American Journal of Agricultural Science
2017; 4(3): 29-36
http://www.aascit.org/journal/ajas
ISSN: 2381-1013 (Print); ISSN: 2381-1021 (Online)

American Association for Science and Technology

Keywords
Hexopliod,
SDS-PAGE,
HMW-GS,
LMW-GS,
Glutenin

Received: February 1, 2017
Accepted: March 17, 2017
Published: June 7, 2017

Advances in the Detection of Genetic Diversity in Bread Wheat (*Triticum aevestivum* L.) Using SDS-PAGE Analysis

Naeem Khan[1, *], Shahid Ali[2]

[1]Department of Plant Sciences, Quaid-e-Azam University, Islamabad, Pakistan
[2]Department of Botany, Hazara University, Mansehra, Pakistan

Email address

naeemkhan@bs.qau.edu.pk (N. Khan)
[*]Corresponding author

Citation

Naeem Khan, Shahid Ali. Advances in the Detection of Genetic Diversity in Bread Wheat (*Triticum aevestivum* L.) Using SDS-PAGE Analysis. *American Journal of Agricultural Science*. Vol. 4, No. 3, 2017, pp. 29-x.

Abstract

Wheat is the most essential food crop and taxonomically belongs to family poaceae. Bread wheat is a Hexapliod species with 2n=6x=42 chromosomes having high genetic diversity, the genetic variation enables plant species to adapt to changing environments and provide resistance against different diseases. The scientists are highly interested to study the genetic diversity among wheat varieties morphologically (plant height, tiller number, spikes number, main ear length, spike lets number, grain number, grain weight, seed hardness, sedimentation value, and grain yield etc) biochemically (phenol color reaction and through different other chemical substance) and at molecular level (protein content, RNA and DNA variation). It has been revealed that morphological and biochemical variations are correlated to environmental condition (stress and favorable condition). The simple SDS-PAGE (Sodium dodecyl sulfate -polyacrylamide gel electrophoresis) method was adopted to show genetic variation in wheat which helps in classification and making a dendrogam with different cluster groups, that is vital for both plant breeder and germplasm curators. The high molecular weight-gultenin subunits (HMW-GS) and low molecular weight-gultenin subunit (LMW-GS) were analazyed through SDS-PAGE techniques. The variation in glutenin subunits was due to different allelic combination in various loci in different chromomes the glutein have important baking qualities. This study was aim to collect information related to wheat genetic diversity and the use of SDS-PAGE study of wheat endosperms protein that is valuable for assessment of genetic variability and cultivars identification that help in wheat breeder programs.

1. Introduction

Genetic diversity is defined as the total genetic variation of plants, animals, or other organisms which control all the characteristics with in species or in population each character is restricted to specific allele of a gene in a gene pool [1]. It is the diversity, which enables species to adapt to changing environments and provides an indemnity against mysterious future needs or conditions, thereby help to maintain the stability of farming system at the local, national and world wide level. In a review of the use of biodiversity [2] revealed that the genetic variation provide defense against the diseases and environmental deformities. Clay [3] referred genetic diversity is the difference in

expression of individual gene (polymorphism) and helps the population to adapt to their ever-changing environments, more the variation, better the chance of the individuals to adapt the new environment and produce offspring to continue into the subsequent generation.

1.1. Agro Biodiversity

Diversity existing in biological system, plants and animals is now mentioned as "agro biodiversity". Efforts are required to conserve the genetic variation of the biological system for global food security. The conference was convened by FAO in Leipzig, Germany, and urged the entire nation to take a worldwide act for the protection of genetic diversity and application of genetic resource for agriculture and food [4]. Miller and Koebner [5] stated that there were several factors due to which the genetic materials cannot utilization by breeding programs and traditional farming communities. The limitation are due to the absence of specific characterization and evaluation data; lack of information, procedure and materials to conserve the present genetic diversity of biological system in both ex-situ and in-situ environment; the conservation policies aren't applied at National level; difficulty to provide the required life environment and to the handling of large collections; through ex-situ techniques small range of species are use for research purposes; lack of market incentives etc. Enormous evidence showed that presently modern agricultural activities led to direct negative effect on living biodiversity at all levels: ecosystem, species and genetic, and on both natural domesticated diversity". What is now required in a watchful assortment of modern agricultural technique with traditional variants to indemnify and persisted the food production for many coming years.

1.2. Wheat (*Triticum aestivum* L.)

Bread wheat is the world's principle food crop and taxonomically belongs to family poaceae, tribe triticeae drum, sub tribe Triticinae Holm, genus Triticum L. and section Speltoidea Flaks [6]. Bread wheat is hexaploid species with 2n=6x=42 chromosomes which can be assigned to 16000 Mb [7]. In Pakistan the wheat cultivation is confined about 80% in watered condition and merely 20% is in rain fed areas. And approximately 35% of agricultural soil is used for wheat growth [8].

