INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 14, No. 4, p. 27-33, 2019
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Estimation and conformation of HMW glutenin loci in Pakistani *Barley* Lines detected through polyacrylamide gel electrophoresis

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Article published on April 28, 2019

Key words: Barley, Selected lines, Glutenin protein, SDS-PAGE.

Abstract

Glutenin, is consider the major storage proteins in the family of Poaceace which is also consider the main contents in wheat cereal of this family, to commercialize the ancient domesticated cereal like *barley* in the race of industrial crop and determining the uses of *barley* flour quality and resemblance of *barley* elite lines elasticity and dough quality, experimental assay was designed in the Laboratory of Plant Molecular Biology Department of Botany, university of Malakand on 100 elite lines of the *barley*.to detect High Molecular weight Glutenin protein extracted from the *Barley* seeds on the basis of Molecular weight, the Glu loci in *barley* 100 lines which score from the gel of polyacrylamide classify into nine different size (120, 105, 90, 80, 65, 45, 30, 20 and 10 KD) bands which further was classified into five different groups on the basis of variability present in selected barley lines and also in their Molecular weight; absence/presence and intensity of bands were observed. Largest group was observed is group 1 have 41% of elite lines in which 120, 105, 90, 80 and 65 KD molecular weight bands were recorded present. And some of the lines were found unknown which are not related any of these five group respectively, which clearly determine the novel allelic subunit and also show variation from the gene pool in these lines, further evaluation of these lines are needed to track these loci in this cash crop.

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Introduction

Exploration and genetic evaluation of ancient cash crops are beneficial to the modern agriculture to evaluate the landraces/accessions and lines of the crop through different modern tools, which broaden the gene-pool for the plant breeders and enhance crop performance and fulfill the requirement of the agriculture and industry, resemblance with the statement aim is to identify the High Molecular weight (HMW) Glutenin protein in *barley* and determine the intensity of the specified quality protein in the selected hundred Lines. *Barley* (*Hordeium vulgare* L.) is among the main grains in providing man and livestock food resources, globally. This plant is planted in regions where other plants could not grow well due to the low rainfall, high altitude, soil salinity, cold and heat. About one third of lands on Earth and 85% of lands in Iran are located in arid regions (Badripur *et al*., 2004). Barley is the second most important winter cereal in Pakistan. About two third of the area devoted to barley in the country is rain fed and one third is irrigated. It offers great opportunity to bring the marginal areas under cultivation where other crops cannot be grown successfully. It is among the major crops used for food, feed and malt in the world (Ahmad *et al.,* 2008). Barley is mainly used for human and livestock food and preparing malt. The cultivation area for barley in 2006 was over 56million hectares and its yield was more than 137 million tons. In Iran, its yield is about 1.7 million hectares and it is the main pervasive crop compatible with stress conditions. Knowledge regarding the amount of genetic variation in germplasm arrays and genetic relationships between genotypes are important considerations for efficient conservation and utilization of germplasm resources (Russel *et al.,* 1997; Davila *et al.,* 1998; and Manjunatha *et al.*, 2006). In the context of plant improvement, this information provides a basis for making decisions regarding selection of parental combinations that will maximize gain from selection and maintain genetic diversity. Information on the amount of genetic variation present and the location of the genetic determinants of diversity may be useful for germplasm conservation and targeting gene discovery efforts (Sorrels & Wilson, 1997; Jana, 1999; Hou *et al*., 2005). Albumins, which are soluble in water and comprise mostly enzymatic proteins; globulins, which are soluble in dilute salt solutions and generally occur in protein bodies (i.e., they can be considered as storage proteins in the strict sense); prolamins, which are soluble in aqueous ethanol solutions and are also found in protein bodies as true storage proteins (Chmelík *et al.,* 2002). Prolamins are the main protein storages in barley which are called hordeins that form 35-50% of seeds' nitrogen. (Kirkman *et al.,* 1982). Glutelins, which are soluble in alkaline or acid solutions, or in detergents and are probably mainly structural proteins, although some of them may have metabolic functions (Chmelík *et al.,* 2002). The high-molecular-weight (HMW) subunits of glutenin are coded by genes at three genetically unlinked loci, *Glu-Al, Glu-Bl* and *Glu-Dl,* which occur on chromosomes lA, 1B and lD, respective1y (Payne *et al*., 1984). Each locus exhibits extensive allelic variation (Payne *et al*., 1981). And the allelic protein subunits can be easily distinguished by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), while in our this study these allelic subunits were also determine through page electrophoresis in barley which divide the selected lines of barley to different groups on the base of Glu quality protein. In previous studies, crosses were made between varieties with contrasting HMW subunits of glutenin, and the segregating progenies at later generations were tested for bread making quality by the SDS-sedimentation test and for glutenin subunit composition by SDS-PAGE (Payne *et al*., 1979; Payne *et al*., 1981). Therefor the present studies conducted to further evaluation of these lines are needed to track these loci in this cash crop.

