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Estimation of Genetic Diversity in Genetic Stocks of Common Wheat (*Triticum aestivum* L.) Using SDS-PAGE

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Abstract:

Common bread wheat (Triticum aestivum L.) is an allohexaploid species (2n = 6x = 42, having AABBDD genome). Globally, wheat is a staple food for human population due to its bread making quality. The bread making quality of wheat is controlled by seed storage proteins. In the present study water soluble seed storage proteins were extracted from ditelosomic and deletion lines of group 5 homoeologous chromosomes. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out using 12.5% resolving gel (3.0M Tris pH 9, 0.4% SDS and 4.5% stacking gel (0.4M Tris pH 7.0, 0.4% SDS). Each individual protein band was considered as a locus / allele. Alleles were scored as present (1), absent (0) and a bivariate (1-0) data matrix was generated. In a total, 69 alleles were scored in thirteen genotypes giving an average of 5.3 alleles per genotype. High amount of genetic diversity ranging from

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0-100% was estimated in available wheat genotypes. One comparison (Del5AL12-- Del 5AS-3) showed complete homozygosity (GD=0%). Two comparisons (Del 5AS10--Del 5DL-1 and Dit 5Bl--Del 5BL6) showed 100 % genetic distance. Bivariate data was also used to construct a dendrogram using computer program Popgene ver. 32. The genetic stocks of hexaploid wheat of different lines were clustered in 4 groups (A, B, C and D) comprising 4, 3, 3, and 3 genotypes, respectively. Genetic stocks Del5AL12 and Del 5DL-1 were most distantly related and hence it is recommended that these two lines should be crossed to create a breeding population with maximum genetic diversity which will be useful for the development of new improved varieties of wheat.

Key words: Wheat, Seed Storage Proteins, SDS-PAGE, Genetic Diversity, Phylogenetic analysis.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop that provides more than 60% proteins to the world population [1, 2]. Wheat is unique in all cereal grains due to its major contribution in agricultural crops [3] and was extensively cultivated by human being in the past history [4]. Bread wheat is allohexapliod, containing three distinct but genetically related (homoeologous) genomes A, B and D [5]. Each genome includes seven pairs of metacentric or submetacentric chromosomes (1A-7A, 1B-7B, 1D-7D). Triplication of genome enabled to produce genetic stocks of wheat including nullisomic, tetrasomic and ditelesomic lines [6, 7]. These lines have been widely used for mapping of wheat chromosomes [8]. Development of deletion lines [9] has facilitated the physical mapping of wheat chromosomes. Evaluation and characterization of crop populations and cultivars diversity is extremely essential stair in plant expansion programs [10]. There are two major groups of wheat proteins: Metabolic and Wheat Storage proteins. Metabolic Proteins are essential for the growth and development of the wheat seedling consists of Albumins (22%) and Globulins (15%), while Wheat Storage

Proteins (Gluten) are essential for dough quality. Gluten is divided into Gliadines (MW 30-80, kDa) and Glutenins (MW 12-130 kDa). Gliadines are non-aggregating proteins and are responsible for the extensibility in dough while Glutenins are aggregating proteins, involved in dough strength and quality [11, 12]. Glutenins are sub-divided into two groups, the High Molecular Weight Glutenins (HMW-GS) 70-90 kDa which forms the macro molecules and LMW-GS (20-45 kDa) which form the Gluten complex [12]. Present study was carried out based upon Gluten, to estimate genetic distance among genetic stocks (ditelesomic and deletion lines) by using SDS-PAGE.

Materials and Methods

Plant Materials

Seeds of 13 genetic stocks of common wheat including deletion (Del) and ditelosomic (Dit) lines of homoelogous group 5 chromosomes of common wheat 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. Following lines were used during SDS-PAGE including, Ditelosomic 5BL, 5AL, 5DS and deletion lines viz; Del 5AL12, Del 5AS-3, Del 5AL-23, Del 5AS-10, Del 5DL-1, Del 5BS-4, Del 5BL-4, Del 5BL-6, Del 5DS-2 and Del 5BL-1.