2. Method

2.1. Molecular Characterization

To explore the genetic diversity on the basis of protein, SDS-PAGE can be carried out. For SDS-PAGE analysis, seeds of each genotype can be crushed into fine powder with mortar and pestle. Add protein extraction buffer (400 µl) to 0.01g of seed flour and mix well with Automatic Lab-Mixer. The extraction buffer contain the following final concentration: 0.5M Tris HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol blue (BPB) can be added to the protein extraction buffer as tracking dye to watch the movement of protein in the gel. The SDS-PAGE of total seed protein can be carried out in the discontinuous buffer system. Acrylamid gel concentration 12.5% and 6 µl of sample can be used for analyzing germplasm. After staining and de-staining, the gel can be dried using gel drier

2.2. Detection of Proteins

After the process of electrophoresis the gel can be transferred to tray containing staining solution where shaking of gel can be done for 40 minutes, followed by de-staining until the background of gel disappear. Keep the de-stained gel in distilled water in a refrigerator to make clear the polypeptide bands.

3. Molecular Characterization of Wheat

Sodium dodecyl sulfate polyacrylamide gel electrophoresis has become one of the most widely used techniques to separate and characterize proteins. This technique offers two distinct advantages. Polypeptides migrate according to molecular weight on SDS gels so, that molecular weight of polypeptides may be easily and rapidly estimated. At the same time SDS solubilizes many insoluble proteins so the SDS gel electrophoresis has become the technique of choice for resolving mixture of insoluble proteins, especially membrane proteins.

3.1. SDS-PAGE Analysis

SDS-PAGE analysis, is a technique mostly used in molecular biology and bio-technology for separation of biological macromolecules, frequently proteins and/or nucleic acids, based on their electrophoretic mobility. Which is the function of the length, conformation and charge of the molecule. Since, last decade there have been noteworthy improvement in the genetic characterization of the gluten proteins in hexaploid wheats, by using improved approaches of protein fractionation and the suitable genetic stocks. The gliadins and HMW subunits of glutenin are easily determined by SDS-PAGE and display wide pattern difference amongst diverse cultivars.

Smith and Smith [9] considered morphological classification as first stage in depiction and classification of crop germplasm. Presently, large amount of cultivated crops are grown-up for many purposes. All these cultivated and domesticated plants were highly adapted to that environment which was suitable for growth and development of that specie and these species are actual source of genetic variation. Though, this genetic variation can be use efficiently when this diversity is assessed, computed and ordered in some way [10]. A variety of procedures involving

morphological, biochemical and molecular are currently being lent to evaluate the pattern of genetic diversity in different crop species, conventionally, plant/cultivars have been identified primarily on well recognized morphological and physiochemical descriptors. It is necessary to quantify and classify diversity in germplasm, for both plant breeders and germplasm curators. Quantitative morphological trait appeared to provide a good source of information for determining phenotypic variation when the germplasm included a large number of accessions [11].

Weltzien and Fischbeck [12] reported that the harvest index of local barley landraces was near the optimum under different environments. Plant height and time to flowering were intermediate whereas, lodging susceptibility and protein content were higher under favorable growing conditions. Variation among yield components was greater between than within populations. Under the stress environments, significant amount of genetic variation was found among landraces. Anwar [13] described the proportion of total variance due to differences among agro-types within landraces was greatest. Various reasons were advocated for the occurrence of greater diversity in wheat, such as isolation from other wheat germplasm, primitive farming system, heterogeneous environment, field mixture and cross-fertilization. Ciaffi et al. [14] identified 44 different high molecular weight glutenin subunits patterns in *T. dicoccoides*, these glutenin subunits control by the grouping of 15-alleles are found in A genome and 19 in genome B, respectively. The newly allelic discovery improves the technology to know better about the genetic variation in wheat. It also enhance the basic research on the connection amongst industrial assets and the amount of functionality of high molecular weight and low molecular weight glutenin subunits. Tahir et al. [15] conducted experiment to show that high molecular weight glutenin subunit in a hexa-ploid wheat specie together from Baluchistan, Pakistan through SDS-PAGE.

Fabrizius et al. [16] evaluated the effect of genetic diversity on hetroses as well as trans-gressive separation amongst progeny after a cross. It was revealed that genetic diversity amid parents might donate absolutely to hetrotic and trans-gressive separation. The correlation among agronomic traits of wheat indicated that the genes on chromosomes 3A regulatory to these traits, might be pleiotrophic [17]. In this study the lesser association amid plant height and seed weight presented little connection among these traits. Grain yield showed low correlation with the stability parameters showing the possibility of attaining high yield and stability. Only a few correlations in wheat landraces were found to be significant for kernel weight.

Moghaddam et al. [18] compared 31 bread wheat genotypes derived from landraces with modern cultivars to estimate genetic variation. Large differences were found amongst the genotypes when all the characters were considered. The comparison showed that landraces were, on average, later in maturity, taller in height and having maximum number of spikes per plant but lowers the number of grains/spike, grain yield/plant, 1000- grain weight and harvest index. Whoever, some landrace had mean valve similar to those of modern cultivars. Elizabeth et al. [19] investigated nineteen sesbania accessions to characterize on morphological and agronomic data were 74.4% and 74.0% respectively. The cluster analysis performed with the eight selected characters classified the accessions into 5 groups. Zhu et al. [20] used Chinese and Australian wheat varieties to study glutenin subunits and its kinship to northern type Chinese steamed bread quality. They found high impact of flour protein content bread quality.