Materials and methods

The experiment was conducted by using one hundred elite lines of barley seed of selected lines accessions was get from the Gene bank of (PGRI) Plant Genetic Resource Institute, National Agriculture Research Center (NARC), Islamabad, Pakistan. The study was conducted in the laboratory of Plants Molecular Biology,

2. Department of Botany and University of Malakand, Pakistan. For the specific seed storage protein extraction, single seed of single line was ground to fine powder with the help of mortar and pestle. 0.3g powder of each sample was added to the eppendorf tube.

HMW-GS extraction

The grains (20g) were grinded into flour and 20mg was weighted in 1.5ml Eppendorf tube. The 300 micro liter of protein extraction buffer [28.5% sample buffer (7% SDS, Tris-HCl 0.01 M (pH 6.8), 30% glycerol, 0.001% Commassive blue), 5% 2-ercaptoethanol] was added to each E. tube and extracted for thirty minutes at room temperature (25°C) with occasional vortexing. The samples were incubated for 2 hours at 27°C in water-bath and then centrifuge at 13000rpm for 10 minutes under 25°C. Before electrophoresis, all samples were heated at 80°C for 20min.

Electrophoresis

Electrophoresis was conducted using 15% polyacrylamide gel (composition of resolution gel: 3.0M Tris-HCl pH9.0, 0.4% SDS and staking gel 0.4M Tris-HCl pH 7.0, 0.4% SDS). The electrode buffer containing 0.025M Tris, 129M Glycine and 0.125% SDS was poured into the electrophoresis tank. Then 10µl of the supernatant was loaded in each well of 15% PAG. Electrophoresis was run at 50V until the blue line passed through the bottom of gel plates. The gels were than stained and de-stained for data scoring.

Data analysis

The HMW-GS were identified using the numbering system of Payne & Lawrence, (1983), while quality scores were assigned using the scoring system (Payne *et al*., (1987). Alleles were assigned quality scores of 1 (the poor quality) to 4 (the high quality) and individual scores for each allele present in a genotype were then pooled to provide the overall quality score for that a genotype. The generated data matrix was preceded through un-weighted pair group method on arithmetic averages (UPGMA) using PC-ORD software in order to find out the genetic relationship of the genotypes.

Results

High Molecular weight Glutenin protein extracted from the Barley seeds was detected on the basis of Molecular weight into nine different size (120, 105, 90, 80, 65, 45, 30, 20 and 10 KD) bands. The glutenin protein was classified into five different groups on the basis of variability present in selected barley lines and also in their Molecular weight; absence/presence and intensity of bands were observed. We classified barley lines into various groups based on diversity in glutenin polymorphism.

