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Estimation of Genetic Diversity in Genetic Stocks of Hexaploid Wheat Using Seed Storage Proteins

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Abstract: Bread wheat (*Triticum aestivum* L.) is an allohexaploid specie, consist of three genomes AABBDD having 2n = 6x = 42 chromosomes. The wheat is a staple food of human beings due to its bread making quality which is composed of seed storage proteins of wheat especially High Molecular Weight Glutenins (HMW-GS). During present research, HMW-GS were analyzed in genetic stocks of common wheat consist of Nullisomic-tetrasomic, ditelosomic and deletion lines of group 3 homoeologous chromosomes by Sodium Dodecyle Sulpahate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Protocol for protein extraction and separation was optimized. The protein profiles were used to estimate genetic distances and Phylogenetic relationships among the genetic stocks were evaluated. Genetic stocks showed different banding patterns and each protein band was considered as a locus/allele. Alleles were scored as present (1) and absent (0) to generate bivariate 1-0 data matrix. A total of 45 alleles were amplified. Genetic distance among the genetic stocks ranged from 0-72%. A dendrogram was constructed using computer program Pop Gene version 3.2. Genetic stocks of wheat were clustered in 3group A, B and C comprising 4, 4 and 1 genotypes, respectively. Maximum differences were observed among Dit-3BS and NT-3B3D and hence it is recommended that these 2 genetic stocks should be crossed to obtain maximum genetic diversity in the segregating population of wheat.

Keywords: Group 3 homoeologous chromosome, genetic diversity, seed storage proteins, SDS-PAGE, wheat

INTRODUCTION

Wheat belongs to the grass family among the largest family of plants. It had been cultivated for over 1000 years (Kerby and Kuspira, 1987). Wheat is one of the most important food grains consumed directly by humans because of its nutritional value and adapted in 43 countries (Wies, 1987). There are 3 cytological classes of wheat viz; diploid (Triticum monococcum, T. urartu, T. tauschi etc. 2n = 2x = 14), Tetraplod (Triticum dicocoides, 2n = 4x = 28) and hexaploid (Triticum aestivum, 2n = 6x = 42). About 95% of wheat crop grown in the world is hexaploid. Bread wheat (Triticum aestivum L., 2n = 6x = 42, consist of (AABBDD) genome of an allohexaploid species composed of three related genomes A, B and D, each containing seven pairs of chromosomes (1A-7A, 1B-7B, 1D-7D) (Sears, 1966). Sears (1966) established that each chromosome in hexaploid wheat has a homoeologue in each of the other 2 genomes. Sears (1966) described the effects of aneuploidy for each wheat chromosome, including the nullisomics, monosomics, telocentrics and isochromosomes and produced nullisomic tetrasomic lines. Bread making quality of wheat is controlled by water holding capacity seed storage glutenins (Payne *et al*., 1987). High Molecular Weight Glutenin Subunits (HMW-GS) are endosperm proteins, mainly concerned with bread wheat quality (Liu *et al*., 2008; Shewry *et al*., 2003). Because HMW-GS play an important role in dough making quality of bread. The HMW-GS allele at Glu-A1, Glu-B1 and Glu-D1 loci are present on long arm of chromosomes 1A, 1B and 1D respectively (Payne *et al*., 1987). Genetic diversity is one of the key factor for the improvement of many crops plants including wheat. An improvement in the efficiency of genetic gain by selection can be obtained when the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity/distance estimates among genotypes are helpful in selection, yield performance and disease resistance of wheat parents to be used in breeding programs (Van Becelaere

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et al., 2005). The study of genetic diversity is also important for varietal identification, proper purity maintenance, for the implementation of plant variety protection rights and export under WTO regulations (Zhu *et al*., 2000).

Genetic diversity estimates based on morphological traits suffered that such traits are limited in number and are influenced by environment (Maric *et al*., 2004). Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment. These are abundant that do not require previous pedigree information (Bohn *et al*., 1999). Among the biochemical markers, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) has been widely used due to its simplicity and effectiveness for estimating genetic diversity (Shuaib *et al*., 2007; Sultana *et al*., 2007) due to its simplicity and effectiveness. Seed storage proteins have also been recommended as reliable genetic markers for characterizing wheat varieties based on bread-making quality (Fufa *et al*., 2005). In the present studies high molecular weight glutenine was analyzed through SDS-PAGE in eight genetic stocks of wheat including Nullisomic-tetrasomic, Ditelosomic and deletion lines of wheat to estimate genetic diversity.