Dotlacil et al. [21] used 123 European varieties and winter wheat cultivars to study the earliness, Morphological and some agronomic characters of all these landraces in three-year (1997-99) field experiments, in Czech Republic. Simultaneously, by using SDS PAGE techniques high molecular weights Glu-alleles were located on specific chromosomes were identified in these cultivars. Within these selected genotypes, 224 Glu-lines has been showed to carry 3 allelic blend at 1A chromosome; 10 allelic groupings at 1B chromosome; and 3 allelic groupings at 1D chromosome individually. Comparatively, infrequent alleles were 2* at 1A and 3 + 12 at 1D, as well as alleles 8, 6, 9, 7, 13 + 16 and 17 + 18 at 1B. Some alleles are specific to certain cultivars like allele 20 at 1B was showed only in cultivars from DNK, CHE, and EST. allele 2* at 1A locus was found mostly in cultivars from Eastern, southeastern, and Central Europe. The central Europe cultivars were having characteristic allelic combination 17 + 18 at 1B chromosome. However, the gluten subunits are not specific to the geographic characterization of cultivars. The composition of Glu-alleles caused some influence on the characteristic of the cultivars like earliness of cultivars, Spike length, the number of spike, and some glutein related allele effect the weight of the grain per spike. Crude protein configuration was minimalized in that germplasm when gluten subunits at 1A locus were absent (0). The valve of gluten index was noticeably higher (59.2) in cultivars bearing allelic combination 5 + 10 at 1D.

Gupta et al. [22] performed genetic divergence analysis among twenty four advanced cultivars including four standard controls of bread wheat was carried out through study of plant height, tiller number, spikes number, main ear length, spike lets number, grain number, grain weight, seed hardness, phenol color reaction, protein content, sedimentation value, and grain yield. All the cultivars were grouped in four clusters. Intra cluster distance was highest for clusterШ, which included the largest number of cultivars. ClusterП showed the largest inter-cluster distance with clusterШ followed by clusterIV and clusterП. ClusterI and П were highly divergent. Hybridization program could be suggested between cultivars falling under these clusters, as this would give greater heterotic responses. Jagdish and Harsh [23] conducted a study to assess the genetic variability present in accessions of wheat (*T.aestivum*) collected from national and international sources. Twenty-five genotyped were selected and grain growth rate was assessed based on the grain weight/heat degree-days during grain growth.

Considerable genetic variability for grain growth rate was recorded for this trait. In general, popular genotypes grown in hot environment showed better grain growth rate. The association of grain growth rate with 100-seed weight or individual grain weight was more apparent compared to that with grain yield per se. The grain yield was highly influenced by grain-growth duration in the test genotypes rather than grain-growth rate. It was interpreted that high grain-growth rate at the beginning of the grain filling period coupled with considerably longer grain-growth duration could enable selection of genotypes suitable for late-sown condition. Khan et al. [24] passed seven Pakistani wheat cultivars thorugh SDS-PAGE, to fractionate the high molecular weight glutenin subunits and to explore the genetic diversity within species. The mitotic division it was indicate that the chromosomal number in six varieties was 2n=42. In variety sargab-92 the chromosomal number is irregular, showing 2n=40 as well as 41 instead of 42 chromosomes In six Pakistani cultivars the banding patterns of HMW glutenin subunits show no variability but the genetic changeability was revealed by the genotype Sargab-92, in which 4 extra bands had been recognized.

Kumar and Shukla [25] evaluated 30 diverse genotypes of bread wheat for yield related traits to estimate genetic parameters of variation. A significant amount of variability for days to primordial initiation, days to ear emergence, plant height, number of tillers/plant, number of effective tillers/plant, ear length/plant, number of spiklets/spike, number of kernels/spike, biological yield/plant, 1000-kernal weight and harvest index. Singh et al. [26] studied genetic diversity in terms of ten characters (i.e. numbers of days to flowering, number of days to maturity, plant height, spikes/plant, spike length, spikelets/spike, grains/spike, grain weight/spike, 1000-grain weight and yield/plant) of 80 bread wheat cultivars. Significance differences among the cultivars for the 10 characters were observed. The genotypes were congregated into 11 clusters. Cluster X and XI were comprised of only one genotype, each which recorded the shortest plants and highest grain weight per spike, respectively. In general, clusterVII, VIII, IX and X were divergent from each other and the remaining clusters. ClusterIII, VII, I, I V, III, VI exhibited low inter cluster distances indicating closeness to each other compared to the remaining cluster. The maximum inter cluster distance was recorded in cluster X and VII Cluster IV, IX and X showed highest inter-cluster distances.

Anwar et al. [27] collected 127 cultivars from different regions of Pakistan to identify the high molecular weight of gluteinin by using (SDS-PAGE) techniques. Through these techniques 13 different glutenin subunits were observed in the selected cultivars. Of these alleles spotted at all the Glu-I loci, 3-belonged to Glu-AI, five each to Glu-BI and Glu-DI loci; Subunit Null organized by locus amongst three and five higher molecular weight glutenin subunits. The occurrence of alleles in the complete set of quality score calculated according to [28] ranged between 4 and 10 in the landraces examined. Bhattacharya et al. [29] evaluated protein quality and functional properties of 219 hexaploid wheat landraces from nine different provinces of Iran. Significant differences in means for protein quality characteristics were observed among the province of origin, suggesting that the selection of more accessions from provinces with higher mean would be worthwhile strategy for selecting valuable genetic resources. The land races represented valuable genetic resources that could be used to develop new bread wheat cultivars with improved end use quality traits. A hierarchical analysis of variance revealed that 28% of the variation in the average diversity estimated was due to variation among regions of collection.

