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GENETIC DIVERSITY ANALYSIS IN DURUM WHEAT BASED ON SEED STORAGE PROTEIN MARKERS

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Abstract

Tetraploid wheat (Triticum durum L.), an allotetraploid species with the AABB genome (2n = 4x = 28), is a low-gluten wheat widely utilized in the production of bakery products such as flatbreads, pasta, noodles, couscous, and macaroni. The present study was undertaken to evaluate the extent of genetic variability among a global collection of T. durum accessions under the local climatic conditions of Azad Jammu and Kashmir. A total of 161 accessions were analyzed for seed storage protein diversity using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Substantial polymorphism was observed, indicating a high degree of genetic variability within the collection. These findings highlight the potential of T. durum germplasm as a valuable genetic resource for breeding programs aimed at developing improved cultivars with desirable agronomic and quality traits.

Keywords: *Cluster analysis, Durum wheat, Germplasm, Genetic diversity, SDS-PAGE, Total seed protein, Triticum durum*

INTRODUCTION

Wheat is one of the most important food crops globally, and among its types, durum wheat holds a special place as it is primarily used in the production of pasta, noodles, and bread. The annual world production of durum wheat is estimated at approximately 25,360 thousand tons (2). Understanding the extent of genetic variation within durum wheat is essential for its improvement, as it provides the foundation for breeding new and superior varieties. The present research is the first report on the estimation of genetic distance in durum wheat germplasm using total seed protein marker systems.

Traditionally, morphological and cytogenetic markers have been employed to estimate genetic distance. However, these approaches have significant limitations: morphological characters are strongly influenced by environmental conditions, while cytogenetic studies are often lengthy and technically demanding (9, 10). In earlier work, genetic variation in Iranian durum wheat varieties was assessed using High Molecular Weight Glutenin Subunits (HMW-GS) (5). The study concluded that variation at HMW-GS loci can serve as a useful marker system for evaluating genetic distances in durum wheat. Similarly, Gashaw et al. (6) analyzed Ethiopian durum wheat cultivars and reported that geographic distances and genetic distances are not necessarily correlated.

In Pakistan, no prior study has reported on the genetic diversity of durum wheat. Therefore, the present research aimed to analyze genetic variation in a global collection of durum wheat accessions, using seed storage proteins as the basis for diversity assessment.

MATERIALS AND METHODS

PLANT MATERIAL

A total of 161 accessions of durum wheat (*Triticum durum* L.) were obtained from the Plant Genetic Resource Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. These accessions were originally collected by the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, and represent diverse geographical regions including Pakistan, Syria, Egypt, and Cyprus.

PROTEIN EXTRACTION

The protocol initially described by Payne *et al.*, (1987) with minor modifications was used to study total seed storage proteins. Seeds of each accession were finely ground using a sterile mortar and pestle, and approximately 100 mg of powder was transferred into a 1.5 mL Eppendorf tube. Protein extraction buffer (12) was added, and the samples were incubated at room temperature for 5–6 hours with vortexing at hourly intervals. Following extraction, the tubes were placed in boiling water for 5 minutes, then centrifuged at 7000 rpm for 5 minutes. The supernatant containing extracted proteins was transferred to fresh tubes and stored at 4 °C until further use.

SDS-PAGE ANALYSIS

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed using a vertical gel electrophoresis apparatus (Bio-Rad, USA). 38 mL Tris buffer (pH 8.8), 25 mL acrylamide solution, 700 µL 10% SDS, 1.5 mL 10% APS, and 30 µL TEMED were mixed and poured between glass plates. The gel was allowed to polymerize at room temperature for 1 hour. 3.5 mL distilled water, 1.3 mL acrylamide solution, 600 µL Tris buffer (pH 6.8), 50 µL 10% SDS, 1.5 mL 10% APS, and 30 µL TEMED were mixed and layered on top of the separating gel. Wells were created with a comb, and the gel was allowed to polymerize for ~30 minutes. For each sample, 10 µL of extracted protein was mixed with 2 µL loading dye and loaded into wells. Electrophoresis was carried out at a constant voltage of 70 V for 3–4 hours until the tracking dye reached the bottom.

GEL STAINING AND VISUALIZATION

Upon completion of electrophoresis, gels were stained for 2 hours in a solution containing 0.2% (w/v) Coomassie Brilliant Blue (CBB) R-250 dissolved in 40% (v/v) methanol and 10% (v/v) acetic acid. Gels were destained in 20% (v/v) methanol and 5% (v/v) acetic acid with gentle agitation on a Double Shaker Mixer (DH-10) until the background became clear and protein bands were distinct. Excess stain was absorbed by tissue paper pieces added intermittently to the destaining solution. Gel images were captured using a Uvitech Gel Documentation System.

DATA SCORING AND DENDROGRAM CONSTRUCTION

Protein bands were scored as present "1" or absent "0" for each accession, generating a binary data matrix. The similarity coefficients among accessions were calculated using Jaccard's coefficient. Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to construct a dendrogram, which was generated using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) software version 2.1.

RESULTS AND DISCUSSION

A total of 161 durum wheat genotypes were analyzed using total seed storage protein profiles. All gels were carefully examined, and only consistently scorable protein alleles were considered for genetic diversity analyses. Each distinct protein band was treated as a single locus/allele, and scored as present (1) or absent (0). This bivariate (1–0) data matrix was subsequently used to estimate genetic distances.