MATERIALS AND METHODS

The genetic stocks of common wheat seeds were kindly provided by Dr. John Raupp, wheat Genetic Resource Centre, Kansas State University, USA and Prof. Dr. J. Dubcovsky, Department of Agronomy and Range sciences university of California Davis, USA. In order to extract HMW-Glutenins, seeds of the genetic stocks were cut in halves with a sharp razor. Total proteins were extracted from embryo-less half of single wheat seed using protocol of Payne *et al*. (1987). Half seeds were crushed into fine powder and poured into a 1.5 mL eppendorf tube. To extract the total seed storage protein, 4 mL water was mixed with 0.5 mL 2-mercaptoethanol (ME) and 1.5 mL protein Extraction Buffer (EB) to make a total volume of 6 ml. 500µl of the extraction mixture (EB + ME + Water) was added to the flour in each eppendorf tube and proteins were extracted at room temperature for about 2-3 h. During this period, tubes were vortexed 3-4 times. After the completion of extraction procedure, tubes were placed in boiling water for 5 min and stored at 4°C until used. SDS-PAGE gels were run on Bio-rad protein vertical gel Electrophresis apparatus. A 12.5% resolving gel (3.0 M Tris pH 9, 0.4% SDS and 4.5% stacking gel (0.4M Tris pH 7.0, 0.4% SDS) was prepared and polymerized chemically by addition of 17 µL of N, N', N', N' Tetramethylene Diamine (TEMED) and 10% Ammonium Persulphate (APS). Electrode buffer solution was poured into the bottom pool of the apparatus. Gel plates were placed in the apparatus carefully to prevent bubbles formation at the bottom of gel plates. Then electrode buffer (0.025 M Tris, 1.29 M Glycine, 0.125% SDS) was added to the top pool of the apparatus. 10 µL of the extracted proteins were loaded with the micropipette in the wells. The apparatus was connected with constant electric supply and electric current of 70 V was applied. The gels were run till the tracking dye "Brilliant blue R250" (BPB) reached the bottom of the gel. Gel were stained in staining solution for 30 min and destained in destaining solution until clear background was obtained. After destaining the gels was photographed using gel documentation system "Uvitech".

RESULTS AND DISCUSSION

Eight genetic stocks of wheat viz; Ditelosomic 3BS, Dit3AL, Dit3DL, Dit3DS, NT3D3A, NT3A3B, Del3AS-4 and NT 3B3D were used during present research work to estimate genetic distances among all possible combinations using SDS-PAGE. SDS-PAGE analysis of the genetic stocks is shown in Fig. 1. Various genetic stocks showed different banding patterns. In lane number 4 and 9 same genetic stock (Dit 3DS) were used to check the reliability of the procedure. It is evident from the Fig. 1 that similar banding profile was obtained in both the samples which indicate that the procedure used during present research was reliable and there were no mistakes in preparation/loading of the samples. According to Nei and Li (1979) each protein band was considered as a locus/allele. Alleles were scored as present (1) or absent (0) and Bivariate 1-0 data matrix was generated (data not shown). Total of 45 alleles were amplified in eight genetic stocks. UPGMA procedure was used to estimate genetic distances among all the possible combinations (Table 1). Genetic distances ranged from 0-72%. Highest genetic distance (GD = 72%) was observed for comparisons viz; Dit3DS-NT3B3D and NT3D3A-NT3B3D. Minimum genetic distance (GD = 0%) was observed for six comparisons viz; Dit 3AL-Dit3DL-Dit 3AL-Del 3AS-4, Dit 3DS, NT3D3A, Dit3DS, Dit3DS,