Jan et al. [30] studied nine wheat cultivars during the previous eight years. A randomized Complete Block Design (RCBD) with 3-replicates was used during each year. Highly genetic differences were observed among cultivars for biomass, Straw and grain yield, spike /m2, grain/spike, and 1000 2-1cyrain weight during both years. Cultivars were highly consistent in performance across year for plant height ($P<0.01$), biomass ($P<0.01$), Grain/spike ($P<0.01$) and 1000-grain weight (P (0.01) and Fakhre-Sarhad was selected as a best cultivar. In the correlation study very significant and positive correlation of plant height vs. biomass ($P<0.01$), and biomass vs. straw yield ($P<0.01$) was contributing component ($P<0.02$), but relationship among the yield components was not existent. Kamal et al. [31] conducted experiment on modern wheat cultivars to evaluate its growth and yield qualities under rain fed and irrigated condition. The wheat varieties showed significant differences ($P<0.05$) with respect to the plant height and 1000-grain weight, grain and straw yields, protein, amylose, ash and potassium contents in grains. In the study, best performance variety was also selected based on highest grain yield, protein and amylose content. Through irrigation process many characteristics is improved like grain and straw yields, protein content, amount of amylose, P, K and S contents in grains as compared to rain fed conditions. In standard cultivars Akbar, Aghrani, Barkat, and Kanchan are the promising varieties than the other with respect to grain and protein yields.

Khan [32] conducted an experiment to observe the banding patterns of high molecular weight (HMW) glutenin subunits through SDS-PAGE of the 7 hexaploid wheat genotypes it was characterized that 6 wheat genotypes were similar no genetic variation was found but great variability was found in sargab-92, where some additional bands had been observed. Lad et al. [33] carried out association and path coefficient examination in 24 diverse genotypes of wheat to understand the relationship and contribution of 9 characters towards grain yield. The genotypic and phenotypic coefficients of variation were highest for yield/plant, productive tillers, grain per spike and grain weight per spike. The grain yield exhibited highly significant and positive correlation with productive tillers/plant, spiklets per spike, grains per spike and grain weight per spike at both phenotypic and genotypic levels.

Pawar et al. [34] considered the genetic variability for yield and for yield components in 50 wheat cultivars. Genetic

variability was detected in the cultivars in relations of number of days to 50% flowering, plant height, number of productive tillers/plant, spike length, number of spikelets and grains/ear, grain weight and yield. The coefficient of variation (CV) was higher for plant hight, both genotypicaly and phenotypically. Upadhyaya [35] evaluated 1704 accessions of groundnut for various morphological and agronomic characteristics to calculate the morphological differences and to identify position of diverse descriptor characters. The significant traits studies showed that 12 phenotypic descriptors and 15 agronomic characters were essential in illumination of multivariate polymorphism. The experimental study clearly showed the important differences in morphology and agronomic traits of selected groundnut collection. The mean pod length, 100-seed weight and yield/plant was higher in hypogea group than in the fastigiata while, it was contrary for plant height.

Renato et al. [36] revealed that all storage protein in seeds and low-molecular-weight glutenin subunits (LMW-GS) of wheat endosperm are composed by several polymorphic units during germination time these endospermic proteins are degraded and provide energy to the growing embryo. On the basis of their structural properties, glutenin provide important characteristics to the wheat flour. The high-molecular-weight glutenin subunits have ability to form intermolecular disulphide bonds due to this internal bond formation, glutenin polymers are formed which regulate the processing properties of wheat bread.

Jakubauskiene and Juodeikiene [37] conducted experiment to show the association amongst different protein and the class of baked ring-shaped rolls. Different wheat cultivars were grown in Lithuania to evaluate its flour protein composition by using SDS-PAGE. Analysis of swelling techniques based on the echolocation was used to analyze the excellence of baked good. The following parameters are used to evaluate the flour of selected varieties: Proteins content and high and lower molecular glutein subunits all these contents have greatest effects in the quality of the ring-shaped rolls.

Li et al. [38] conducted experiment on 205 accession of emmer wheat, these cultivars were taken from different areas of china and Europe and studied the genetic diversity of the (HMW-GS) encoded by Glu-1 loci by using SDS-PAGE combined with two-dimensional gel electrophoresis and acidic capillary electrophoresis. Maximum variations in HMW-GS composition were recorded. For the formation of HMW.GS about 40 alleles (6 for Glu-A1 and 34 for Glu-B1) and 62 subunit combinations (genotypes) were investigated, some of which were not early identified.

Popa et al. [39] studied the properties of forty Romanian wheat (*Triticum aestivum L.*) cultivars by endospermic protein using SDS-PAGE techniques to uncover the homogeneity and heterogeneity and to also assemble the basic information on HMW-GS variation of the selected wheat genotypes. All fractionized germplasm were divided into two categories homogenous and heterogeneous. In all 40 genotypes only five genotypes were showing no variation and found homogenous, their protein content fractionized using SDS-PAGE techniques. The reaming 35 cultivars show clear variation in protein patterns and are found heterogeneous. The variations in heterogeneous landraces are not specific to HMW-GS but also in other proteins bands. For HMW-GS in Romanian wheat thirteen different alleles were identified at the three Glu-1 loci. Xueli et al. [40] conducted experiment to analyze HMW-GS and LMW-GS composition in selected genotypes, 270 European spelts, 15 Iranian spelts and 25-bread wheat cultivars. All these cultivars were analyzed by gel electrophoresis. The result exposed a total of 22 HMW-GS alleles (4 at Glu-A1, 11 at Glu-B1 and 7 at Glu-D1) and 32 allele combinations amid three Glu-1 loci. The dendrogram assembled by high molecular and lower molecular glutenin subunit bands suggesting the great variation between European spelts and common wheat and form separated different cluster groups. In all Iranian splet accessions exhibited similarity in HMW-GS alleles, because of a wider polymorphism and its dendogram cluster group patterns is differed from European Splets. Central European spelt wheat's is a significant genetic reservoir for refining common wheat quality.