The group 1 includes barley lines with 120, 105, 90, 80 and 65 KD molecular weight bands. Majority of the lines belong to the group 1, about 41% of the 100 selected barley lines belong to this group (Fig. 2). 120, 105, 90, 80 and 65 KD molecular weight bands were recorded present and 45, 30, 20 and 10 KD molecular size bands were found absent. The high intensity bands are 90, 80 and 65 KD molecular weight bands and 120 and 105 KD molecular weight bands are medium intensity bands. Group 2 includes those lines, which contain 105, 90 and 80 KD molecular weight bands. All of the polymorphic bands are high intensity bands, 120, 65, 45, 30, 20 and 10 KD molecular weight bands were observed absent but 120 KD molecular size band was present in group 1, about 13% of the lines belong to the group 2. Group 3 includes those lines, which contain 120, 105, 90, 80, 65, 20 and 10 KD molecular weight bands. In this group 45 and 30 KD bands were absent, 20 and 10 KD molecular weight bands were found present but these two bands were observed absent in group 1 and group 2. The 120 and 105 KD molecular weight bands are high intensity bands and 90, 80 and 65 KD molecular size bands are medium intensity bands, 20 and 10 KD molecular weight bands are low intensity bands. This is the 2nd largest group having 14 % of the lines belonging. Group 4 include those accessions, containing 90, 80, 65 and 20 KD molecular weight band, 120, 105, 45, 30 and 10 KD molecular weight bands were found absent in the group 4, the 120 and 105 KD molecular weight bands were present in group 1, and 45 KD molecular weight band was present in group 5.

High intensity band is 90 KD and 80, 65 and 20 KD molecular weight bands are medium intensity bands. The group 5 include those accessions, which consist 105, 80, 65, 45 and 10 KD molecular weight bands 120, 90, 30 and 20 KD bands were observed absent, 120 and 90 KD bands were present in group 1 and group 3 and 90 KD band was found present in group 1, 2, 3 and 4 also. In this group 105, 80, 65 and 45 KD molecular size bands are high intensity bands and 10 KD molecular weight band is low intensity band. There are some unknown accessions which are not related to these groups about 9% accessions of the 100 selected lines shown in (Fig. 5).

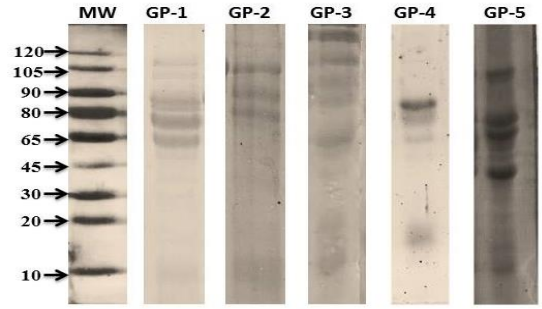

Fig. 1. Loci pattern in the groups.

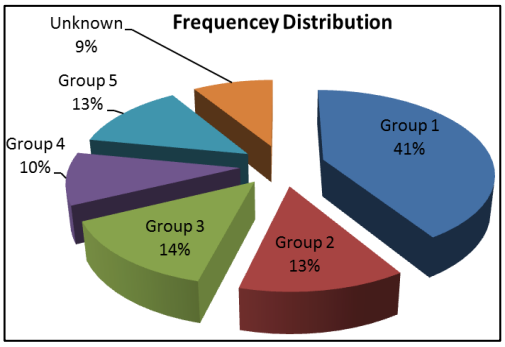

Fig. 2. Frequency chart for the groups MW= Molecular Weight, GP= Group.

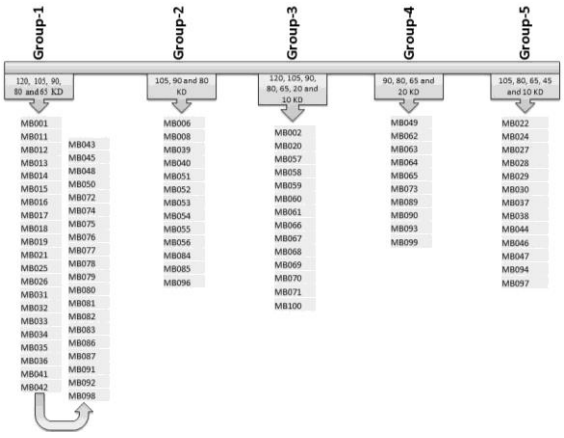

Fig. 3. Accessions numbers of the selected barley lines belong to their groups.

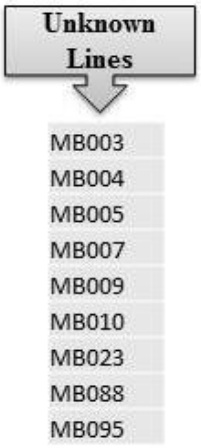

Fig. 4. Accession numbers of the unknown barley lines.