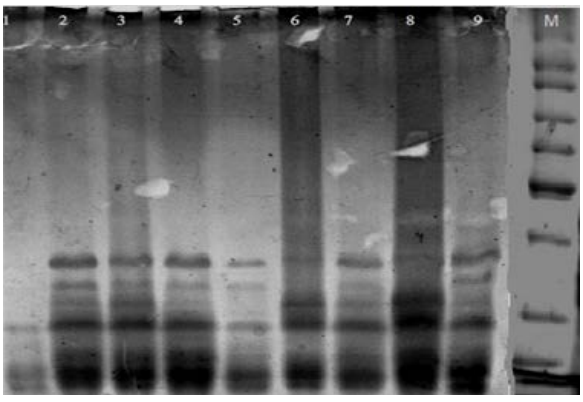

Fig. 1: Profile of nine genetic stocks of common wheat using SDS = PAGE 1 = Dit 3BS, 2 = Dit 3AL, 3 = Dit 3DL, 4 = Dit 3DS, 5 = NT 3D3A, 6 = NT 3A3B, 7 = Del 3AS-4, 8 = NT 3B3D, 9 = Dit 3DS, M = Molecular weight marker

Table 1: Estimates of genetic distances in eight genetic stocks of common wheat using UPGMA procedure

Alleles	1	2	3	4	5	6	7	8
2	0.34							
3	0.34	0.00						
4	0.20	0.17	0.17					
5	0.20	0.17	0.17	0.00				
6	0.40	0.34	0.34	0.50	0.50			
7	0.34	0.00	0.00	0.17	0.17	0.34		
8	0.67	0.57	0.57	0.72	0.72	0.40	0.57	
9	0.20	0.17	0.17	0.00	0.00	0.50	0.17	0.72

1 = Dit 3BS, 2 = Dit 3AL, 3 = Dit 3DL, 4 = Dit 3DS, 5 = NT 3D3A, 6 = NT 3A3B, 7 = Del 3AS-4, 8 = NT 3B3D, 9 = Dit 3DS

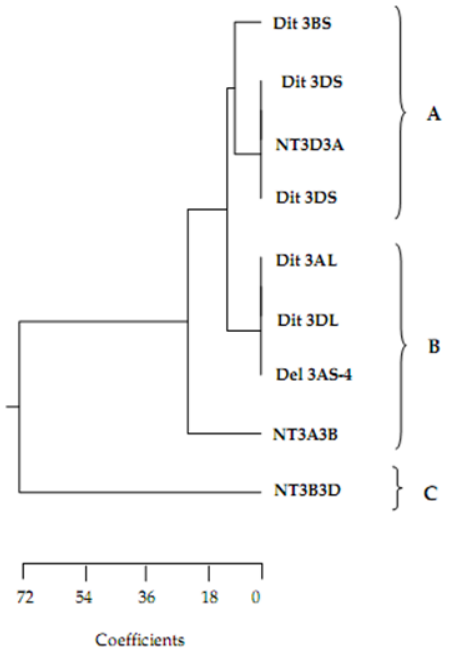

Fig. 2: Dendrogram constructed for eight genetic stocks of wheat using data obtained from SDS-PAGE

Nt3D3A, Dit3DS, Dit3DL, Del3AS. In rest of the cases, 1, 3, 3, 2, 6, 3 and 9 comparisons showed 67, 57, 50, 40, 34, 20 and 17%, genetic distances, respectively. The bivariate data was also used to construct dendrogram using computer program Popgene ver 32 1.31 (Yeh *et al*., 1999). The dendogram of eight genetic stocks of wheat using SDS-PAGE data is present in Fig. 2. Eight genotype were clustered in 3 groups A, B and C comprising 4, 4 and 1, genotypes, respectively. Maximum difference was observed among Dit 3BS and NT 3B3D and hence these 2 genotypes should be crossed in a breeding program to obtain maximum diversity in the segregating population.

CONCLUSION

From these studies it is concluded that High Molecular Gluten (HMG) seed storage protein profiles could be a useful biochemical markers in genotype recognition, registration of novel varieties, pedigree analysis as well as in the estimation of genetic diversity and classification of adapted cultivars, by this means improving the efficiency of wheat breeding programs in cultivar development.

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