Gaines et al. [41] quantified the specific protein content such as (HMW-GS 5+10) and (LMWGS KS2) in wheat mill brooks of 33 wheat by using 2D and SDS-PAGE. The SDS sedimentation volume was similar to the total protein content in the millstreams, which were particular guides of exact loaf volume and bread asset. Therefore, the glutenin proteins is meaningfully significant for refining the dough quality appropriate for bread and Chinese noodles.

Al Khanjari et al. [42] characterized various wheat germplasms from Oman and collected wheat spikes from different cultivated areas. The morphological evaluation of 15 qualitative and 17 quantitative characters exposed difference among Omani wheat genotypes. Overall, the phenotypic data showed the high diversity among the Omani wheat. Simple phenotypic characters can be used to show the genetic diversity among the selected genotypes. Shoaib et al. [43] evaluated thirteen Pakistani wheat varieties together from diverse ecological zones of Pakistan. Samples were evaluated for variation in seed storage protein by SDS-PAGE and physiochemical characteristics including thousand grain mass (TGM), moisture, minerals, phosphorus and ash contents. On the basis of molecular characteristics the variety Fakhre-Sarhad and Wafaq-01 was positioned best varieties having higher number of HMW-GS, but these varieties needs improvement in physiochemical status. While Watan and Gndam-711, Saleem-2000, Zakht and Chudry-97 contain less number of HMW-GS as compared to Fakhre-Sarhad variety and placed in second position, thus they are in need of improvement in protein level. The SDS-PAGE based genetic diversity was evaluated, the result showed comparatively significant variation in HMW-GS bands of different wheat varieties.

Wiser [44] revealed that in wheat different kinds of protein are present but glutein show a critical role and defining the baking properties and conferring the water absorption capacity, elasticity, viscosity and cohesivity on bread. On the

basis of their solubility in aqueous alcohol the gultenin protein is divided into two categories: soluble glutenins and insoluble glutenins. Both these types of proteins consists many partially closely related amino acid components for example glutamine and praline contents. Glutenin proteins are mainly monomeric and approximately its molecular weight is around 28,000-55,000 and the glutenin can be divided into different types the primary protein structure is converted into alpha/beta-, gamma-and omega-type. In the complex structure disulphide bonds are either present or absent as a cross-links. Various types of bonding such as ionic bonds, hydrogen bonds and hydrophobic bonds are present which is necessary for their aggregation and provide specific structure and properties to bread quality.

Caballero et al. [45] performed experiments on *Triticum Urartu* and found that this specie is a wild 2n wheat recognized as donor of the A genome in the durum and bread wheats. Triticum urartu has its normal occurring zone in the Fertile Crescent. Nine genotypes and its inhabitants from the Fertile Crescent zone (five Lebanese and four Turkish) were analyzed to show the diversity among allele codes for the glutenins (Glu-Au1 and Glu-Au3) and gliadins (Gli-Au1 and Gli-Au2). The maximum genetic diversity is identified in Turkish populations as compared to the Lebanese populations. The greater values of allelic differences showed that, these genotypes have high level of diversity, and a real risk of extinction. Martin et al. [46] in their study find out that Triticum urartu is a diploid wheat specie that has been identified as the donor of the A genome in polyploid wheats. This species were study to analazyed the gliandins by acid-PAGE in accessions collected from Iraq, Armenia, Iran, Lebanon, Turkey and former-Soviet Union. It was found that approximately Twenty-six different alleles for each locus (Gli-Au1 and Gli-Au2) involve in the synthesis glaindins subunits. The high genetic diversity is recognized but this variation between species is in danger of erosion given no random combination. Consequently, the extinction of the cultivars means loss of the genetic variation in species. The given studies provide new idea according to the gentic diversity in seed storage protein (glutein) synthesized by the Au genome, these results is also helpful for breeder to improve the wheat baking qualities. The molecular and morphological studies may be used to understand the genetic variation and used to show the genetic relations between bread wheat genotypes with great accuracy [47].

Shewry et al. [48] conducted experiment and determined all protein by using SDS-PAGE techniques. The proportion of wheat glutein protein is calculated from the extracted proteins and expressed as arbitrary units. Rapid increase in protein content was observed between 12 and 35 dpa followed by a slow increase until 42 dpa. The proportion of glutein proteins was evaluated by scanning and quantifying from the pertinent bands when the total proteins content was fractionized by SDS-PAGE. The bands present in the top of the gel showed (HMW) subunits of glutein, similarly the bands present in the lower end of the gel shown the (LMW) of gluteinn subunits.

