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Characterization of promising wheat lines by seed storage protein through sodium Dodecyl sulphate polyacrylamide gel electrophoresis

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Abstract

Wheat storage proteins accounted for up to 60% of the total grain proteins. They form gluten proteins, which make a visco-elastic network enables dough to be processed into bread, pasta and other products. A major effort of a plant breeder is the constant improvement of the best available genotypes for further enhancement in their yield potential either directly or through improvement of various factors which contribute indirectly to high yield. In order to study genetic variation of wheat relatives, electrophoretic patterns of seed storage proteins 45 LINES including some checks improved cultivars were fractionated by SDS-PAGE. The results showed some close relationship between *T. urartu*, *T. dicoccum* and bread wheat in the case of glutenin and gliadin. Therefore It was speculated that progenitor of A genome of cultivated wheat could be *T. urartu* strongly. A high level of polymorphism was detected in the glutenin and gliadin subunits of the wild wheat relatives, showing some similarities with cultivated bread wheat, useful breeding perspectives. The electrophoresis proved to be a suitable method to discriminate wheat variety and species. Also results of this study confirmed that the genetic variation amongst seed storage proteins of wild relatives were considerable. The wild progenitors are important genetic resources and therefore observed genetic variability could be use in any selection strategies.

Keywords: SDS, Storage protein, gel.

Introduction

Bread wheat (*Triticum aestivum* L. em. Thell), an allohexaploid ($2n=6x=42$), is the primary food crop of worldwide importance. It is also a crop where conventional plant breeding has paid rich dividend, as epitomized by the Green Revolution. It is the major staple food source for a large part of world population. Thus, well planned and dynamic wheat breeding programmes are needed to evolve high yielding and versatile varieties to meet the increasing demand of the people. The quality of wheat is considered very important factor in domestic as well as global markets for making different value added products (Werner et al. 1992). To improve the grain quality for value added products, cultivars with specific quality parameters are very essential. It is agronomically and nutritionally most important cereal essential for the food security, poverty alleviation and improved livelihoods. Wheat is supreme among cereals largely because its grain contents proteins with unique chemical and physical properties. Variation is important for improvement of different quality traits in plant breeding (Nevo et al. 1987) [6]. Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as morphological traits, end-use quality traits, and molecular markers. It helps in selecting genetically diverse lines. The present study was undertaken to evaluate the genetic diversity in set of forty wheat Lines using SDS-PAGE. In this study SDS-PAGE of grain storage proteins was performed in order to investigate genetic diversity among different wheat lines on the basis of the molecular weight of storage protein.

Material and Method

The present studies were carried out in the Department of Genetics and plant Breeding, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. 45 lines of wheat were used and study through SDS-PAGE. The purpose of SDS-PAGE is to separate proteins

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according to their size, and no other physical feature. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight). SDS gel electrophoresis of samples having identical charge per unit mass due to binding of SDS results in fractionation by size.

Extraction of proteins from seeds The seed storage proteins were extracted from wheat seeds using procedure following: Two gram seeds of each sample were crushed in 2ml extraction buffer then centrifuged for 10 min. at 10,000g at room temperature. The supernatant was collected and stored in refrigerator for electrophoresis.

Gel preparation Ten per cent separating gel was prepared and poured immediately in the glass plate cassette and a little amount of water was added to avoid zigzag surface and removed after 15 min. After polymerization of separating gel (12 per cent), stacking gel (5 per cent) was poured into glass plates and after inserting combs left undisturbed for 30 minutes to complete polymerization.

Sample preparation for electrophoresis 50 µl of supernatant of each sample was taken and equal amount of sample buffer was mixed and heated at 90 °C in water bath for 10 min. and after cooling these were loaded to gel.

Sample loading to gel and electrophoresis after transfer of glass cassette (gel cell) to electrophoresis assembly having electrode buffer, 50 µl of prepared sample from each sample were loaded in each individual well. Gel was electrophoresed at 60 volt constant voltage. When the dye reached the bottom of the separating gel, current was switched off. It takes 10-12 hours.

Staining and destaining Gel wall was dismantled from glass plates and marked by cutting at the corner and transferred to staining tray containing 500 ml staining solution. After overnight staining of gel, it was transferred to destaining solution tray and destained upto clear background of gel which takes 5-6 hours. After destaining the photograph of the gel was taken and saved for analysis.

Gel Analysis The distance between the origin and the tracking dye of gel was divided into three equal zones. In each of the zones, the total number of bands were counted and further classified into following groups according to their thickness and intensity. Thick i.e. relatively thick as compared to other protein bands. Medium i.e. in between the thick and thin protein bands. Faint i.e. slight appearance of protein bands.

Relative mobility (R_m)

Total length of lane was measured in cm on the gel and relative mobility (R_m) of each band was calculated according to formula given below:

$$R_f = \frac{\text{Distance between origin and migrating band}}{\text{Distance between origin and tracking dye}}$$

Data Analysis of Electrophoretic Gel patterns

The gel patterns were extrapolated on the basis of relative mobility of each protein band.

Jaccard's Coefficient (J.C.)

