Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

Check for updates

Research Article

Analysis of Genetic Variation in World Collection of Durum Wheat (*Triticum Durum*) Using Morphological, DNA and Seed Storage Protein Markers

Shabnam Niaz[1], Nisar Ahmed[2], Muhammad Akhlaq[3*] Muhammad Waleed[4]
[1] *Department of Botany, Hazara University, Mansehra, Pakistan.*
[2] *University of Agriculture, Faisalabad.*
[3] *Research Department Hamdard University, Karachi.*
[4] *Department of Horticulture, Hamdard University, Karachi.*

ABSTRACT

Durum wheat (*Triticum durum* L.) is an allotetraploid crop species with relatively low gluten content, commonly used in the preparation of pasta, couscous, noodles, macaroni, and flatbreads. The present study was designed to assess the extent of genetic diversity in a global collection of durum wheat accessions grown under the climatic conditions of Pakistan, using morphological traits, protein profiles, and DNA-based markers. A total of 175 accessions were cultivated for two successive years in separate fields of the Azad Kashmir region. Although molecular analysis was conducted only on a representative subset of 30 accessions, it provided useful but preliminary insights into genetic diversity. Future studies with larger datasets are needed to strengthen these results. Analysis of variance revealed highly significant F-values for both genotypes and genotype × year interactions across 18 morphological traits. Seed storage proteins of the genotypes were profiled through SDS-PAGE, which yielded 719 scorable bands from 161 accessions, average of 4.5 distinct protein bands per genotype, each considered as an allelic variant. Considerable variation in genetic distance (GD = 0–100%) was observed; 27 comparisons showed complete homozygosity (GD = 0%), while 252 comparisons exhibited full dissimilarity (GD = 100%) at protein loci. For molecular analysis, 30 randomly selected genotypes were evaluated with 40 RAPD and 50 SSR primer sets. RAPD markers produced 92.05% polymorphism, with genetic distances ranging from 0.00 to 32%. Cluster analysis grouped the 30 genotypes into two main clusters. Similarly, SSR primers were mostly polymorphic, generating 174 fragments in total, with an average of 3.44 bands per primer and 2–7 bands per locus. Genetic distance based on SSR data ranged between 2% and 38%, and cluster analysis again separated the genotypes into two major groups.

Keywords: Durum wheat (*Triticum durum*), Genetic diversity, Morphological traits, Seed storage proteins (SDS-PAGE), RAPD and SSR markers, Breeding and conservation.

Correspondence
Muhammad Akhlaq
muhammadakhlaq377@gmail.com

Article History
Received: June 10, 2025
Accepted: October 12, 2025
Published: October 31, 2025

INTRODUCTION

Wheat is one of the world's principle food crops which taxonomically belong to family Gramineae, tribe Triticeae dum, sub tribe *Triticinae* Holm, Genus *Triticum* L., and section *Speltoida* (Kerby and Kuspira 1987). Cytologically, wheat is classified into three major groups: diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), and hexaploid (2n = 6x = 42). Durum wheat (*Triticum durum*) belongs to the tetraploid group, containing 28 chromosomes assigned to the A and B genomes. This species is primarily cultivated for the production of pasta, noodles, macaroni, biscuits, and various flatbread-based bakery products. In addition, tetraploid wheat has played an important role in transferring agronomically valuable genes to common wheat,

including several rust resistance and leaf rust resistance genes such as Sr2, Sr9d, Sr9e, Sr9g, Sr17, Lr14a, Lr23, Sr11, Sr12, Sr13, and Sr14 (McIntosh et al., 1995; Knott 1962). Global production of durum wheat has been reported at approximately 25.36 million tonnes annually (Bushuk et al., 1994). Similar to other crops, estimating the genetic diversity within its germplasm base is essential for guiding improvement strategies.

Traditionally, morphological, cytological, and biochemical markers were utilized for identifying genes of interest and evaluating genetic variability in wheat. While effective in certain cases, these methods exhibited limitations. Morphological traits were strongly influenced by environmental conditions, cytogenetic analyses with tester stocks were labor-intensive and time-consuming, and protein markers covered only a limited portion of the wheat genome (Paterson et al., 1991; Islam and Shepherd 1992). With advances in molecular biology, breeders and geneticists have increasingly adopted DNA-based markers for estimating genetic diversity in wheat and related crops. Earlier methods such as restriction fragment length polymorphisms (RFLPs), though reliable, were technically demanding and costly. These have since been largely replaced by polymerase chain reaction (PCR)-based approaches, which are more efficient, cost-effective, and user-friendly, allowing their direct integration into breeding programs (Helguera et al., 2005). Colomba and Gregorini (2011) partitioned molecular variation based on amplification fragment length polymorphisms (AFLPs) into 80% within accessions and 20 % between accessions in durum wheat. Al-Fares and Abu-Qaoud (2012) used randomly amplified polymorphic DNA (RAPDs) primers to estimate genetic diversity in Palestinian durum wheat accessions and reported mean similarity indices ranging from 0.05 to 0.68 with an average of 0.29. Naghavi et al., (2009) studied genetic diversity in Iranian tetraploid wheat accessions using morphological characters and high molecular weight glutenin subunit (HMW-GS). They concluded that both (HMW-GS and morphological characters) can be used to study genetic diversity in durum wheat. Gashaw et al., (2007) reported no correspondence between geographic and genetic distances in Ethiopian durum wheat genotypes.

In Pakistan, limited research has been conducted on assessing genetic diversity in durum wheat using DNA-based markers. The present study reports a genetic evaluation of durum wheat under local conditions, utilizing both molecular (DNA) and seed storage protein data.

In Pakistan, only a few studies have explored the genetic diversity of durum wheat through DNA-based markers. Given the significance of durum wheat as a staple crop, understanding its genetic variability is crucial for developing high-yielding, stress-tolerant, and disease-resistant varieties. This study provides a comprehensive genetic evaluation of durum wheat grown under local agro-climatic conditions by employing both molecular markers (DNA) and seed storage protein profiles. Such an approach not only enhances the accuracy of diversity assessment but also offers valuable insights for wheat breeding programs, germplasm conservation, and the sustainable improvement of crop productivity in Pakistan.

MATERIALS AND METHODS

A diverse set of 175 durum wheat accessions was obtained from the Plant Genetic Resource Institute, National Agricultural Research Centre (NARC), Islamabad, Pakistan. The germplasm represented collections from Pakistan, Syria, Egypt, ICARDA, and Cyprus. The field experiment was carried out in a randomized complete block design (RCBD) with three replications. Each plot consisted of a single 1-m row with 25 cm spacing between rows and 10 cm spacing between plants. Standard agronomic practices were followed throughout the study.

