrast, the rest of the genotypes reside in group G (Fig. 2).

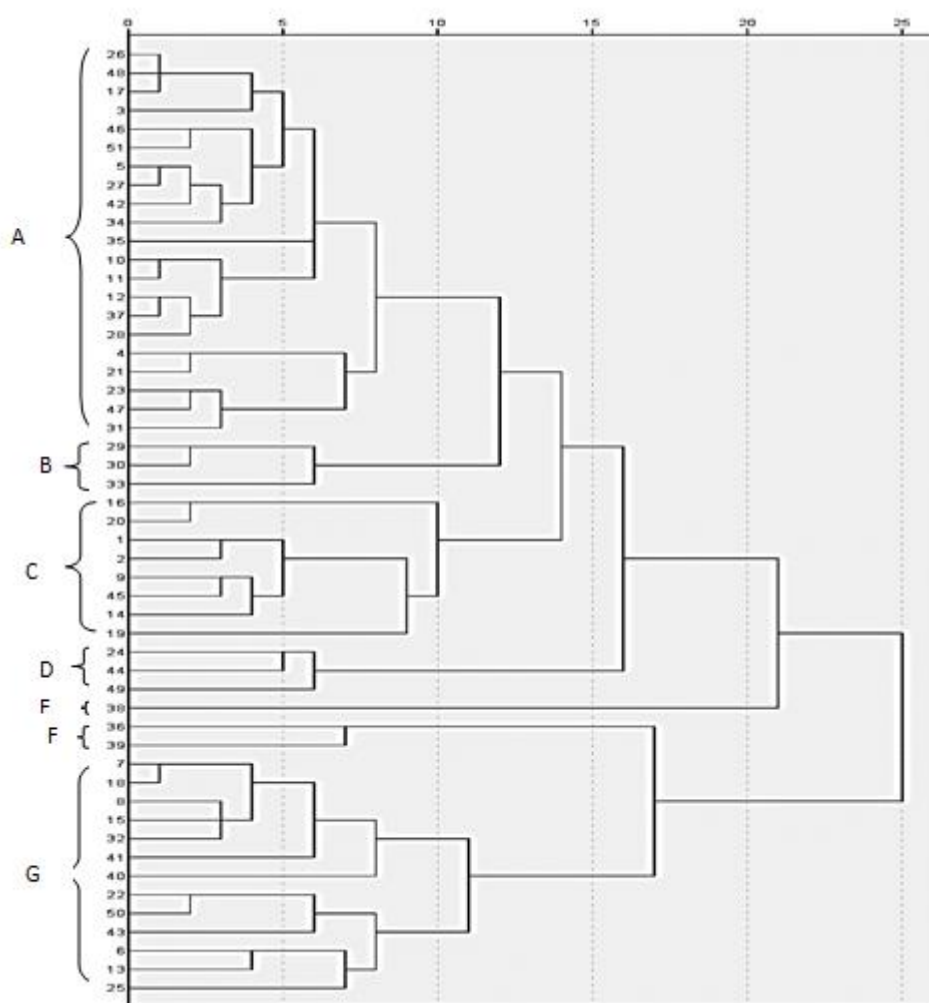


Fig. 2. Morphological dendrogram representing different clusters.

The present research work following (Ashraf 2010) demonstrated that different molecular markers, currently available for tagging of different traits, are helpful for Marker-assisted breeding techniques in wheat in stress conditions and are intensively used to create stress-tolerant lines in different crops. The current study is also in close agreement with (Muhammad et al. 2016; Khan et al. 2016 and Shuaib et al., 2020), as these results suggest the usefulness of morphological studies complemented with molecular markers to enhance drought tolerance in wheat in drought conditions.

Molecular marker screening for drought tolerance

All the 51 genotypes were screened out for drought tolerance based on molecular markers. Total of seven drought markers were selected as wmc-97, wmc-105, wmc-147, wmc-154, wmc-169, wmc182 and wmc-219. The only reliable bands were included in scoring; the faint bands were not considered (Table 3). The molecular fragment size was matched with a standard molecular ladder of 100 bp (gene link Inc. USA).