Nisar et al. [49] conducted experiment and collected 29 wheat germplasm from that area of Pakistan that has been never studied and it was suggested that high genetic diversity between genotypes were present, these genotypes also showed significant response against rust disease. New wheat cultivar were identified, resistant to rust disease, further investigations of these new alleles posses rust response through molecular markers is required. Highly Polymorphism was observed in HMW glutenin loci, the glutenin is important for bread-making quality. Hence this variation is important for breeder to develop new cultivar with good bread-making quality. Zhang et al. [50] Conducted experiment to study the composition and function of (LMW) glutenin subunits. It was clear that these protein coded by a group of gene set located at Glu-A3,Glu-B3, Glu-D3, loci on short arm of a chromosomes and showed high allelic diversity. SDS-PAGE analysis was used to characterized the genetic and protein composition of LMW-glutenin subunits.

Hashjin et al. [51] studied that the morpho-protein patterns of genetic diversity increases the efficacy of germplasm conservation and development. Sixteen allelic compositions were identified in the genotypes for high molecular weight glutenin subunits. The three alleles were present at the Glu-A1 locus and 8 alleles at Glu-B1. The null allele was observed more frequently than the 1 and 2 alleles. Two alleles, namely 17 + 18 and 20, represented more frequent alleles at Glu-B1 locus. The genetic variability in Glu-A1and Glu-B1 loci were 0.42 and 0.81, respectively. The cluster analysis based on morphological traits and HMW-GS clustered the genotypes in to six and seven groups, respectively. The results showed maximum genetic variation among selected genotype. The plants belong to diverse clusters can be used for hybridization to produce valuable recombinants in the segregating generations for the improvement of durum wheat.

3.2. Glutenin Composition of Wheat and SDS-PAGE Analysis

SDS-PAGE was used to study the glutenin composition of bread wheat. The pioneer studies of Bietz and Wall [52] showed that two types of subunits were present, the low molecular weight (10,000-70,000 Da) and high molecular weight glutenin subunits (80-130 Kda). Li et al. [53] reported high genetic diversity in high molecular weight glutenin subunits, while Benmoussa et al. [54] suggested that deletion and insertions with repetitive regions are responsible for these variations in subunit length. Similarly Yahata et al. [55] and Popa et al. [39] also reported homogeneity and heterogeneity in the LMW and HMW sub unit by using SDS-Polyacrylamide gel electrophoresis.

4. Conclusion

Bread wheat is a Hexapliod species with 2n=6x=42 chromosomes and having high genetic diversity. Environmental variations may affect the morphological and biochemical variations among wheat verities. The SDS-

PAGE technique was carried out for the examination of high molecular weight-gultenin subunits (HMW-GS) and low molecular weight-gultenin subunit (LMW-GS). Glutenin having important banking qualities but the variations in glutenin subunits was attributed to different allelic combinations at various loci in different chromosomes.

References

[1] Cromwell, E., Cooper, D., Mulvany, P., 1999. Agricultural Biodiversity and Livelihood: issues and entry points: Paper for DFID Linking Policy and Practice on Biodiversity Project (LPPB), ODI, FAO, ITDG.

[2] Browning, J. A., 1988. Current thinking on the use of biodiversity to buffer small grains against highly epidemic and foliar pathogens: problems and future prospects." P. 76-90 IN CIMMYT, Breeding strategies for resistance to the rust of wheat. Mexico. D. F. CIMMYT.

[3] Clay, J., 1991. Cultural survival and conservation: Lessons from the past 20 years in: Common Wealth of Australia: Biodiversity series paper No.1. deptt. of Environment, Sports Territories, Canberra.

[4] FAO., 1996. Report of the International Technical Conference on Plant Genetic Resources. Leipzing Germany.

[5] Miller, T. E., Koebner, R. M. D., (eds).2000. Proceedings of the Seventh International Wheat Genetic Symposium, Cambridge. Institute of Plant Sciences Research, Cambridge.

[6] Kerby, K., J. Kuspira., 1987. The phylogeny of the polyploid wheats Triticum aestivum (bread wheat) and Triticum turgidum (macaroni wheat)." *Genome*29.5: 722-737.

[7] Lagudah, E. S., Dubcovsky, J., Powell, W., 2001. Wheat genomics. Plant Physiol. Biochem. 39: 335-344.

[8] Hassan, M., Wasiullah, Iqbal, J., Ilyas, M., 2003. Evaluation of Wheat Varieteis under the Agro-climate Condition of Barani Agricultural Research Station, Kohat. Pak. J. Agron. 2(1): 8-12.

[9] Smith, J. S. C., Smith, O. S., 1989. The description and assessment of distances between inbred lines of maize: the utility of morphological, biochemical and genetic descriptors and scheme for testing of distinctiveness between inbred lines. Maydica. 34:151-161.

[10] Amurrio, J. M., De Ronand, A. A., Zeven, A. C., 1995. Numerical taxanomy of Iberian pea landraces based on quantitative and qualitative characters. Euphytica. 82:195-205.

[11] Van-Beuningen, L. T., Busch, R. H., 1997. Genetic diversity among North American Spring.

[12] Weltzien, El., Fischbeck, G., 1990. Performance and Variability of Local Barley Landraces in Near‐Eastern Environments." *Plant Breeding* 104.1: 58-67.

[13] Anwar, R., 1993. Distribution of wild and primitive wheats in Pakistan. Biodiversity and Wheat Improvement. Damania, A. B. (ed.) A Wiley-Sayce Publication. Pp. 417-422.