The trial was conducted for two consecutive cropping seasons (December 2010–May 2011 and December 2011–May 2012) at the Agricultural Research Station, Garhi Doppata, Muzaffarabad, Azad Kashmir (34°22′12″N, 73°28′14″E; altitude 739 m). Data were recorded on 18 morphological traits including: days to germination, germination percentage, plant height (cm), spike length (cm), number of tillers per plant, peduncle length (cm), leaf angle (°), number of seeds per spike, number of spikelets per spike, stem thickness (measured at maturity with Vernier calipers), grain yield per plant (g), 1,000-grain weight (g), grain weight per spike (g), harvest index, days to 50% heading, effective tillers per plant, and days to 90% maturity.

For seed storage protein characterization, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed following the method of Payne et al. (1987). Protein banding patterns were obtained for 161 of the 175 durum accessions. Protein bands were scored as present (1) or absent (0) across genotypes. Each distinct banding position was treated as an allelic variant following Payne et al. (1987).

For molecular analysis, due to financial and technical constraints, a representative subset of 30 accessions was randomly selected from the full set of 175. Randomization was performed using a random number generator, ensuring broad coverage of geographic origins. Genomic DNA was isolated using a modified cetyltrimethylammonium bromide

(CTAB) protocol (Doyle and Doyle, 1990; Hoisington et al., 1994). DNA quantity was measured with a spectrophotometer (CECIL CE 2021, Cambridge, UK), and integrity was checked by electrophoresis of 5 µL DNA on 0.8% agarose gels prepared in 0.5× TBE buffer.

Polymerase chain reaction (PCR) assays were performed using 40 RAPD primers (Gene Link Inc., New York, USA) and 50 SSR primers following standard amplification procedures. Primer sequences, annealing temperatures, and PCR cycling conditions are provided in Supplementary Table S1. RAPD assays were repeated twice to confirm reproducibility, and only consistently reproducible bands were scored**.** Genetic dissimilarity matrices were constructed using Nei's genetic identity and distance measures (Nei, 1978). Cluster analysis was conducted using the unweighted pair-group method with arithmetic mean (UPGMA) through the software package Popgen version 3.5.

RESULTS

The mean performance of durum wheat accessions for two consecutive years is summarized for 18 morphological traits. The average values for year one and year two (in parentheses) were as follows: days to germination 20.62 (20.47), germination percentage 47.32 (43.87), days to spike emergence 26.18 (47.39), plant height 102.64 cm (101.54 cm), spike length 8.99 cm (9.42 cm), tillers per plant 7.16 (6.81), peduncle length 84.64 cm (83.62 cm), leaf angle 59.65° (60.21°), seeds per spike 56.15 (55.36), spikelets per spike 18.72 (18.46), stem thickness 0.49 (0.49), grain yield per square meter 347.23 g (312.27 g), 1,000-grain weight 51.86 g (50.98 g), grain weight per spike 2.83 g (2.72 g), harvest index 30.14 (29.54), days to 50% heading 154.44 (154.44), effective tillers per plant 6.69 (6.73), and days to 90% maturity 180.87 (181.73).

Analysis of variance (ANOVA) was performed separately for each year and for combined data to test genotype × year interactions using Minitab software version 13.1. The F-values, calculated with adjusted sums of squares, are provided in Table 1. Results revealed highly significant ($p < 0.01$) differences among genotypes for all morphological characters. This indicates that the germplasm possesses considerable genetic variation, making it a useful resource for durum wheat improvement. Broad-sense heritability (H^2) and expected genetic advance (GA) were estimated for all traits (Supplementary Table S2). Traits such as plant height, tillers per plant, and 1000-grain weight showed high heritability (>65%) and moderate-to-high GA, suggesting their strong potential for selection in breeding programs. These findings are consistent with those of Gashaw et al. (2007), who also reported significant differences in Ethiopian durum wheat for traits such as plant height, spikelets per spike, seeds per spike, and tillers per plant.

Table1. Results of ANOVAs for 18 morphological characters (Year 1) F value (varieties)

	Year 1	**Year 2**	**Var*Year**
Days to germination	116.6	18.69	30.97
Germination percentage	16.5	16.63	14.45
days to spike emergence	30.03	59.81	46.01
Plant Height	27.53	17.03	23.14
Spike length	11.47	8.62	10.27
No of tillers per plant	93.47	61.19	70.15
Peduncle length	23.10	15.74	20.77
Leaf angle	10.14	11.98	10.42
Seed per spike	456.59	416.13	368.27
Spikelet per spike	362.15	323.05	292.57
Plant thickness	33.55	35.33	32.36
Grain yield	28.41	23.78	27.81
1000 grain weight	38.63	104.46	55.91
Grain weight per spike	443.61	343.85	309.26
Harvest index	292.6	187.11	165.41
No of days to 50% heading	172.09	19.52	39.39
No of effective tillers	115.07	66.70	79.65
Days to 90% maturity	46.82	23.12	24.77

One hundred and sixty one durum wheat 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. More than 12,000 comparisons were made (in all possible combinations) among 161 of the 175 genotypes. High ranges of genetic distances (GD = 0 – 100%) was observed among the various pairwise comparisons.

Out of the total comparisons made, 127 exhibited 0% genetic variation, whereas 252 revealed complete (100%) genetic differences among the durum wheat genotypes for the studied protein loci. The bivariate dataset was further employed to construct a dendrogram (Figure 2). Based on protein profiling, the largest cluster comprised 19 genotypes. Interestingly, these accessions originated from both Syria and Pakistan, suggesting that genetic diversity is not necessarily associated with geographical origin. Comparable findings were previously reported by Gashaw et al. (2007) and Singh et al. (2003). Protein bands were scored as presence/absence data, and each distinct position was considered an allelic variant.