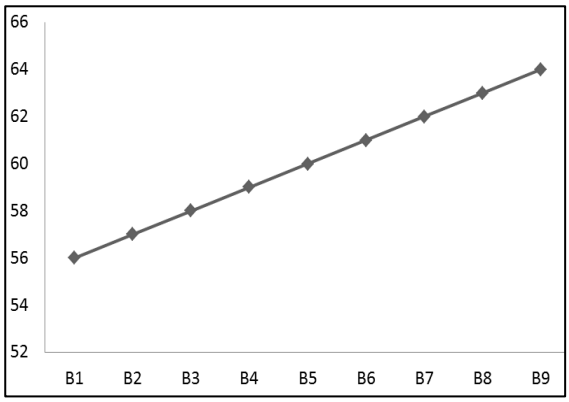

Fig. 5. Frequency distribution for genetic diversity within bands.

Genetic diversity within banding pattern

SDS PAGE was used to find out of the genetic diversity existed in the elite line of barley on the basis of Glutenin protein study it was found that lines revealed significant level of genetic divergence.

In this study 15% polyacrylamide gel was formed and the sample was load to the gel 10 microliter with the help of micropipette which gives the best result a total of 9 reproducible bands were observed. The entire band loci were polymorphic.

A total of 56% genetic diversity was found the utmost level of variation was found in B1 (0.44%) followed by B2 (0.43%), B3 (0.42%), B4 (.41%) and B5 (0.40%) polymorphism respectively. Similarly B6 (.39%) revealed low level of i.e.B7 (.38%), B8 (.37%) and B9 (.36%) respectively table (1).

Table 1. Total genetic diversity present in 09 reproducible bands of Glutenin protein in elite lines of barley.

Band	*F*	Present%	Absent%	TGD%
B1	56	56	44	0.44
B2	57	57	43	0.43
B3	58	58	42	0.42
B4	59	59	41	0.41
B5	60	60	40	0.4
B6	61	61	39	0.39
B7	62	62	38	0.38
B8	63	63	37	0.37
B9	64	64	36	0.36

Dendogram tree based on glutenin protein binary data matrix divided lines of barley into multiple clusters on the basis of variation present in their glutenin protein profile (Fig. 6).

It was observed that dendogram delineated genotypes into two linkages i.e. L-I and L-II.

Two linkages are consists of 4 clusters. The linkage I consists of 3 clusters and similarly linkage 2 contains 1 cluster. The linkage 1 contain cluster 1 which composed of 55 lines such as (MB001, MB011, MB012, MB013, MB014, MB015, MB016, MB017, MB018, MB019, MB021, MB025, MB026, MB031, MB032, MB033, MB034, MB035, MB036, MB041, MB042, MB043, MB045, MB048, MB050, MB072, MB074, MB075, MB076, MB077, MB078, MB079,

MB080, MB081, MB082, MB083, MB086, MB087, MB091, MB092, MB098, MB002, MB020, MB057, MB058, MB059, MB060, MB061, MB066, MB067, MB068, MB069, MB070, MB071 and MB100), cluster 2 contain 14 lines (MB006, MB008, MB039, MB040, MB051, MB052, MB053, MB054, MB055, MB056, MB084, MB085, MB096 and MB009), cluster 3 contain 14 lines (MB003, MB007, MB088, MB005, MB059, MB062, MB064, MB065, MB073, MB089, MB090, MB093, MB099 and MB063).

Cluster 4 consists of linkage II containing 17 lines (MB004, MB010, MB023, MB095, MB022, MB024, MB027, MB028, MB029, MB030, MB037, MB038, MB044, MB046, MB047, MB094 and MB097).

