



RESEARCH PAPER

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Molecular and agronomic characterization of durum wheat

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Abstract

Triticum durum is an allotetraploid species ($2n=4x=28$, genomically AABB) and is used to produce pasta, noodles, couscous and macaroni etc. Present research was aimed to analyze the amount of genetic diversity in a world collection of durum wheat (175 accessions) using morphological, seed storage protein and DNA markers. Highly significant F values for genotypes and genotype by year interaction were observed for 18 morphological characters. A total 719 protein alleles (using SDS-PAGE) were observed. High range of genetic distances (GD ranging from 0 – 100%) was observed among accessions. One hundred twenty seven comparisons showed complete homozygosity (GD = 0 %) for total seed storage proteins while 252 comparisons showed 100 % genetic differences for the protein loci. Genetic distance among durum accessions using Randomly Amplified Polymorphic DNA (RAPD) markers ranged from 0 to 32%. While genetic distance among durum accessions using Simple Sequence Repeat (SSR) primer sets ranged from 2 to 38 %. It has been concluded that the accessions of durum wheat have sufficient genetic diversity among their morphological, seed storage protein and DNA polymorphisms which can be used for breeding better durum wheat genotypes.

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Introduction

Triticum durum ($2n=4x=28$, genomically AABB) is the only source to produce products like pasta, noodle and macaroni. Review of the available literature shows that only 5 % of the wheat grown in the world is of tetraploid (durum) while rest 95% is of hexaploid type (Bushuk *et al.*, 1994; Hancock, 2004). Because of its commercial importance in the production of bakery products, durum wheat always get attractive price in market as compared to bread wheat. For the improvement of durum wheat (like any other crop species) it is prerequisite to have an estimate of already existing genetic diversity in the germplasm. Because of its commercial importance, a lot of work on exploration and utilization of genetic diversity of durum wheat has been started all over the world. Colomba and Gregorini (2011) used Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat primers (SSR) to investigate genetic diversity in durum accessions of Italian origin. They reported that AFLP based molecular variation partitioned into 80% variations within the accessions and 20 % variation between the accessions. For SSR analysis, they reported 73% variation within accessions and 27 % variation between accessions. Al-Fares and Abu-Qaoud (2012) while using RAPD primers for estimating genetic diversity in Palestinian durum wheat concluded that RAPD assays are simple, rapid and reliable tools for evaluating genetic variability among durum wheat. Gashaw *et al.*, (2007) reported that good opportunity exists for improvement of grain yield of durum wheat through hybridization of genotypes, which can only be elaborated through marker assisted selection. In Indian sub continent, not much work has been reported on the estimation of genetic diversity in durum wheat using DNA based markers (Saleemi *et al.*, 1982). Present research reports genetic analysis of world collection of durum wheat germplasm based on agronomic and molecular markers (DNA and seed storage protein).

Materials and methods

A comprehensive world collection of durum wheat germplasm comprising 175 accessions was obtained

from Plant Genetic Resource Institute, National Agricultural Research Centre, Islamabad, Pakistan. The accessions belong to Pakistan, Syria, Egypt, ICARDA, and Cyprus. Accessions were Planted for 2 years (2010-2011 and 2011-2012) at Agricultural Research Station, Garhi Doppata, Muzafrabad, Azad Kashmir (Latitude/longitude: $34^{\circ}22'12''N$ $73^{\circ}28'14''E$, Decimal coordinates, 34.3700 73.4708, Altitude, 739 m). Randomly Complete Block (RCBD) with 3 replications was used as experimental design. Standard cultural practices were carried out during growth periods.

Data were recorded on 18 morphological characters viz; days taken to germinate, germination percentage, Plant height (cm), Spike length (cm), Number of tillers per plant, peduncle length (cm), leaf angle (degree), number of seeds per spike, number of spikelets per spike, plant thickness (measured with vernier clipper at maturity), grain yield per plant (g), 1000 grain weight (g), grain weight per spike (g), harvest index, number of days to 50% heading, number of effective tillers per plant and number days to 90% maturity. Total seed storage protein analysis was carried out using SDS-PAGE as described by Payne *et al.*, (1987) DNA analyses using RAPD and SSR primers were carried out using procedures described by Hoisington *et al.* (1994) and Doyle and Doyle, (1990). Statistical analysis of molecular data was done using formula described by Nei and Li, (1979).