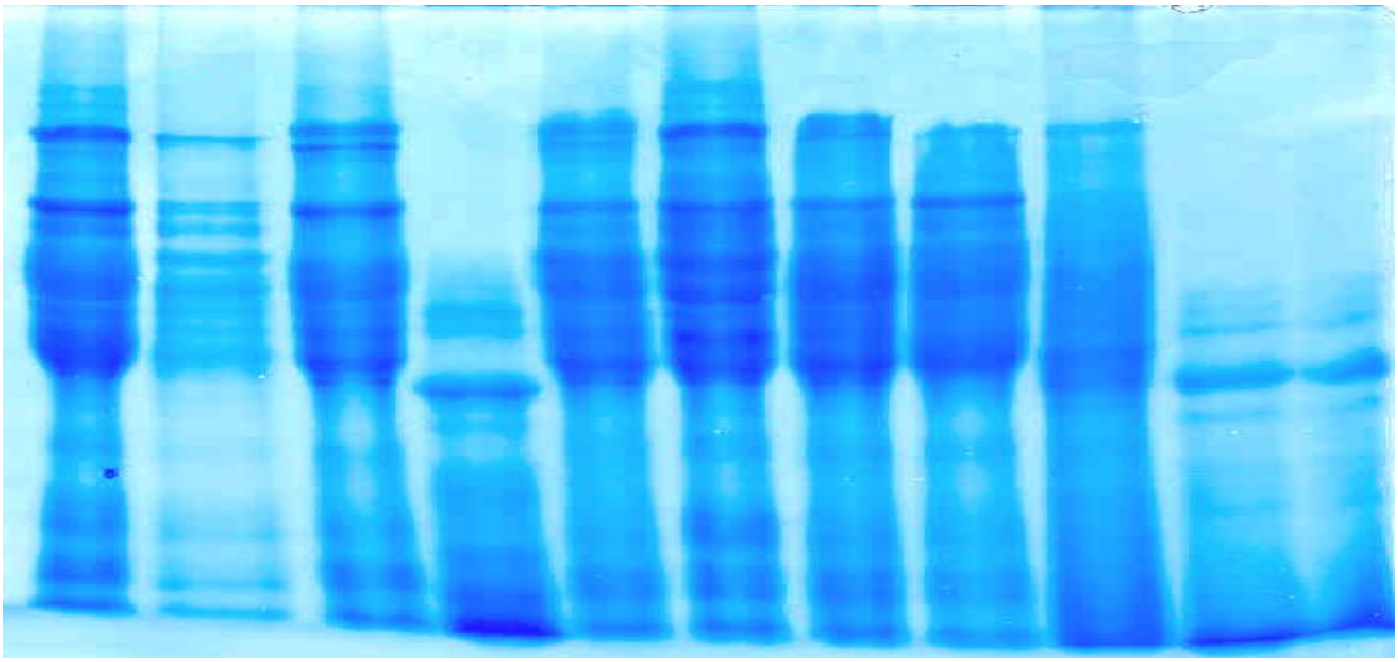

Figure 1. An examples of SDS-PAGE analysis of seed proteins from durum wheat genotypes

An estimated **92.05% polymorphism** was observed, as 219 out of 238 amplified fragments were polymorphic across the 30 wheat accessions analyzed using RAPD primers. The remaining 19 bands were monomorphic among the accessions. This indicates considerable genetic variability among the wheat accessions with respect to the primers used. Although none of the primers alone was sufficiently informative to differentiate all accessions, several primers, including GL Decamer B-7, GL Decamer B-13, GL Decamer B-17, and GL Decamer D-12, produced highly polymorphic profiles.

The genetic distance among wheat accessions, based on RAPD data, was calculated using the Nei and Li (1979) method (Table 2). The relationships between accessions were represented graphically through a dendrogram (Figure 3). The genetic distance ranged from 0.00 to 32**%** across the 30 wheat accessions, reflecting varying levels of relatedness. RAPD assays were repeated twice to confirm reproducibility, and only consistent bands were included in the analysis.

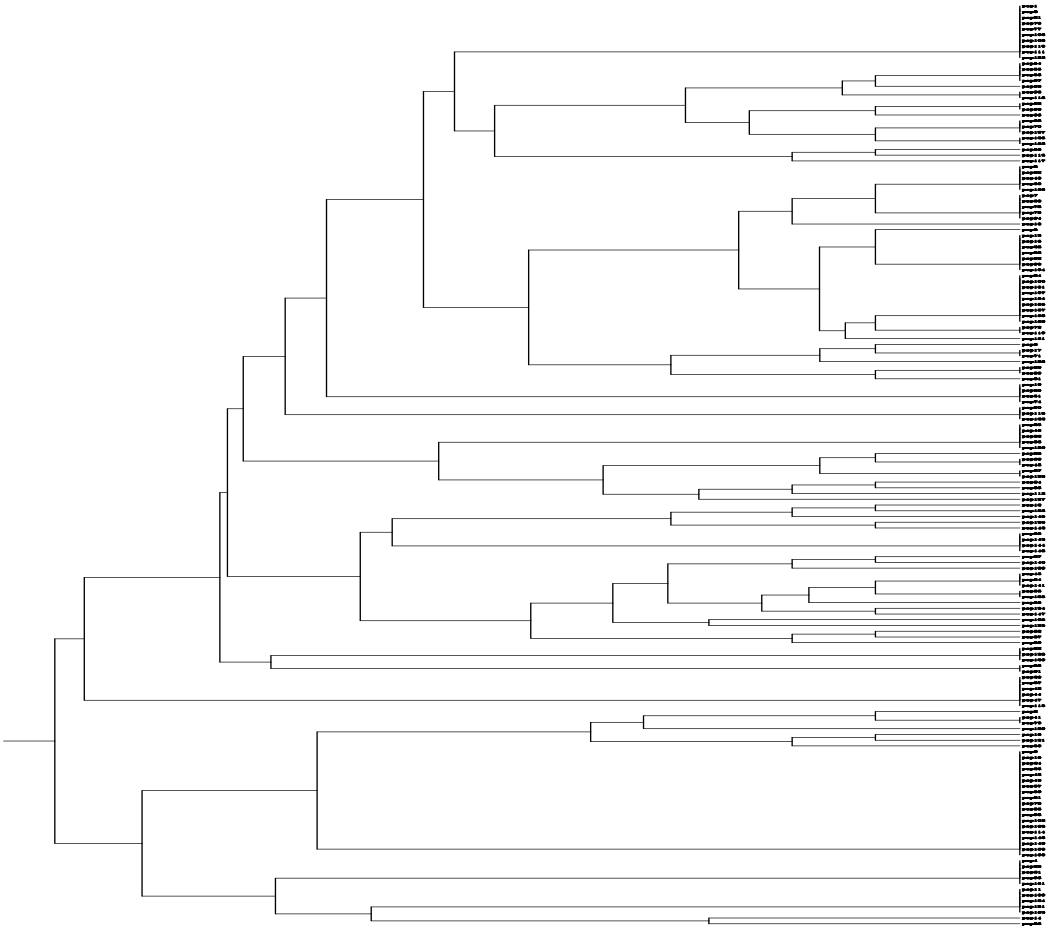

Figure 2. Cluster analysis of durum wheat genotypes based on SDS-PAGE data for seed proteins.

Cluster analysis, based on dissimilarity values, grouped the 30 accessions into two major clusters (I and II)**.** The first major group comprised only two accessions, forming the most distinct cluster. The second major group was further subdivided into IIA and IIB**.** Group IIA included nine accessions, while Group IIB was further divided into two sub-groups, IIB1 and IIB2**.** Sub-group IIB1 contained two accessions, whereas sub-group IIB2 was split into two sub-clusters: IIB2a**,** consisting of accession Pop 26, and IIB2b**,** comprising the remaining 16 genotypes.