[14] Ciaffi, M., Lafiandra, D., Porceddu, E., Benedettelli, S., 1993. Storage protein variation in wild emmer wheat (*T. turgidum* spp. Dicoccoides) from Jordan and Turkey. I. Electrophoretic characterization of genotypes. Theor. Appl. Genet. 86: 474-480.

[15] Tahir, M., Turchetta, T., Anwar, R., Lafiandra, D., 1996. Assessment of genetic variability in hexaploid wheat land races of Pakistan based on polymorphism for HMW glutenin subunits. Genet. Resources and Crop Evolution. 43: 211-220.

[16] Fabrizius, M. A., Busch, R. H., Khan, K., Huckle, L., 1998. Genetic diversity and heterosis of spring wheat crosses. Crop Sci. 38:1108-1112.

[17] Shah, M. M., Baenziger, P. S., Yen, Y., Gill, K. S., Moreno-Sevilla, B., Haliloglu, K., 1999. Genetic analysis of agronomic traits controlled by wheat chromosome 3A. Crop Sic. 39: 1016-1021.

[18] Moghaddam, M., Ehdiae, B., Waines, J. G., 1998. Genetic variation for and interrelationships among agronomic traits in land races of bread wheat from southwestern Iran. J. Genet. & Breed. 52: 73-81.

[19] SA., Elizabeth, AV., Schammacs, E. A., Vencovsky, R., Martin, P. S., Bandel, G., 2001. Germplasm characterization of Sasbania accessions based on multivariate analysis. Genetic Resource and Crop Evolution. 48: 79-90.

[20] Zhu, J., Huang, S., Khan, K., Brien, L. O., 2001. Relationship of protein quantity, quality and Dough Properties with Chinese Steamed Bread Quality. J. Cere. Sci. 33(2): 205-212.

[21] Dotlacil, L., Gregova, E., Hermuth, J., Stehno, Z., Kraic, J., 2002. Diversity of HMW Glu alleles and evaluation of their effects on some characters in winter wheat land races and old cultivars. Czech Journal of Genetics and Plant Breeding. 38 (3-4): 109-116.

[22] Gupta, R. S., Tiwari, D. K., Doel, S. S., Singh, R. P., 2002. Genetic divergence in bread wheat (*Triticum aestivum L. em Thell*). New Botanist. 60:237-243.

[23] Jagdish, R., Harsh, C., 2002. Rate of grain growth in advanced wheat (*Triticum aestivum*) accession under late sown environment. Indian J. Agri. Sciences. 72(10): 581-585.

[24] Khan, M. F., Schumann, E., Weber, W. E., 2002. Characterization of Pakistani wheat varities for General Cultivation in the Mountanious Regions of Azad Kashmir. Asian J. Plant. Sci. 1(6): 699-702.

[25] Kumar, P., Shukla, R. S., 2002. Genetic analysis for yield and its attributed traits in bread wheat under various situation. JHKVV research journal 2000, 36 (1-2): 95-97.

[26] Singh, P., Mishra, P. K., Billore, M. M., Bhawsar, R. C., 2002. Studies on genetic diversity in Wheat (*Triticum aestivum*) Adv. In plant Sci. 15 (1): 301-305.

[27] Anwar, R., Masood, S., Khan, M. A., Nasim, S., 2003. The high molecular weight glutenin subunit composition of wheat (*Triticum aestivum L.*) land races from Pakistan. Pak. J. Bot. 35(1): 61-68.

[28] Payne, P. I., 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread making quality. Ann. Rev. Plant Physiol. 38: 141-153.

[29] Bhattacharya, M., Jafari Shabestari, J., Qualset, C., Corke, H., 2003. Diversity of protein quality traits. Cereal research communications. 31: 193-200.

[30] Jan, I., Usman, M., Iftikhar., Khalil, H., Jan, T., 2003. Performance of recently released wheat cultivars. Asian J. Pl. Sci. 2(8): 627-632.

[31] Kamal, A. M. A., Islam, M. R., Chowdhury, B. L. D., Talukder, M. A. M., 2003. Yield performance and Grain Quality of Wheat Varieties Grown under Rain fed and Irrigated Conditions. Asian J. Pl. Sci. 2(3): 258-260.

[32] Khan, M. F., 2003. Evaluation of Hexaploid Wheat Genotypes by using DNA isolation and gel-electrophoresis. Asian J. Plant. Sci 2(2): 212-215.

[33] Lad, D. B., Banger, N. D., Bhor, T. J., Mukhekar, G. D., Birader, A. B., 2003. Correlation and path coefficient in wheat. J. Maharashtra Agric. Univ. 28(1): 23-25.

[34] Pawar, S. V., Patil, S. C., Naik, R. M., Jambhale, V. M., (2003). Genetic variability and heritability in wheat. Jour. Maharashtra Agric. Uni. 27(3): 324-325.

[35] Upadhyaya, H. D., 2003. Phenotypic diversity in groundnut (*Arachis hypogaea L.*) Core collection assessed by morphological and agronomical evaluations. Genetic Resources and Crop Evolution. 50: 539-550.

[36] Renato, D. O., Masci, S., 2004. The low-molecular-weight glutenin subunits of wheat gluten. J. Cere. Sci. 39(3): 321-339.