The molecular markers screening for drought resistance also confirmed that the genotypes 11861, 11860, 10850, 10815, 10819, 11866, and 10825 had amplified the maximum number of drought resistance genes as 7, 7, 7, 6, 6, 6, and 6, respectively. The current study is in close agreement with previous results of (Kirigwi et al. 2007, Muhammad *et al.* 2016; Muhammad et al., 2020; Khan *et al.* 2016), who found the association of molecular markers with some quantitative traits in wheat genotypes. Therefore, 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 for drought-stress environments. The minimum number of drought-tolerant genes was found in 11868, 10853, 11873, 10826, 10874, and 11864 are considered to be drought susceptible.

S No	Genotype	WMC219 180bpbp	WMC169 167bp	WMC97 184bp	WMC105 350BP	WMC147 152bp	WMC154 147bpS	WMC182 159bp
1	11868	+	-	-	-	-	-	-
2	10854	-	-	-	-	-	-	-
3	10853	+	-	-	-	-	-	-
4	10850	+	+	+	+	+	+	+
5	10849	+	-	+	+	+	+	-
6	10825	+	+	+	+	+	-	+
7	10845	-	-	+	+	+	+	-
8	10847	-	-	-	+	-	+	-
9	11862	+	-	+	+	+	+	-
10	11860	+	+	+	+	+	+	+
11	11861	+	+	+	+	+	+	+
12	11866	+	+	+	+	+	+	-
13	11809	-	-	-	-	-	-	-
14	11873	-	-	-	-	-	-	+
15	10842	+	-	+	+	+	+	-
16	10841	-	-	+	-	+	-	+
17	10833	-	-	+	+	+	+	-
18	10843	-	-	+	-	+	-	+
19	10848	-	+	+	+	+	+	-
20	10832	-	-	+	-	+	-	+
21	10829	+	-	+	-	+	-	+
22	10852	+	+	+	+	-	-	+
23	10834	-	+	-	+	+	-	+

24	10831	+	+	+	-	+	-	-
25	10835	-	-	-	-	-	-	-
26	10830	-	-	-	-	-	-	-
27	11865	-	-	-	-	-	-	-
28	10826	-	-	-	-	-	-	+
29	10822	-	-	+	-	+	-	+
30	11881	+	+	+	+	+	-	-
31	10828	-	-	+	-	+	-	+
32	10824	+	+	+	-	+	-	-
33	10874	-	-	-	-	-	-	+
34	10827	+	+	+	-	+	-	-
35	11864	-	-	-	-	-	-	+
36	10820	-	-	+	+	+	-	+
37	11867	+	+	+	+	-	-	+
38	11882	-	-	-	-	-	-	-
39	10818	+	+	+	-	-	-	-
40	10821	+	-	+	+	-	+	-
41	10819	+	+	-	+	+	+	+
42	11879	-	+	-	-	+	-	+
43	11878	-	+	+	-	-	+	+
44	10814	-	+	+	-	-	+	+
45	11875	-	-	-	-	-	-	-
46	10730	+	+	+	-	+	-	+
47	10815	+	+	+	-	+	+	+
48	10816	-	+	+	-	-	-	+
49	10813	-	+	+	-	-	-	+
50	11877	+	-	-	-	-	+	-
51	10738	-	-	+	-	-	+	-

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

Conclusion

The present research concluded that 11861, 11860, 10850, 10815, 10819, 11866, and 10825 have shown more resistant genes and are recommended for rainfed areas of Pakistan. The morphological parameters could be used for screening wheat germplasm for drought. Marker-assisted selection (MAS) is an advanced technique for screening drought tolerance. MAS is a cost-effective and more reliable technique and could be utilized in modern research.

Acknowledgment

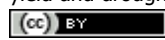
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