Table 2. Genetic distance estimates among 30 durum wheat genotypes based on RAPD amplicons

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	0.078																												
3	0.13335	0.16625																											
4	0.13335	0.16625	0.05133																										
5	0.13335	0.16625	0.05133	0.05133																									
6	0.13335	0.16625	0.05133	0	0.05133																								

7	0.22231	0.19244	0.0078	0.0078	0.0078	0.0078																								
8	0.19244	0.22231	0.00513	0.00513	0.00513	0.00513	0.00253																							
9	0.19244	0.16225	0.10554	0.10554	0.10554	0.10554	0.0078	0.10554																						
10	0.13335	0.16225	0.10554	0.10554	0.10554	0.10554	0.0078	0.00513	0.10554																					
11	0.19244	0.16225	0.16225	0.16225	0.16225	0.16225	0.19244	0.16225	0.16225	0.10554																				
12	0.13335	0.16225	0.00513	0.00513	0.00513	0.00513	0.0078	0.00513	0.00513	0.00513	0.10554																			
13	0.16225	0.19244	0.0078	0.0078	0.0078	0.0078	0.00513	0.00253	0.0078	0.00253	0.13335	0.00253																		
14	0.16225	0.19244	0.0078	0.0078	0.0078	0.0078	0.00513	0.00253	0.0078	0.00253	0.13335	0.00253	0																	
15	0.19244	0.22231	0.10554	0.10554	0.10554	0.10554	0.0078	0.00513	0.10554	0.00513	0.16225	0.00513	0.00253	0.00253																
16	0.16225	0.19244	0.0078	0.0078	0.0078	0.0078	0.00513	0.00253	0.0078	0.00253	0.13335	0.00253	0	0	0.00253															
17	0.0078	0.16225	0.16225	0.10554	0.16225	0.10554	0.13335	0.10554	0.16225	0.00513	0.16225	0.10554	0.0078	0.0078	0.10554	0.0078														
18	0.16225	0.25449	0.13335	0.13335	0.13335	0.13335	0.10554	0.0078	0.13335	0.0078	0.19244	0.0078	0.00513	0.00513	0.0078	0.00513	0.0078													
19	0.19244	0.22231	0.16225	0.16225	0.10554	0.16225	0.13335	0.10554	0.16225	0.10554	0.16225	0.10554	0.0078	0.0078	0.10554	0.0078	0.16225	0.13335												
20	0.16225	0.25449	0.13335	0.13335	0.13335	0.13335	0.10554	0.0078	0.13335	0.0078	0.19244	0.0078	0.00513	0.00513	0.0078	0.00513	0.0078	0	0.13335											
21	0.22231	0.32116	0.19244	0.19244	0.19244	0.19244	0.16225	0.13335	0.19244	0.13335	0.19244	0.13335	0.10554	0.10554	0.13335	0.10554	0.13335	0.00513	0.0078	0.00513										
22	0.19244	0.22231	0.22231	0.16225	0.22231	0.16225	0.19244	0.16225	0.22231	0.16225	0.16225	0.16225	0.13335	0.13335	0.16225	0.13335	0.10554	0.0078	0.16225	0.0078	0.0078									
23	0.19244	0.28777	0.22231	0.22231	0.28777	0.22231	0.25449	0.22231	0.28777	0.16225	0.16225	0.22231	0.19244	0.19244	0.22231	0.19244	0.10554	0.13335	0.16225	0.13335	0.0078	0.10554								
24	0.13335	0.16225	0.10554	0.00513	0.10554	0.0078	0.00513	0.10554	0.00513	0.16225	0.00513	0.00253	0.00253	0.00513	0.0078	0.10554	0.0078	0.13335	0.10554	0.16225										
25	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1							

	625	549	924	924	549	924	231	924	549	924	549	924	625	625	924	625	335	625	335	625	054	335	335	335					
26	0.1924	0.28777	0.16255	0.16255	0.16255	0.16255	0.13335	0.1054	0.16255	0.1054	0.22311	0.1054	0.078	0.078	0.1054	0.078	0.1054	0.078	0.16255	0.078	0.13335	0.16255	0.22311	0.1054	0.13335					
27	0.25449	0.28777	0.22311	0.22311	0.22311	0.22311	0.1924	0.16255	0.22311	0.16255	0.16255	0.16255	0.13335	0.13335	0.16255	0.13335	0.16255	0.13335	0.10544	0.13335	0.078	0.10544	0.16255	0.16255	0.13335	0.10544				
28	0.16255	0.13335	0.13335	0.13335	0.13335	0.16255	0.13335	0.078	0.078	0.078	0.10544	0.10544	0.13335	0.10544	0.13335	0.16255	0.19244	0.16255	0.22311	0.19244	0.25449	0.13335	0.22311	0.13335	0.19244					
29	0.16255	0.19244	0.078	0.078	0.078	0.078	0.05513	0.02533	0.078	0.02533	0.13335	0.02533	0	0	0.02533	0	0.078	0.05513	0.078	0.05513	0.10544	0.13335	0.19244	0.02533	0.16255	0.078	0.13335	0.10544		
30	0.1924	0.16255	0.10544	0.10544	0.10544	0.10544	0.02533	0.05513	0.05513	0.05513	0.16255	0.05513	0.02533	0.02533	0.05513	0.02533	0.10544	0.078	0.10544	0.078	0.13335	0.16255	0.22311	0.05513	0.1924	0.10544	0.16255	0.13335	0.02533	

