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Assessment of genetic diversity by seed storage proteins in wheat germplasms

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Abstract

The wealth of genetic diversity in 15 wheat (*Triticum aestivum* L.) local cultivars collected from various areas of Bajaur agency, Pakistan was investigated through biochemical characterization (SDS-PAGE). For biochemical characterization, Sodium Dodecyl Sulphate Polyacrylamide gel Electrophoresis was carried out. The seed proteins were resolved on 12.25% polyacrylamide gel. Total 7 bands were observed in all fifteen genotypes. Among which 4 bands were monomorphic while 3 bands were polymorphic. Except for some minor differences in the intensity of the bands, major differences were not recorded in the protein profiles. Maximum 44% genetic diversity was observed in the collected lines. The linkage distances ranging from 00 to 44%. Cluster analysis exhibited moderate level of genetic diversity.

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Introduction

Wheat (*Triticum aestivum* L.) is the world's most widely cultivated cereal crop. It has been considered the first strategic food crop in the winter seasons. Wheat is the primary staple food of Pakistan. Total area under wheat cultivation is 8,358 thousand hectares with a total production of 21,612.3 thousand tones and an average yield of 2,586 kg/ha (Anonymous, 2006). Varieties have been a landmark in the genetic improvement of wheat, as it resulted in increase in its potential for grain yield. Information about genetic diversity and genetic relatedness among elite material is a fundamental element in plant breeding (Zhu et al., 2000). Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up DUS (distinctness-uniformity-stability) test for candidate cultivar (Chan and Susan, 1997). For acquiring plant breeder's rights (PBR), varieties of agricultural importance have to be tested for distinctness (D), uniformity (U) and stability (S) (DUS testing) (Ardley and Hoptroff, 1996). Evaluation of genetic diversity in wheat has been on differences in morphological and agronomic traits or pedigree information (Bernard *et al*., 1998). A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and segregating populations. These methods have relied on pedigree, morphological agronomic performance, biochemical and molecular (DNA-based) data (Mohammadi and Prasanna, 2003). Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. A large number of germplasm lines can be characterized for biochemical markers in a short period of time. In addition the data reflects more truly the genetic variability as biochemical markers are direct product of genes and the environment does not influence their expression (Masood et al., 2000). Among biochemical techniques SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm (Javaid et al., 2004; Anwar et al., 2003.). The analysis of storage protein variation in wheat has proved to be a useful tool not only for diversity studies but also to optimize variation in germplasm collections (Masood *et al*., 2000). SDS-PAGE can be used as a promising tool for distinguishing cultivars of particular crop species (Jha and Ohri, 1996). The main objective of our research was to evaluate the potential of SDS-PAGE technique to assess the genetic diversity and relatedness among some Pakistani wheat genotypes based on protein profiles and to develop an optimized and efficient operational system for their use.

Material and methods

Plant sample

Grains of fifteen wheat germplasms were collected from different ecological and unexplored regions of Pakistan during 2006. The samples were stored in labeled glass bottles to ensure safety, in the Department of Biotechnology, University of Malakand, for analysis.

SDS-PAGE electrophoresis

Storage proteins were extracted from wheat following the method of Laemmli (1970) using 12.25% polyacrylamide gel. The grains were grind to fine powder and 10 mg was weighed in 1.5 ml microtube. Add 400 ml protein extraction buffer (Tris-HCl 0.05 M, pH 8), 0.02% SDS, 30.3% urea, 1-2% Bromophenol blue) to each micro tube, kept overnight at 40°C and centrifuged at 13000 rpm for 10 min. The supernatant contain dissolved extracted protein ready for experiment purposes, which could be kept for longer time at 4°C. Electrophoresis was performed at a constant current of 70 V at room temperature for the time required for the tracking marker dye (Bromophenol blue) in extraction buffer to migrate off the gel. Gels were stained overnight with 6% (w/v) TCA (trichloroacetic acid) solution containing 4% (v/v) methanol and 0.022% (w/v) commasie blue R-250. De-staining was carried out in acetic acid methanol solution for overnight.

Data analysis

The bands of HMW-GSs on SDS-PAGE gels were read using HMW-GS methodology and the nomenclature

described by Payne and Lawrence, 1983.

Evaluation of variation in the endosperm proteins was performed by the calculation of the individual band frequency for each germplasm. Electrophoregrams for each variety were scored and the presence (1) or absence (0) of each band was scored. The clustering of germplasm was based on Jacquard´s coefficient by the Un-weighted Pair Group Method with Arithmetic Average (UPGMA) using the software package program STATISTICA vir-6 (Sneath and Sokal, 1973).