Protein Extraction

The total seed proteins were extracted from the embryo-less half of single wheat seed using modify protocols of [13]. Seeds were crushed to a fine powder and poured into a 1.5 ml eppendorf tube. 4 ml water was mixed with 0.5 ml 2-mercaptoethanol (ME) and 1.5ml 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 hrs. During this period, tubes were vortexed 3-4 times. After the completion of extraction procedure, tubes were placed in boiling water for 5 minutes and stored at 4ºC until used.

Electrophoresis

SDS-PAGE gels were run on Bio-rad protein vertical gel Electrophresis apparatus. A 12.5% resolving gel (3.0M Tris pH 9, 0.4% SDS and 4.5% stacking gel (0.4M Tris pH 7.0,0.4% SDS) were prepared and polymerized chemically by addition of 17 µl of tetramethylenediamine (TEMED) and 10% Ammonium persulphate. Electrode buffer solution was poured into the bottom pool of the apparatus. 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 protein were loaded with the micropipette into the wells of the gels. The apparatus was connected with constant electric supply and electric current of 70V was applied. The gels were run till the tracking dye "Brilliant blue R250" (BPB) reached the bottom of the gel. Gels were stained in staining solution for 30 minutes and destained in destaining solution until clear background was obtained. After destaining the gels were photographed using gel documentation system "Uvitech".

Results and discussion

Improvement in quality and quantity of wheat has always been on the top priority of wheat breeders and geneticists. It has been proposed that variation within wheat varieties are due to the occurrence of diverse allelic combinations in each variety [14]. Genetic diversity is routinely used for getting success in wheat enhancement and their diversity can be estimated by different methods [15]. Different banding pattern in wheat proteins through SDS-PAGE analysis were previously determined by [16]. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a reliable,

easy and relatively quick procedure that has been widely used for the estimation of genetic variability in the crops of commercial importance like wheat [13]. Among the seed storage proteins of wheat, High Molecular Weight Glutenin Subunits (HMW-GS) play most important role in defining bred making qualities of wheat [13, 17]. During the present research work, HMW-GS of wheat were analyzed through SDS-PAGE. Genetic diversity using SDS-PAGE in high and low molecular weight glutenin subunit bands were observed by constructing dendrogram in European spelts wheat [18]. Thirteen genetic stocks of hexaploid wheat including ditellosomic (Dit) and deletion lines developed by [7] and [9] were used. The Protein profile of the thirteen genetic stocks is presented in Fig 1. Various genetic stocks showed different banding patterns following [19] procedure each individual protein band was considered as a locus / allele. Alleles were scored as present (1) or absent (0), a bivariate (1-0) data matrix was generated presented in Table 1. The most imperative locus that differentiates bread wheat is Glu-D1. Allelic variation at the Glu-D1 locus had a superior impact on bread-making quality than the difference at the Glu-A1 and Glu-B1 loci [20, 3].

About total 69 alleles were scored in thirteen genotypes giving an average of 5.3 alleles per genotype. Unweighted Pair Group of Arithmetic Mean (UPGMA) principle was used to estimate genetic distances among all the possible combinations the genetic estimates are given in table 2. High amount of genetic diversity (GD) ranging from 0- 100% were estimated in the different lines. One comparison (Del5AL12 -- Del 5AS-3) showed complete homozygosity (GD=0%). On the other hand two comparisons Del 5AS10—Del 5DL-1 and Dit 5BL---Del 5BL6 showed 100 % genetic divergence. The Bivariate data were also used to construct a dendrogram by using computer program Popgene ver. 3.2. A total of 13 genetic stocks of hexaploid wheat were clustered in 4 groups viz; A, B, C and D (Fig. 2). Groups A, B, C and D comprised 4, 3, 3, and 3

genotypes, respectively. Genetic stock viz; Del5AL12 and Del 5DL-1 were most distantly related among the group of 13 accessions. Wheat genotypes whose bootstrap values are below than 50% designate that the positions of these genotypes may alter if extra marker systems are used or additional genotypes are mixed up in the scrutiny [21].

The analysis of wheat HMW glutenin subunits is helpful for genetic diversity findings and is also used for the optimization of differences in the germplasm collections and, to breed wheat cultivars with superior bread-making quality [22]. It is recommended that these two lines (Del5AL12 and Del 5DL-1) should be crossed to create a breeding population with maximum genetic diversity which will be useful for isolating better genotypes of common wheat.