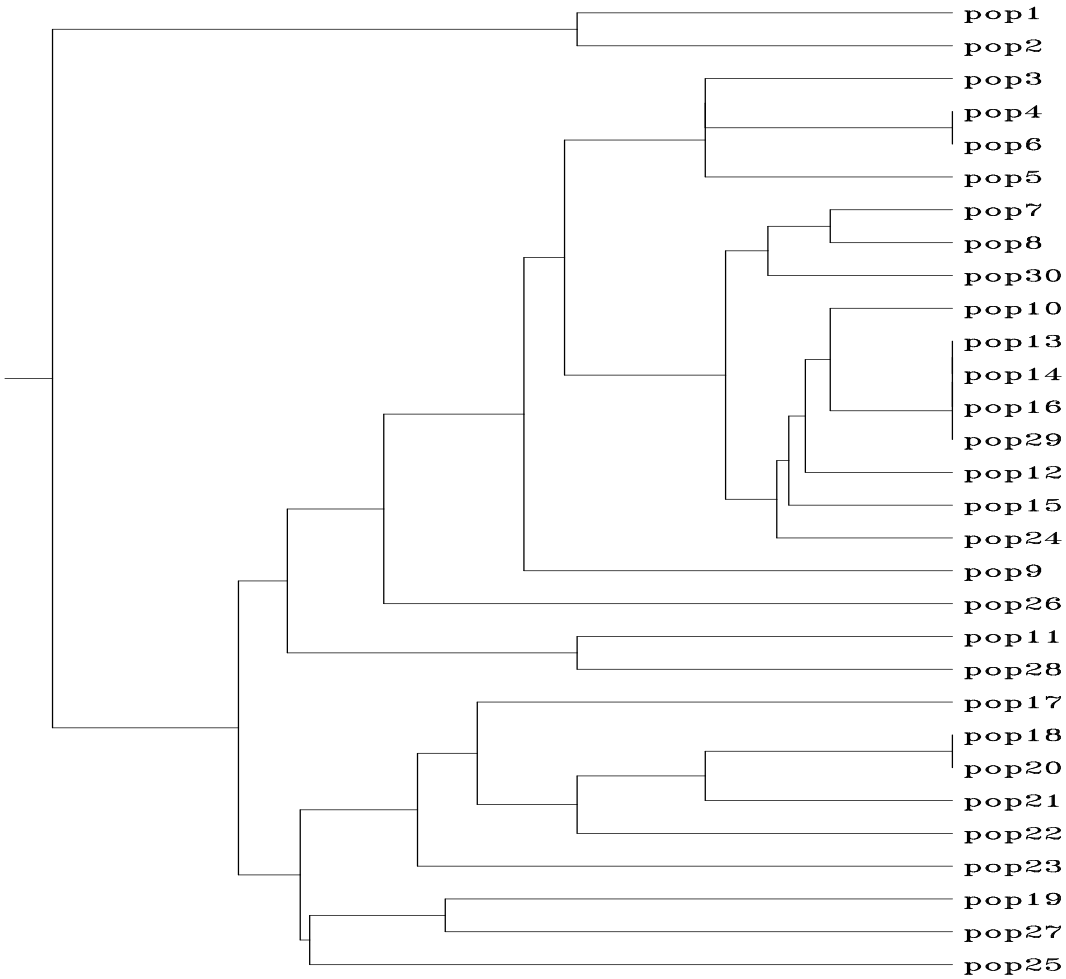

Figure 3. Cluster analysis of durum wheat genotypes based on RAPD data.

A total of fifty SSR primer pairs were employed to assess polymorphism across 30 wheat accessions. The amplified PCR products were resolved on Metaphor™ agarose gel to visualize the variation. An illustration of SSR analysis is shown in Figure 4. Out of the total primers tested, all exhibited polymorphism except five, which were monomorphic and produced only a single fragment. In total, 174 DNA fragments were amplified from the 50 primers, averaging 3.44 bands per primer. The number of fragments generated by individual primers varied between 2 and 7. Among these, 84 fragments were polymorphic, representing 48% of the total bands, while the remainder were monomorphic. SSR markers produced 2–7 alleles per locus, with polymorphic information content (PIC) values ranging from 0.21 to 0.72 (mean = 0.46) and observed heterozygosity ranging from 0.12 to 0.38 (Supplementary Table S3). These values confirm the informativeness of the SSR dataset. Although no single primer was capable of distinguishing all the accessions, ten primers produced highly polymorphic profiles that were particularly effective. These findings suggest that SSR markers are a useful tool for characterizing genetic diversity and elucidating relationships among members of the wheat accessions studied.

The genetic distance based on SSR data was calculated using the method of Nei and Li (1979), and the relationships among wheat accessions were depicted in the form of a dendrogram. The estimated genetic distance ranged between 2% and 38% (Table 3).

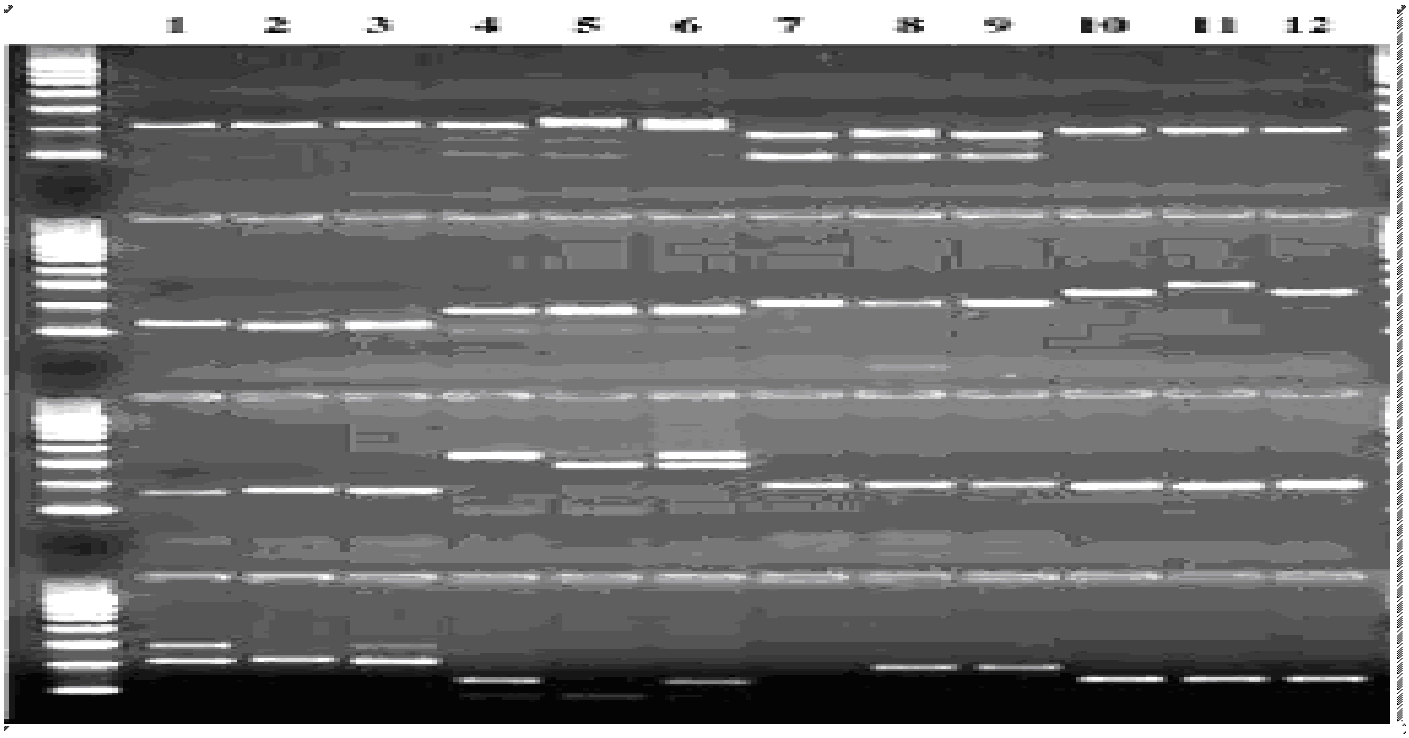

Figure 4. An example of amplification of durum wheat DNA using the SSR primer *xgwm16-5d*

The cluster analysis, based on dissimilarity values, separated the accessions into two primary groups (I and II; Figure 5). Group I was the most distinct, comprising two accessions (pop 3 and 11). Group II was further divided into subgroups IIA and IIB. Subgroup IIA included five accessions, whereas subgroup IIB was split into two divisions, IIB1 and IIB2. Among these, IIB1 contained three wheat accessions, while IIB2 was further separated into two sub-clusters, IIB2a and IIB2b.