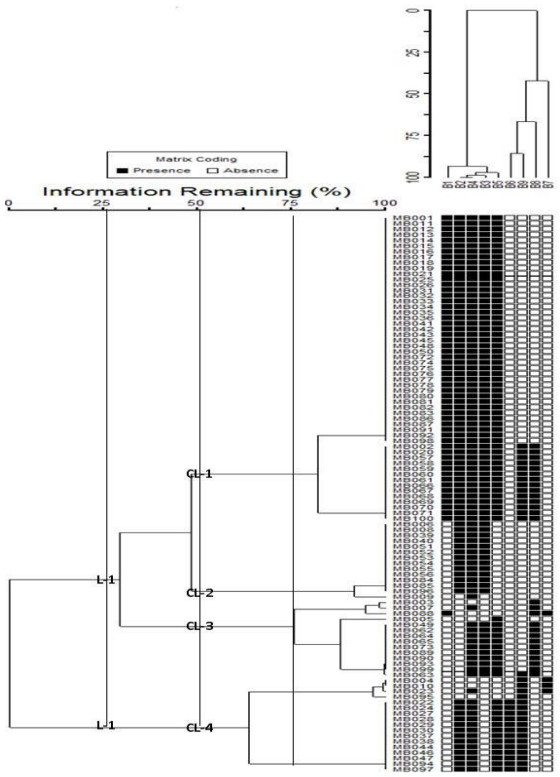

Fig. 6. Dendrogram shown genetic diversity based on Glutenin protein within barley elite lines.

Discussion

Barley is the second most important winter cereal in Pakistan. About two third of the area devoted to barley in the country is rain fed and one third is irrigated. It offers great opportunity to bring the marginal areas under cultivation where other crops cannot be grown successfully. It is among the major crops used for food, feed and malt in the world (Ahmad *et al.,* 2008). Albumins, which are soluble in water and comprise mostly enzymatic proteins; globulins, which are soluble in dilute salt solutions and generally occur in protein bodies (i.e., they can be considered as storage proteins in the strict sense); prolamins, which are soluble in aqueous ethanol solutions and are also found in protein bodies as true storage proteins (Chmelík *et al.,* 2002). Prolamins are the main protein storages in barley which are called hordeins that form 35-50% of seeds' nitrogen. (Kirkman *et al.,* 1982). Glutelins, which are soluble in alkaline or acid solutions, or in detergents and are probably mainly structural proteins, although some of them may have metabolic functions (Chmelík *et al.,* 2002). The high-molecular-weight (HMW) subunits of glutenin are coded

by genes at three genetically unlinked loci, *Glu-Al, Glu-Bl* and *Glu-Dl,* which occur on chromosomes lA, 1B and lD, respective1y (Payne *et al.*, 1984).

The analyses of HMW-GS are often effective not only for diversity studies, but for identifying wheat genotypes with bread making quality. It is the predominant sub unit in wheat landraces (Lagudah *et al.,* 1987; Gross & Guo, 1993) and it has been reported that more than 60% wheat landraces of Pakistan possessed 'null' allele at *Glu*-A1 locus (Tahir *et al.,* 1996; Anwar *et al.,* 2003).

In the present study the analysis of HMW glutenin in barley lines was classified on the bases of Molecular weight Marker using SDS-PAGE. Total of 100 lines were used for the evaluation of the high molecular weight glutenin. High Molecular weight Glutenin protein extracted from the Barley seeds was detected on the basis of Molecular weight into nine different size (120, 105, 90, 80, 65, 45, 30, 20 and 10 KD) bands. The glutenin protein was classified into five different groups on the basis of variability present in selected barley lines and also in their Molecular weight; absence/presence and intensity of bands were observed. We classified barley lines into various groups based on diversity in glutenin polymorphism. Group 1 is the largest group which include about 41% of 100 lines, 120, 105, 90, 80 and 65 KD molecular weight bands were found present and 45, 30, 20 and 10 KD molecular weight were recorded absent in group 1. Similarly, 13% of the 100 lines belong to group 2. All the polymorphic bands which present in group 2 are high intensity bands and 120 KD molecular weight band was absent in group 2. Group 3 is the 2nd largest group in present investigation which has 14% of 100 lines belonging. Group 4 include 10% lines and group 5 has 13% respectively. About 9% lines were found unknown who could not related to present five groups on the bases of molecular weight.

Conclusion

Our study concluded that, HMW Glu protein is locus specific protein in wheat and barley. This is first dissertation to assess diversity in barley through Glu protein which indicate considerable level of variation in their banding pattern, which will provide further opportunity to plant breeders to select genetically persistent barley lines on the base of Glu loci which indicate that further molecular level exploration of these lines are needed.to explore Glu gene specific Molecular markers are recommended to find and diagnose these loci in the genome of barley which further help in barley crop utilization.

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