Results

Genetic diversity evaluation

In this study SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight of gluten subunits and investigate genetic diversity among wheat germplasms of different localities of Bajawar, Pakistan. The electrophorogram showing proteins banding pattern of different wheat varieties are given in Figures 1. A total of 7 bands were obtained among which bands number 1, 2, 6 and 7 of the HMW-Gs were monomorphic while 3, 4 and 5 were polymorphic (Figure 1).

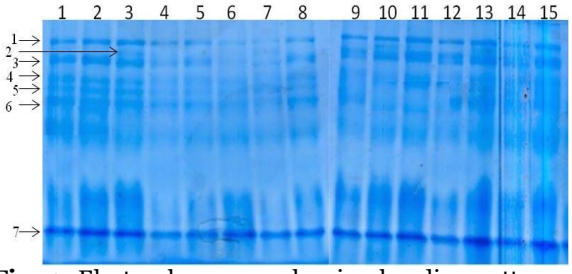

Fig. 1. Electrophorogram showing banding patterns of 16 germplasms of wheat proteins.

Cluster analysis

In cluster analysis for total polypeptide bands, the whole germplasms are divided into two groups or lineages (L) at 44% genetic diversity. Linkage 1 is divided into 2 cluster having 13% genetic diversity. Cluster I (CI) consists of three genotypes collected from Manoo, Enzarai, Safarai Cluster II (CII) also consists of three genotypes collected from Pashat, Selai Patai, Bagh. Linkage 2 is further divided into 3 clusters i.e. cluster III, IV and V with 30% genetic distance. Cluster III (CIII) consists of one genotype collected from Kotkai. Cluster IV (CIV) consists of two genotypes collected from Kamar, Levesam. Cluster V (CV) is the largest group consists of five genotypes collected from Qazafi, Danqool, Jarr, Rashakai, Khar, Nawagai.

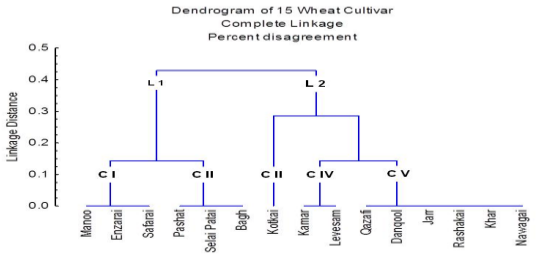

Fig. 2. Dendrogram of wheat cultivars.

Discussion

Level of distribution, areas sampled and plant characteristics such as mode of reproduction, breeding behavior and generation time are some of the important parameters that determine the level of genetic variability. The protein patterns of our genotypes were homogeneous as well as heterogeneous coinciding with the earlier reports (Singh *et al*., 2007; Kang *et al*., 2007 and Popa *et al*., 2003). This heterogeneity could be used in breeding programs for increasing bread-making quality by selection of a glutenin phenotype with a HMW-GS composition associated with good quality. However, other studies showed low degree of heterogeneity between wheat genotypes tested (Lawrence and Shephred, 1980; Mohd *et al*., 2007; Siddiqui and Naz, 2009). This low level of genetic diversity may be attributed to reduce number of varieties used for wheat cropping. Storage protein composition is expected to be a cultivar constant element; being the direct expression of its genotype thus it can provide a useful aid to cultivar identification. These results are in strong agreements by the previous findings of Popa *et al*., (2003) Kang *et al*., (2007) and Tsenov *et al*., (2009). Some of the genotypes formed clusters with 100% genetic similarity showing tight linkages (Fig 1) and thus confirmed the utilization of common exotic breeding lines or sharing of breeding materials at different research stations, conferring loss of genetic diversity. In order to stop genetic erosion, it is necessary to preserve the local wheat germplasm. However it is very difficult to determine the genetic

similarity based on seed storage protein profile to elucidate genetic diversity, therefore DNA based genetic markers such as SSR, RAPD should be used to further analyses of comparing related varieties of wheat to ascertain its genetic identity. The information generated from this study could be utilized to devise an efficient breeding strategy aimed at improving bread making quality and to broaden the genetic base of this important food crop in the respective areas of Pakistan. Since the information obtained reflects the potential usefulness of the wheat germplasm collections therefore efforts are being made to expand the data base by characterizing the remaining germplasm.

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