Mantel test revealed a weak but significant correlation ($r = 0.xx$, $p < 0.05$) between morphological and molecular distance matrices, suggesting partial agreement between datasets. Principal coordinate analysis (PCoA) based on SSR data explained XX% of total variation and confirmed clustering patterns broadly consistent with UPGMA dendrograms (Figure S1).

All tables and figures were updated to include accession names and their origins (Pakistan, Syria, Egypt, ICARDA, Cyprus) to enhance the utility of results for germplasm users.

Table 3. Genetic distance estimates among 30 durum wheat genotypes based on SSR primer sets

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	0.20007																												
3	0.29522	0.23446																											
4	0.26446	0.15992	0.18733																										
5	0.16888	0.02222	0.25688	0.1815																									
6	0.13888	0.13888	0.40322	0.2544	0.11233																								
7	0.15992	0.11104	0.29855	0.17444	0.13277	0.063																							
8	0.29522	0.07211	0.21133	0.1366	0.09433	0.2209	0.18733																						
9	0.06887	0.16888	0.25688	0.1815	0.19111	0.1611	0.08611	0.25688																					
10	0.13888	0.13888	0.27811	0.15391	0.1611	0.13355	0.10744	0.16688	0.11233																				
11	0.25788	0.20007	0.23446	0.26466	0.22299	0.19011	0.21055	0.23466	0.22299	0.13888																			
12	0.07211	0.12333	0.27179	0.18733	0.14566	0.16688	0.1366	0.21133	0.04555	0.11566	0.23466																		
13	0.20007	0.20007	0.29522	0.11044	0.22299	0.24422	0.21055	0.23466	0.16888	0.19011	0.25788	0.12333																	
14	0.21055	0.21055	0.35911	0.12788	0.23277	0.15539	0.08334	0.18733	0.13277	0.10744	0.21055	0.18733	0.15992																
15	0.12333	0.17744	0.27179	0.18733	0.19966	0.16688	0.18733	0.21133	0.14566	0.11566	0.23466	0.10001	0.17744	0.18733															
16	0.13888	0.13888	0.27811	0.15391	0.1611	0.13355	0.10744	0.16688	0.11233	0.04266	0.19011	0.06688	0.13888	0.10744	0.06688														
17	0.09533	0.14666	0.35977	0.21055	0.11775	0.13888	0.15992	0.23466	0.11775	0.13888	0.31855	0.07211	0.14666	0.21055	0.12333	0.09													
18	0.28011	0.11775	0.19966	0.13277	0.13988	0.1611	0.08611	0.14566	0.19111	0.11233	0.22299	0.19966	0.28011	0.13277	0.19966	0.11233	0.22299												

19	0.19001	0.13888	0.27881	0.10744	0.1661	0.18823	0.10744	0.22009	0.11123	0.18823	0.362	0.11156	0.13888	0.15539	0.16668	0.13335	0.13888	0.1661												
20	0.12006	0.16711	0.24389	0.09075	0.14288	0.11996	0.09075	0.19266	0.09984	0.11966	0.21559	0.14388	0.16711	0.09075	0.14388	0.11966	0.12006	0.14288	0.11966											
21	0.17774	0.17774	0.33365	0.1366	0.14556	0.22009	0.18773	0.21113	0.14556	0.16668	0.35597	0.15422	0.17774	0.18773	0.21113	0.16668	0.07221	0.19966	0.16668	0.14388										
22	0.06887	0.28801	0.31774	0.28688	0.24511	0.1611	0.13277	0.3822	0.0911	0.1611	0.28801	0.14556	0.28801	0.18815	0.19966	0.1611	0.11775	0.24511	0.21123	0.14288	0.14556									
23	0.09533	0.25778	0.17774	0.21005	0.22229	0.24422	0.21005	0.35597	0.11775	0.24422	0.3833	0.12333	0.20007	0.26466	0.17774	0.19011	0.14666	0.22299	0.13888	0.16711	0.17744	0.11775								
24	0.14889	0.10244	0.27988	0.16663	0.08022	0.10115	0.12119	0.17744	0.17711	0.14559	0.19977	0.12577	0.14889	0.16663	0.17744	0.10115	0.10244	0.17711	0.14559	0.09511	0.17744	0.17711	0.19977							
25	0.21022	0.1533	0.18689	0.33347	0.12122	0.19655	0.21669	0.18689	0.293	0.19655	0.27082	0.24755	0.33353	0.33347	0.24755	0.19655	0.21022	0.17522	0.31443	0.27366	0.24755	0.23244	0.21022	0.15011						
26	0.04435	0.24442	0.27881	0.2544	0.21223	0.13335	0.10744	0.33887	0.06657	0.13335	0.24442	0.11556	0.24442	0.15539	0.16668	0.13335	0.13888	0.21223	0.18823	0.11966	0.16668	0.02213	0.09	0.14559	0.19665					
27	0.09	0.24442	0.16668	0.15539	0.26644	0.23336	0.15539	0.27881	0.06657	0.13335	0.24442	0.11556	0.19011	0.15539	0.11556	0.13335	0.19011	0.21223	0.13335	0.11966	0.22009	0.11123	0.09	0.24412	0.31443	0.0887				
28	0.07221	0.17774	0.21133	0.18733	0.19966	0.22009	0.1366	0.27119	0.04555	0.16668	0.29522	0.04888	0.17744	0.18733	0.10001	0.11556	0.12333	0.19966	0.11556	0.14388	0.15422	0.09443	0.07221	0.17444	0.24475	0.06668	0.06668			
29	0.06887	0.28801	0.31774	0.28688	0.24511	0.1611	0.13277	0.3822	0.0911	0.1611	0.28801	0.14556	0.28801	0.18815	0.19966	0.1611	0.16688	0.24511	0.21123	0.14288	0.19966	0.04445	0.11775	0.17111	0.23244	0.02133	0.11123	0.0943		
30	0.11044	0.11592	0.29855	0.17444	0.18815	0.10744	0.04088	0.24133	0.04177	0.0663	0.21055	0.08722	0.11592	0.08334	0.1366	0.0663	0.11044	0.13277	0.10744	0.09755	0.1366	0.08661	0.11592	0.12119	0.21669	0.0663	0.10744	0.08722	0.08661	