Table-1. Bivariate (1-0) data matrix for thirteen genetic stocks of hexaploid wheat using SDS-PAGE

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13
A	1	1	0	0	1	0	1	0	0	0	1	0	0
B	1	1	1	0	1	1	1	0	1	0	1	1	1
C	1	1	1	0	1	0	1	0	0	1	1	0	1
D	1	1	1	0	1	0	0	1	1	1	1	1	0
E	1	1	1	0	1	0	1	1	1	1	1	1	1
F	1	1	1	0	0	0	1	1	0	0	0	0	0
G	1	1	1	1	1	1	0	1	1	1	1	0	0
H	1	1	1	1	1	1	0	1	0	1	1	1	1

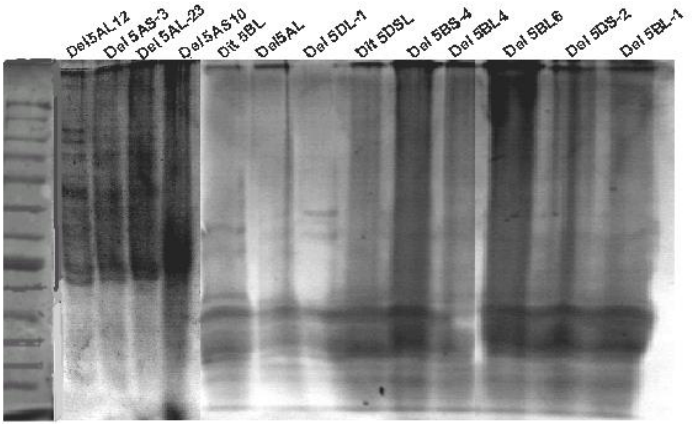

Fig.1. SDS-PAGE of 13 genetic stocks of common wheat

Table-2.Estimates of genetic distances among thirteen genetic stocks of wheat using UPGMA procedure.

1	2	3	4	5	6	7	8	9	10	11	12	13
1	0.00	0.13	0.75	0.13	0.63	0.38	0.38	0.50	0.38	0.13	0.50	0.5
2		0.13	0.75	0.13	0.63	0.38	0.38	0.50	0.38	0.13	0.50	0.50
3			0.71	0.25	0.57	0.50	0.29	0.43	0.29	0.25	0.43	0.43
4				0.71	0.33	1.00	0.60	0.80	0.60	0.71	0.80	0.80
5					0.57	0.50	0.50	0.43	0.29	1.00	0.43	0.43
6						0.86	0.67	0.60	0.67	0.57	0.60	0.60
7							0.75	0.71	0.75	0.50	0.71	0.50
8								0.50	0.33	0.50	0.50	0.71
9									0.50	0.43	0.40	0.67
10										0.29	0.50	0.50
11											0.43	0.43
12												0.40

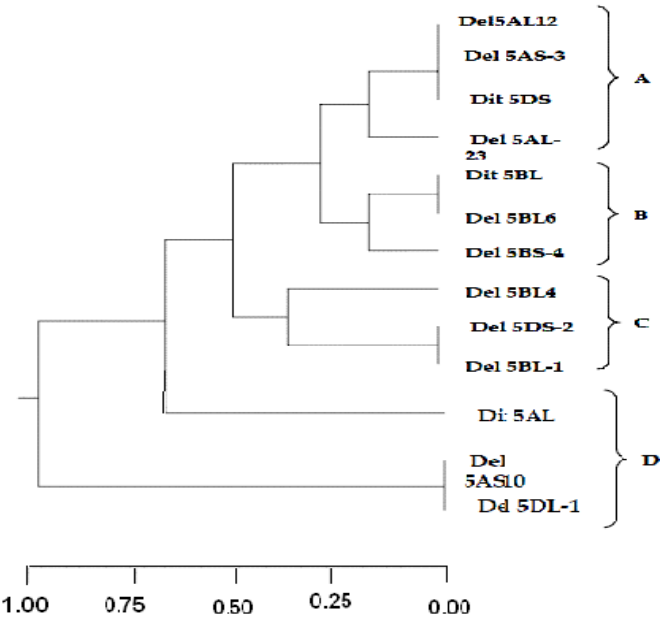

Fig 2. A Dendrogram constructed for 13 genetic stocks of wheat using data generated from SDS-PAGE

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