[37] Jakubauskiene, L., Juodeikiene, G., 2005. The relationship between protein fractions of wheat gluten and the quality of Ring-Shaped Rolls Evaluated by the Echolocation Method. Food Technol. Biotechnol. 43(3): 247-253.

[38] Li, Q. Y., Yan, Y. M., Wang, A. L., Zhang, Y. Z., Hsam, S. L. K., Zeller, F. J., 2006. Detection of HMW glutenin subunit variations among 205 cultivated emmer accessions (*T.turgidium ssp. Dicoccum*). Plant breeding 125 (2): 120-124.

[39] Popa, M., Gregova, E., Kraic, J., newsletter, P.G.R., 2006. Romanian wheat (*T. aestivum L.*) landraces characterized by seed storage-protein electrophoresis. (135): 53-58.

[40] Xueli, A., Qiaoyun, L., Yueming, Y., Yinghua, X., Hsam, S. L. K., Zeller, F. J., 2005. Genetic diversity of European spelt wheat (*Triticum aestivum* ssp. spelta L. em. Thell.) Revealed by glutenin subunit variations at the Glu-1 and Glu-3 loci. Euphytica. 146 (3): 193-201.

[41] Gaines, C., Reid, S., Vander Kant, J. F., Morris, C. F., 2006. Comparison of methods for gluten strength assessment. *Cereal chemistry*, *83* (3), 284-286.

[42] Al Khanjari, S., Hammer, K., Buerkert, A., Röder, M. S. 2007. Molecular diversity of Omani wheat revealed by microsatellites: II. Hexaploid landraces. *Genetic Resources and Crop Evolution*, *54*(7), 1407-1417.

[43] Shuaib, M., I. Khan, Z. Ali, T. Ahmad and A. Zeb (2007). Evaluation of different wheat varieties by SDS-PAGE. Pak. J. Bio. Sci. 10(10): 1667-1672.

[44] Wiser, H., 2007. Chemistry of Gluten Proteins. Food Microbial. 24(2): 115-119.

[45] Caballero, L., Martin, M. A., Alvarez, J. B., 2008. Genetic diversity for seed storage proteins in Lebanon and Turkey populations of wild diploid wheat (*Triticum urartu*).

[46] Martin, M. A., Martin, L. M., Alvarez, J. B., 2008. Polymorphism at the Gli-Au1 and Gli-Au2 loci in wild diploid wheat (*Triticum urartu*). Euphytica 163:303-307.

[47] Eivazi, A. R., Naghavi, M. R., Hajheidari, M., Pirseyedi, S. M., Ghaffari, M. R., Mohammadi, S. A., Majidi, I., Salekdeh, G. H. and Mardi, M., 2008. Assessing wheat (Triticum aestivum L.) genetic diversity using quality traits, amplified fragment length polymorphisms, simple sequence repeats and proteome analysis. *Annals of Applied Biology*, *152*(1), pp. 81-91.

[48] Shewry., Peter, R., Underwood, C., Wan, Y., Lovegrove, A., Bhandari, D., Toole, G., Clare Mills, E. N., Denyer, K., Rowan, A. C. M. l., 2009. Storage product synthesis and accumulation in developing grains of wheat." Journal of Cereal Science 50, 1: 106-112.

[49] Nisar, M., Khan, N., Ahmad, Z., Ghafoor, A., 2011. Genetic diversity and disease response of rust in bread wheat collected from Waziristan Agency, Pakistan." Int. J. Biodivers. Conserv. 3(1):10-18

[50] Zhang., Xiaofei., Jin, H., Zhang, Y., Liu, D., Li, G., Xia, X., He, Z., Zhang, A., 2012. Composition and functional analysis of low-molecular-weight glutenin alleles with Aroona near-isogenic lines of bread wheat." BMC plant biology 12, 1: 1.

[51] Hashjin, R. M., Fotokian, M. H., Sarbrzeh, M. A., Mohammadi, M., Talei, D., 2014. Genetic diversity of durum wheat genotypes using morpho-protein analysis." Cereal Research Communications 42, 4: 677-686.

[52] Bietz J. A., Wall J. S., 1980. Identity of high molecular weight gliadin and ethanol-soluble glutenin subunits of wheat: relation to gluten structure. Cereal Chem. 1; 57(6):415-21.

[53] Li, Q. Y., Y. M. Yan, A. L., Wang, X. L. A, Y. Z. Zhang, S. L. K Hsam and F. J. Zeller. 2006. Detection of HMW glutenin subunit variations among 205 cultivated emmer accessions (*T.turgidium ssp. Dicoccum*). Plant breeding 125 (2): 120-124.

[54] Benmoussa, M., L. P. Vezina, M. Page, S. Yelle, S. Laberge., 2000. Genetic polymorphism in low-molecular-weight glutenin genes from *Triticum aestivum*, variet Chinese Spring. Theor. Appl. Genet. 100 (5): 789-793.

[55] Yahata, E., W. M. Funatsuki, Z. Nishio, Y. Yamamoto, A. Hanaoka, H. Sugiyama, M. Tanida and H. Saruyama. 2006. "Relationship between the dough quality and content of specific Glutenin Proteins in Wheat Mill Streams, and Its Application to Making Flour Suitable for Instant Chinese Noodles", Biosci. Biotechnol. Biochem. 70: 788-797.