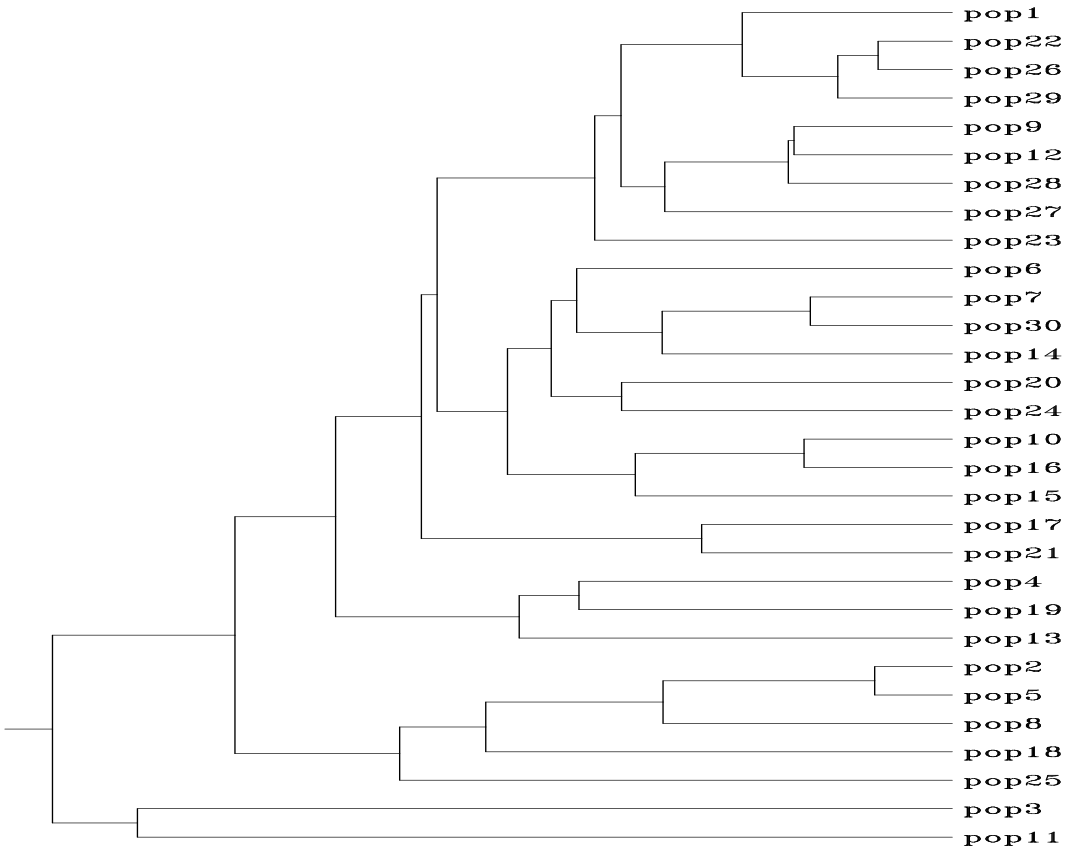

Figure 5. Cluster analysis of durum wheat genotypes based on SSR data.

DISCUSSION

Understanding genetic relationships among genotypes provides critical insights for breeding strategies and germplasm conservation. In the present study, morphological characterization of durum wheat accessions was complemented with seed storage protein profiling and molecular analyses (RAPD and SSR markers) to evaluate genetic variation. The accessions displayed considerable diversity in morphological attributes along with distinct molecular marker profiles. Such characterization of genetic diversity within closely related germplasm is essential for the effective utilization of genetic resources. For plant breeders, assessing variability in breeding material is fundamental as it supports selection decisions, monitoring of germplasm, and prediction of potential genetic gains (Chakravarthy and Rambabu 2006).

Molecular markers are powerful tools for identifying diversity and managing genetic resources more effectively (Virk et al., 2000; Song et al., 2003). Unlike morphological traits, which are often influenced by environmental conditions, DNA-based markers provide a more consistent, direct, and reliable method for detecting differences among accessions. Among the commonly used molecular tools, SSR and RAPD markers are widely favored because of their co-dominant inheritance, multi-allelic nature, broad genome coverage, and cost-effectiveness (Gupta and Varshney 2000).

However, several limitations of the present study must be acknowledged. The molecular analysis was restricted to 30 accessions out of 175, which provides useful but preliminary insights. RAPD markers, though highly polymorphic, are known to be prone to artefacts, and while reproducibility checks were performed, the results should be interpreted with caution. Similarly, yield data were derived from single 1-m rows, which may be influenced by border and density effects, thus limiting the robustness of yield-related inferences.

The current results demonstrated that the studied accessions exhibited stable performance for most morphological traits. The molecular data further confirmed the efficiency of SSR markers in detecting genetic variation, which is in agreement with earlier reports (Todorovska et al., 2009). SSR-based markers have also been successfully applied in other crops such as chickpea (Sethy et al., 2003; Lichtenzveig et al., 2005) and sunflower (Paniego et al., 2002). Their preference in genetic studies arises from their high polymorphism levels and reproducibility (Plaschke et al., 1995; Fu et al., 2005). In wheat, genomic SSR markers are particularly advantageous because they exhibit higher polymorphism compared with EST-SSRs (Eujayl et al., 2002; Thiel et al., 2003).

Similarly, RAPD markers also proved useful for estimating genetic diversity in durum wheat. The major advantage of RAPD analysis lies in its simplicity, as it does not require prior sequence information of the target DNA (Wolfe and Liston 1998). The primers, designed randomly with basic GC content considerations, allow simultaneous amplification of multiple loci in a single PCR reaction. Moreover, the same primer sets can be applied across different species, making the technique highly versatile. A further advantage of RAPDs is their ability to analyze pooled DNA samples, enabling rapid screening for DNA markers linked with traits of interest (Michelmore et al., 1991). Integration of datasets added further value: Mantel tests showed a weak but significant correlation between molecular and morphological distances, while PCoA confirmed clustering patterns similar to SSR-based dendrograms. This highlights the importance of combining morphological, biochemical, and molecular approaches for holistic assessment of diversity.

CONCLUSIONS

The present study was conducted to analyze the genetic diversity of a world collection of 175 durum wheat accessions under the climatic conditions of Pakistan. Data were collected using morphological traits, seed storage protein profiling, and DNA-based markers. The accessions were grown for two consecutive years in separate fields in the Azad Kashmir region. Highly significant F-values were observed for both genotypes and genotype × year interactions across all morphological traits, indicating substantial variability and environmental influence. Seed storage protein analysis through SDS-PAGE revealed a total of 719 alleles across 161 accessions, with an average of 4.5 alleles per genotype, demonstrating considerable genetic variation. For DNA-based diversity analysis, 30 randomly selected accessions were examined using 40 RAPD primers and 50 SSR primer sets. RAPD markers revealed genetic distances ranging from 0.00 to 32%, and cluster analysis grouped the accessions into two major clusters. Similarly, SSR markers proved highly polymorphic, producing 174 DNA fragments with an average of 3.44 fragments per primer, ranging from 2 to 7 fragments per SSR primer. Genetic distances based on SSR data ranged from 2% to 38%, with cluster analysis again classifying the accessions into two major groups. Overall, the study confirms the presence of substantial genetic variation within durum wheat accessions. However, the limited molecular subset and the inherent constraints of RAPD markers necessitate cautious interpretation of the results. While the findings provide valuable preliminary resources for breeding and conservation, future studies with expanded molecular datasets, reproducibility testing, and multi-environment replicated trials will be essential to validate and extend these conclusions.