In total, 719 scorable protein alleles were identified across the 161 genotypes, corresponding to an average of 4.5 alleles per genotype. Genetic distances among genotypes were calculated using Nei and Li's UPGMA (Unweighted Pair Group Method with Arithmetic Mean) formula:

GD=1−dxydx+dy−dxyGD = 1 - \frac{d_{xy}}{d_x + d_y - d_{xy}}GD=1−dx+dy−dxydxy

where:

GD = Genetic distance

dxy = Number of common alleles between two genotypes

dx = Total number of alleles in genotype 1

dy = Total number of alleles in genotype 2

In total, more than 12,000 pairwise comparisons were performed among the 161 genotypes. The results revealed a broad range of genetic distances, from 0% to 100%. Specifically, 127 comparisons showed no genetic difference (0%), while 252 comparisons exhibited complete divergence (100%) for the protein loci studied. The majority of pairwise comparisons (5421 cases) fell within the 41–60% diversity range, indicating a moderate level of genetic variation among most genotypes (Table I).

Table I. Ranges of genetic diversity (G.D.) in pair wise comparisons of durum wheat genotypes based on SDS-PAGE data

G.D range	No. of comparisons
0%	127
1–20%	2080
21–40%	2212
41–60%	5421
61–80%	1864
81–99%	924
100%	252

The bivariate data were further used to generate a dendrogram through the PopGene program (8). Based on protein profile clustering, the 161 genotypes were grouped into three major clusters (Fig.1). Interestingly, accessions originating from different geographic regions—such as Cyprus, Syria, and Pakistan—were distributed across the clusters, suggesting that geographic origin is not strongly correlated with genetic diversity. This finding is consistent with earlier reports by Megha *et al.* (2024), Gashaw *et al.* (2007), and Singh *et al.* (2003) (1, 6, 7), who also observed weak associations between geographic and genetic distances in durum wheat.

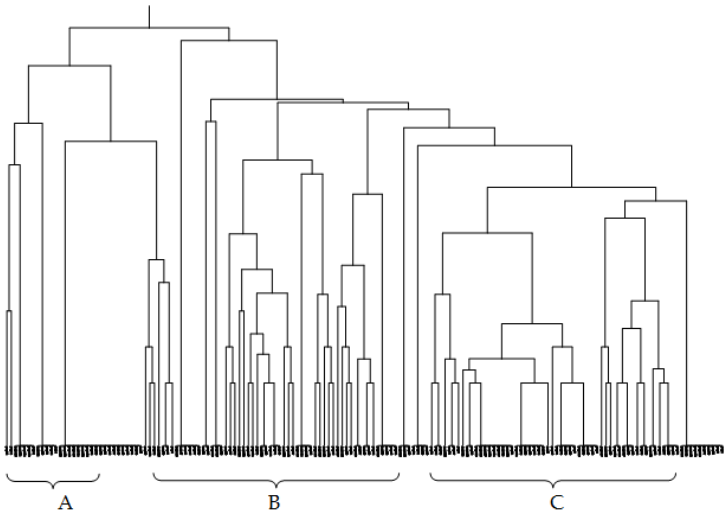

Fig.1. Cluster analysis of 161 durum wheat genotypes based on SDS-PAGE scoring

Comparisons with previous molecular studies further support these observations. Razmjoo and Mohammadi (4) reported 85–100% genetic diversity in durum wheat genotypes using SSR markers. Similarly, El-Rawy and Hasan (3) observed 77.1% diversity among wheat genotypes using SSR primers. These results highlight that both biochemical (protein-based) and molecular (SSR-based) markers are effective in capturing the wide genetic variability present in durum wheat germplasm.

Overall, the present study confirms the existence of substantial genetic variation within durum wheat, emphasizing the importance of exploiting this variability for wheat breeding and genetic improvement programs.

The clustering of 161 durum wheat genotypes into three major groups suggests that protein-based diversity can effectively distinguish between accessions, although the observed lack of association between clustering patterns and geographic origin indicates that genetic diversity is not necessarily determined by collection site. Similar results were reported by Singh *et al.*, (7) and Gashaw *et al.*, (6), who found that durum wheat genotypes from different geographic regions often shared closer genetic affinities than those from the

same region. This observation may reflect historical seed exchange, gene flow, and breeding practices that have blurred geographic boundaries of wheat germplasm.

The results obtained using SDS-PAGE are in line with molecular marker-based diversity studies. For instance, Razmjoo and Mohammadi reported 85–100% diversity among durum wheat genotypes using SSR primers, while El-Rawy and Hasan documented 77.1% genetic variation in wheat with SSR markers (3, 4). Although molecular markers provide higher resolution than biochemical markers, seed storage protein profiling remains a cost-effective and reliable tool for preliminary screening of large germplasm collections. Previous work has emphasized that combining both molecular and biochemical approaches enhances the accuracy of diversity assessments (9, 10).

The high proportion of comparisons showing moderate to high genetic diversity (41–60%) in this study underscores the potential of durum wheat germplasm to contribute to breeding programs aimed at broadening the genetic base. Genetic variability is essential for developing cultivars with enhanced adaptability to biotic and abiotic stresses, such as drought, heat, and evolving pathogens. Earlier studies have highlighted that durum wheat improvement depends on harnessing untapped genetic resources (11, 12). The wide range of diversity observed here indicates the presence of allelic richness that can be exploited to enhance traits such as yield stability, protein quality, and disease resistance.

CONCLUSION

The findings of this study demonstrate a broad spectrum of genetic diversity among durum wheat genotypes, as revealed by seed storage protein profiles. The high proportion of moderate-to-high genetic distances and the lack of correlation with geographic origin highlight the complex evolutionary dynamics of durum wheat germplasm. This diversity represents a valuable resource for breeding programs, offering opportunities to broaden the genetic base, enhance stress tolerance, and improve grain quality traits in future cultivars.

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Authors' contribution:

SN Study designed, research work; MW Write up, data analysis; MA Write up, formatting and critical analysis

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