Jaccard's coefficient (similarity coefficient) was used for calculating the genetic similarity between different mustard strains. Both the strains were considered as similar if this

value was 1 and they were considered genetically different if this value was 0.

$$JC = \frac{\text{No. of common bands}}{\text{No. of common bands} + \text{No. of different bands}}$$

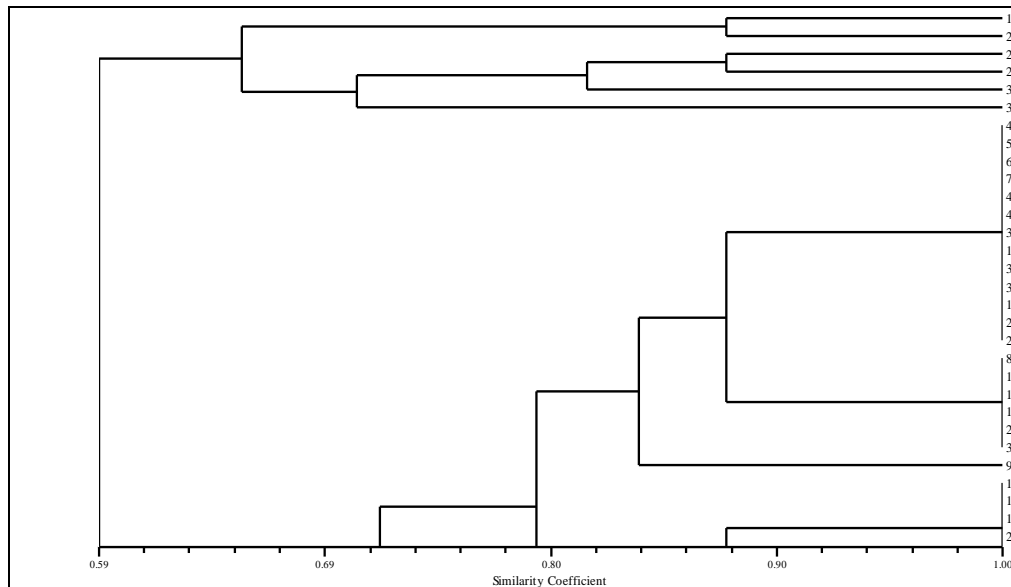
Results and Discussion

SDS polyacrylamide gel electrophoresis (SDS-PAGE) technique was developed for the analysis of wheat endosperm storage proteins. It is used to re-evaluate the glutenin and gliadin composition of intervarietal chromosomal substitution lines of wheat. Gel electrophoresis is a useful method to separate and identify proteins and nucleic acids. In SDS-polyacrylamide gel electrophoresis (SDS-PAGE), proteins are separated largely on the basis of polypeptide length, and so their molecular weight can also be estimated. SDS does however denature the protein, so activity stains cannot be used to identify particular enzymes (Shuaib *et al.* 2007) [9]. Buffer containing sodium dodecyl sulphate (SDS) and mercaptoethanol (ME) were used to extract total protein from the flour or single seeds of forty different wheat (*Triticum aestivum* L.) varieties. A dye-binding analysis for protein was adapted to these extracts. The extracted proteins were separated according to molecular weight (mol. wt) by gel electrophoresis with SDS and the patterns were quantitated by densitometry of the gels, after proteins had been stained with Coomassie brilliant blue R250.

Seed storage proteins are encoded by multigene families with diverse genes (Payne *et al.* 1984) [7]. Although, it is considered as a reliable method for verifying the genetic identity of seed lots, most easily and rather quickly (Nevo, *et al.* 1998) [5]. However, environmental factors can affect the quantitative and qualitative attributes of storage proteins and thus can influence the reproducibility of protein profile on crop varieties. The storage protein is also able to discriminate the morphologically similar genotypes but on the other hand genotypes with variable phenotypic traits showed the similar protein profiles (Neuhoff *et al.* 1988) [4]. Such differences between field and laboratory may be due to the fact that gene sequences that control the expression of seed storage proteins cannot represent the whole genome. Similarity in protein profiles of various genotypes may also be due to the degradation of protein subunits by endogenous seed protease during isolation. Despite of conspicuous morphological differences between accessions they share more or less the same protein profile. The extracted proteins were separated according to molecular weight (mol. wt) by gel electrophoresis with SDS and the patterns were quantitated by densitometry of the gels, after proteins had been stained with Coomassie brilliant blue R250. Molecular weight marker ranged 3500 to 43400 Da was used. Protein Molecular weight Marker are helpful for sizing proteins by polyacrylamide gel electrophoresis (PAGE). Electrophoregrams for each variety were scored and the presence (1) or absence (0) of each band was noted (Damania *et al.* 1983) [3]. The score was entered in a binary data matrix. Based on electrophoresis band spectra, Jaccard's similarity index (JSI) was calculated. Genetic diversity was evaluated via UPGMA cluster analysis by constructing dendrogram of fractions of proteins, which were used for the calculation of similarity coefficients between these lines. The genetic differences between the lines were reflected in to cluster means and the clusters differed from one another but between the cluster, the lines were similar (Cooke *et al.* 1998) [2]. Through this analysis this is proof that

in this study SDS-PAGE of grain storage proteins was performed in order to investigate genetic diversity among different wheat lines on the basis of the molecular weight of storage protein (Bietz *et al.* 1972)^[1]. The genotypes present in one cluster represents 100% similarity while those in different

clusters represents some level of variation among them. The similarity coefficient lies between 50 to 100%. This revealed that most of the lines are similar and they evolved from the same parents directly or indirectly through selection.



Dendrogram showing similarity coefficient lies between 50 to 100% in 45 wheat Germplasm lines

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