Results and discussion

Mean and F values for the morphological characters studies are presented in Table 1. Analysis of variance (using the computer program Menitap version 13.1) showed highly significant ($p < 0.01$) differences among the genotypes for all the morphological characters studied. It indicated that the germplasm under study had enough genetic variability for the morphological characters. The present results supported an earlier finding by Gashaw *et al.*, (2007) who reported highly significant differences for agronomic characters in Ethiopian durum wheat germplasm.

Table 1. F values and means for 18 morphological characters in durum wheat F values (upper line) and Means (lower line).

	Year 1	Year 2	Var*Year
Days to germination	116.6** 20.47	18.69** (20.16)	30.97** --
Germination percentage	16.5** 47.32	16.63** 43.87	14.45** ---
days to spike emergence	30.03** 26.18	59.81** 47.39	46.01** ---
Plant Height	27.53** 102.64	17.03** 101.54	23.14** ---
Spike length	11.47** 8.99	8.62** 9.42	10.27** ---
No of tillers per plant	93.47** 7.16	61.19** 6.81	70.15** ---
Peduncle length	23.10** 84.64	15.74** 83.62	20.77** ---
Leaf angle	10.14** 59.65	11.98** 60.21	10.42** ---
Seed per spike	456.59** 56.15	416.13** 55.36	368.27** ---
Spikelet per spike	362.15** 18.72	323.05** 18.46	292.57** ---
Plant thickness	33.55** 0.49	35.33** 0.49	32.36** ---
Grain yield	28.41** 347.23	23.78** 312.27	27.81** ---
1000 grain weight	38.63** 51.86	104.46** 50.98	55.91** ---
Grain weight per spike	443.61** 2.83**	343.85** 2.72	309.26** ---
Harvest index	292.6** 30.14	187.11** 29.54	165.41** ---
Days to 50% heading	172.09** 154.44	19.52** 154.44	39.39** ---
No of effective tillers	115.07** 6.69	66.70** 6.73	79.65** ---
Days to 90% maturity	46.82** 180.87	23.12** 181.73	24.77** ---

**=Significant at $p < 0.01$

Correlation coefficients were calculated for all the possible combinations using mean values over the two years (Table 2). Significant ($p < 0.05$) negative correlations were observed among days to germination - number of effective tillers per plant; germination percentage - number of tillers per plant; germination percentage - grain yield per square meter; germination percentage - number of days to 90% maturity; days to spike emergence - number of days to 50% heading; days to spike emergence - number of effective tillers per plant; days to spike emergence - number of days to 90% maturity; plant height - peduncle length; number of tillers per plant - grain yield per square meter; number of tillers per plant - number of effective tillers per plant; number of seeds per spike - number of spikelets per spike; number of seeds per spike - grain yield per square meter; number of seeds per spike -1000 grain weight; number of seeds per spike - grain weight per spike; number of seeds per spike - harvest index; number of spikelets per spike - grain yield per square meter; number of spikelets per spike -1000 grain weight; number of spikelets per spike - grain weight per

spike; number of spikelets per spike - harvest index; grain yield per square meter -1000 grain weight; grain yield per square meter - grain weight per spike; grain yield per square meter - harvest index; grain yield per square meter - number of effective tillers per plant; grain yield per square meter - number of days to 90% maturity; grain weight per spike - harvest index; number of days to 50% heading - number of days to 90% maturity. Significant (at $p < 0.05$) positive correlation was observed among days to germination - number of days to 50% heading; days to germination - number of days to 90% maturity; germination percentage - days to spike emergence; germination percentage - spike length; germination percentage - leaf angle; germination percentage - number of days to 50% heading; days to spike emergence - plant height; days to spike emergence - spike length; days to spike emergence - peduncle length; plant height - number of days to 50% heading; spike length - number of days to 50% heading; peduncle length - number of days to 50% heading.

Table 2. Correlation coefficient among 18 morphological characters studied.