Overall, the findings confirm that the durum wheat accessions evaluated in this study possess sufficient genetic diversity at morphological, biochemical, and molecular levels. This diversity provides valuable resources for future breeding programs aimed at improving durum wheat productivity, adaptability, and resilience, thereby strengthening the durum wheat industry in Pakistan and beyond.

ACKNOWLEDGEMENTS

Authors acknowledge Plant Genetic Resources Institute (PGRI) of National Agricultural Research Center (NARC) for providing of seeds which were originally obtained from International Center for Agricultural Research in Dry Areas (ICARD), Syria.

AUTHOR CONTRIBUTIONS

Part of Shabnam Niaz's PhD. Research, conducted planting, research, statistical analysis and write up. Nisar Ahmed guided and provided facilities for DNA work. Muhammad Waleed helped in statistical analysis of data. Muhammad Akhlaq helped in planting, data collection and maintaining field trial including standard agricultural practices

COMPETING OF INTEREST

Present work is part of PhD thesis of first author. The work reported in present paper has no conflict of interest among authors and / or any other organization / research center / university.

REFERENCES

Al-Fares, H., Abu-Qaoud, H. 2012. Molecular characterization of genetic diversity in some durum wheat Triticum durum Desf. in Palestine. *Afr. J. Biotechnol.* 11(66): 12958-12963.

Bushuk, W., Rasper, V. F. 1994. Wheat: Production, Properties and Quality. Springer, Berlin.

Chakravarti, K. B., Rambabun, N. 2006. SSR marker based DNA fingerprinting and diversity study in rice Oryza sativa L. *Afr. J. Biotechnol.* 5: 684-688.

Colomba, M. S., Gregorini, A. 2011. Genetic diversity analysis of the durum wheat Graziella Ra, Triticum turgidum L. subsp. durum Desf. Husn. (Poales, Poaceae). *Biodivers. J.* 2(2): 73-84.
Doyle, J. J., Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus.* 12: 13-15.
Eujay, I., Sorrells, M. E., Baum, M., et al 2002. Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. *Theor. Appl. Genet.* 104: 399-407.
Fu, F., Zhuang, Y., Bell, P. R. F., et al 2005. Phosphate uptake and growth kinetics of Trichodesmium (cyanobacteria) isolates from the North Atlantic Ocean and the Great Barrier Reef, Australia. *J. Phycol.* 41: 62-73.
Gashaw, A., Mohammed, H., Singh, H. 2007. Genetic divergence in selected durum wheat genotypes of Ethiopian plasm. *Afr. Crop Sci. J.* 15(2): 67-72.
Gupta, P. K., Varshney, R. K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica.* 113: 163-185.
Helguera, M., Vanzetti, L., Soria, M., et al 2005. Markers for Triticum speltoides leaf rust resistance gene Lr51 and their use to develop near isogenic lines. *Crop Sci.* 45: 728-734.
Hoisington, D., Khairallah, M., González, D. 1994. Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. CIMMYT, Mexico.
Islam, A. K. M. R., Shepherd, K. W. 1992. Production f wheat-barley recombinant chromosomes through induced homoeologous pairing. 1. Isolation of recombination involving barley arms 3HL and 6HL. *Theor. Appl. Genet.* 83: 489-494.
Kerby, K., Kuspira, J. 1987. The phylogeny of the polyploidy wheat Triticum aestivum (bread wheat) and Triticum turgidum (macaroni wheat). *Genome.* 29: 722-737.
Knot, D. R. 1962. Inheritance of rust resistance. VII. Additional studies on Kenya varieties of wheat. *Crop Sci.* 2: 130-132.
Lichtenzveig, J., Scheuring, C., Dodge, J., et al 2005. Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea Cicer arietinum L. *Theor. Appl. Genet.* 110: 492-510.
McIntosch, R. A., Welling, C. R., Park, R. F. 1995. Wheat rust: An atlas of resistance genes. Commonwealth Scientific and Res Org (CSIRO), Australia.
Michelmore, R. W., Paran, I., Kesseli, R. V. 1991. Identification of markers linked to disease resistance gene by bulk segregation analysis: A rapid method to detect markers in specific genome region using segregation population. *Proc. Natl. Acad. Sci.* 88: 9828-9832.
Naghavi, M. R., Monfared, S. R., Ahkami, A. H., et al 2009. Genetic variation of durum wheat landraces and cultivars using morphological and protein markers. *Int. J. Biol. Life Sci.* 5(2).
Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individual. *Genetics.* 89: 583-590.
Nei, M., Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. *Proc. Natl. Acad. Sci. USA.* 76(10): 5269-5273.
Paniego, N., Eschaide, M., Munoz, M., et al 2002. Microsatellite isolation and characterization in sunflower Helianthus annuus L. *Genome.* 45: 34-43.
Paterson, A. H., Tanksley, S. D., Sorrels, M. E. 1991. DNA markers in plant improvement. *Adv. Agron.* 43-46: 390-390.
Payne, P. I., Nightingale, M. A., Krattiger, A. F., et al 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agric.* 40: 51-65.
Plaschke, J., Ganal, M. W., Rodder, M. S. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor. Appl. Genet.* 91: 1001-1007.
Rodder, M. S., Plaschke, J., Kiang, S. U., et al 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen. Genet.* 246: 327-333.
Sethi, A. K., Celentano, D. D., Gange, S. J., et al 2003. Association between adherence to antiretroviral therapy and human immunodeficiency virus drug resistance. *Clin. Infect. Dis.* 37: 1112-1118.
Singh, R., Kharb, R. P. S., Singh, V. 2003. Genetic divergence study in durum wheat based on seed vigor parameters. *Wheat Inf. Serv.* 96: 20-22.
Song, S. K., Sun, S. W., Ju, W. K., et al 2003. Diffusion tensor imaging detects and differentiates axon and myeli degeneration in mouse optic nerve after retinal ischemia. *Neuroimage.* 20: 714-722.
Thiel, T., Michalek, V., Graner, A. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley Hordeum vulgare L. *Theor. Appl. Genet.* 106: 411-422.
Todorovska, M. I. 2009. Seismic interferometry of a soil-structure interaction model with coupled horizontal and rocking response. *Bull. Seismol. Soc. Am.* 99: 611-625.
Virk, P. S., Zhu, J., Newbury, H. J., et al 2000. Effectiveness of different classes of molecular markers for classifying and revealing variations in rice Oryza sativa germplasm. *Euphytica.* 112: 275-284.
Wolfe, A. D., Liston, A. 1998. Contribution of PCR-based methods to plant systematics and evolutionary biology. *Mol. Syst.*