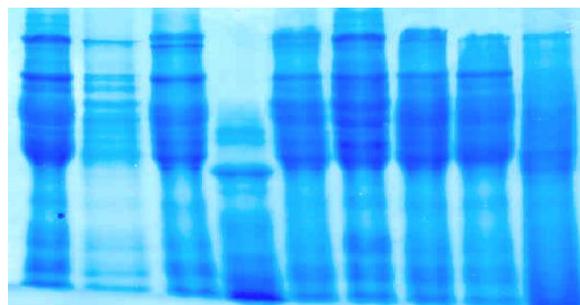
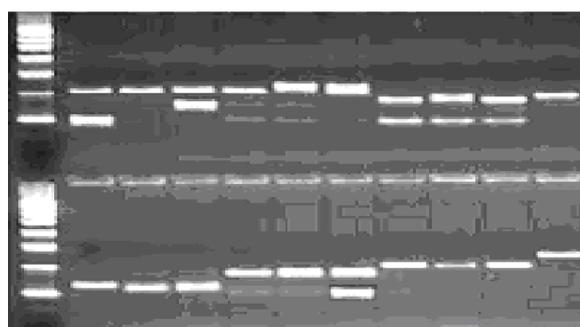
0.03													
-0.13	-0.15												
0.05	-0.05	-0.27											
0.02	-0.16	-0.16	0.11										
0.14	0.18	0.02	0.04	0.07									
0.05	-0.02	-0.23	0.97	0.14	0.06								
0.01	-0.18	0.05	0.04	-0.03	0.05	0.04							
0.09	-0.03	-0.05	0.07	-0.05	0.01	0.09	0.06						
0.09	-0.03	-0.04	0.07	-0.05	-0.01	0.09	0.06	1.00					
0.10	0.09	-0.03	0.02	0.08	-0.01	-0.01	0.07	0.09	0.07				
0.10	0.58	-0.11	0.08	-0.10	0.59	0.11	-0.07	0.49	0.53	-0.07			
-0.03	0.05	0.02	-0.06	0.02	-0.01	-0.06	0.04	0.40	0.49	0.02	0.20		
0.08	-0.03	0.05	0.07	-0.05	0.01	0.09	0.06	1.00	0.90	0.09	0.49	0.40	
0.08	-0.04	0.07	0.11	0.06	0.03	0.13	0.04	0.83	-0.82	-0.02	0.47	0.43	
	0.93												
-0.17	-0.15	0.94	-0.24	-0.151	0.01	-0.21	0.04	-0.04	-0.04	-0.10	-0.12	0.01	
	-0.04	-0.07											
0.14	0.13	0.15	-0.01	0.07	0.97	0.02	0.06	0.01	0.01	-0.04	0.53	-0.01	
	0.01	0.01	0.13										
-0.23	0.18	0.19	-0.07	-0.06	0.10	-0.05	-0.10	0.07	0.07	0.12	0.18	0.03	
	0.07	0.09	0.20	0.07									

Numbers in red indicate significant ($P < 0.05$) values. Var 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18 stands for days to germination, germination percentage, days to spike emergence, plant height, spike length, number of tillers per plant, peduncle length, leaf angle, number of seeds per spike, number of spikelets per spike, plant thickness, grain yield per square meter, 1000 grain weight, grain weight per spike, harvest index, number of days to 50% heading, number of effective tillers per plant and number of days to 90% maturity, respectively.

Table 3. Range of GD based on SDS-PAGE, RAPD and SSR analyses.

Assay Procedure	Genetic distance (%)	
	Minimum Estimated GD	Maximum estimated GD
SDS-PAGE	0	100
RAPD	0	32
SSR	2	38

Germplasm accessions were characterized on the basis of total seed storage proteins using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). An example of SDS-PAGE analysis is presented in Figure 1. High ranges of genetic distances (GD = 0 – 100%) was observed among the various pair wise comparisons (Table 3). One hundred and twenty seven comparisons showed 0% while 252 comparisons showed 100 % genetic differences for the protein loci among the durum genotypes used during present study.

**Fig. 1.** SDS-PAGE analysis of seed storage proteins from durum accessions.**Fig. 2.** Representative gel of amplification of durum wheat accessions using SSR primer set *xgwm16-5d*.

Durum accessions were also used for characterization on the basis of RAPD and SSR primers. An example of PCR assay of durum wheat accession is presented in Figure 2. Most of the primers used during present study were polymorphic. An average of about 3.44 DNA bands per primer per accession were amplified. The number of bands that a primer yielded in the

study ranged from 2 to 7. The genetic distance for RAPD and SSR data using durum wheat accessions was constructed based on Nei and Li (1979). The value of genetic distance ranging from 0.00 to 32% (RAPD based) and 2-38 % (SSR based) was observed among the wheat accessions (Table 3).

The knowledge about the genetic variation among the genotypes provides useful information to address breeding programs and germplasm resource management. In this study, morphological data analysis of the durum wheat cultivars was coupled with seed storage protein and molecular analysis (SSR and RAPD markers) to investigate the genetic diversity among durum wheat accessions. The genotypes showed diverse morphological traits and distinct marker pattern. It has been concluded from the present results that seed storage protein, RAPD and SSR can be used successfully for identification of genetic diversity and the relationship between the members of the collection.

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