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Identification of bread wheat genotypes based on high Mr weight glutenin subunits

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Abstract

The seed proteins of 24 varieties/advanced breeding lines of Indian bread wheat were fractionated using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to determinbe their high Mr glutenin subunit composition. Amongst the 24 varieties/advanced breeding lines, three alleles were identified for the *Glu-A1* locus (subunits 1,2* and null phenotype), three alleles for the *Glu-B1* locus (7+8, 7+9 and 17+18) and two for the *Glu-D1* locus (2+12 and 5+10). These subunits showed high polymorphism. Based on these subunits 9 different groups were observed in 24 genotypes. Two genotypes *viz.*, UP 2441 and CPAN 3004 had distinct group from the remaining genotypes. The genotype UP 2441 showed null, 17+18 and 2+12 and CPAN 3004 revealed 1, 7+9 and 2+12 subunits. This suggested the usefulness of seed protein electrophoresis showing high Mr glutenin subunits in identifying and characterizing the different wheat genotypes.

Key words: Bread wheat, seed protein, electrophoresis, HMW subunit of glutenin

Introduction

The endosperm of the wheat grain usually contains 7 to 15 percent protein and about one half of this is glutenin [1]. The glutenin proteins of wheat endosperm consist of individual subunits that form polymers stabilized by interchain disulfide bonds. The reduced subunits are usually classified as high molecular weight (HMW) or low molecular weight (LMW) based on their molecular weight by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [2]. These high Mr weight glutenin subunits are encoded by the genes at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arm of homoeologous chromosomes 1A, 1B and 1D, respectively. The subunits are highly polymorphic. These subunits are useful marker in the identification of the cultivars in wheat.

Materials and methods

In this study, 24 different genotypes were used (Table 1). Analysis was done in wheat quality lab of Department of Genetics and Plant breeding, GBPUAT, Pantanagar.

Table 1. Distribtition of high molecular weight (HMW) glutenin subunits composition of 24 genotypes

S.No.	Genotypes	*Glu-D1*	*Glu-A1*	*Glu-B1*
1.	UP 2003	5+10	1	17+18
2.	UP 262	2+12	2*	7+8
3.	UP 2338	2+12	2*	17+18
4.	UP 2425	5+10	2*	7+9
5.	UP 2382	2+12	2*	7+9
6.	HD 2329	2+12	2*	7+9
7.	HD 2285	2+12	2*	7+8
8.	WH 542	5+10	2*	7+9
9.	CPAN 3004	2+12	1	7+9
10.	HUW 234	5+10	2*	17+18
11.	PBW 154	2+12	2*	7+8
12.	PBW 226	2+12	2*	7+8
13.	PBW 343	5+10	1	7+9
14.	Raj 3077	2+12	2*	17+18
15.	Raj 3765	2+12	2*	7+8
16.	PBW 373	5+10	1	7+9
17.	Sonalika	2+12	2*	7+9
18.	UP 2418	5+10	2*	7+9
19.	UP 2441	2+12	null	17+18
20.	UP 2450	5+10	2*	7+9
21.	UP 2398	5+10	2*	7+9
22.	K 9607	5+10	1	7+9
23.	K 9545	5+10	2*	17+18
24.	K 9107	5+10	2*	17+18

Seed protein extraction: Total seed proteins were extracted from half kernels with sample buffer (200 µl) containing 4% (w/v) SDS, 15% (v/v) glycerol, 0.001% (w/v) bromophenol blue, 2% (v/v) 2-mercaptoethanol and 0.06M Tris-base, pH 6.8, at 60°C for 1 h and then centrifuged at 12000 g for 10 min. 10 µl of the supernatant were loaded into the sample wells of the gel of SDS-PA6E separation of high Mr glutenin subunits.

SDS-PAGE: The discontinuous SDS-PAGE system used was based on the Laemmli [5] as modified by Lawrence and Shephered [6]. The stacking gel had 30% acrylamide 2.7% C (bisacrylamide-to-acrylamide ratio), 0.2% (w/v) SDS and 3.03% (v/v) Tris base, pH

6.8. The separating gel contained 10% acrylamide, 08% C, 0.2% (w/v) SDS and 9.1% tris base (w/v), pH 8.9. Electrophoresis was performed for 5 h at 40 mA per gel. Staining and destaining were done according to Singh and Shephered [7]. The numbers assigned to high Mr weight glutenin subunits were identified for each genotype according to the numbering system suggested by Payne and Lawrence [8].

Results and discussion

Amongst the 24 wheat genotypes examined three high Mr glutenin weight subunits 1, 2* and null at *Glu-A1* locus and three subunits 7+8, 7+9 and 17+18 at *Glu-B1* locus and 2+12 and 5+10 at *Glu-D1* locus were observed (Table 2).

Table 2. Allelic variants and their frequency at *Glu-1* locus

1A	Geno-types	Frequ-ency (%)	1B	Geno-types	Frequ-ency (%)	1D	Geno-types	Frequ-ency (%)
1	5	20.83	7+8	5	20.83	5+10	12	50
2*	18	75.00	7+9	12	50.00			
null	1	4.16	17+18	7	29.16	2+12	12	50

The genotypes *viz.*, UP 2003, CPAN 3004, PBW 343, PBW 373 and K 9607 were similar for subunit 1 for *Glu-A1* locus. However, 18 genotypes were similar at *Glu-A1* for subunit 2* and only one genotype i.e. UP 2441 showed 'null' phenotype. This indicated that subunits 1, 2* were highly polymorphic at *Glu-A1* locus among the genotypes.

The genotypes UP 2003, UP 2338, HUW 234, K 9545, Raj 3077, K 9107 and UP 2441 were similar for subunit 17+18 at *Glu-B1* locus, while the genotypes UP 262, HD 2285, PBW 154, PBW 22 and Raj 3765 were similar for subunit 7+8 and subunit 7+9 were present in the remaining 12 genotypes. The subunits 17+18, 7+8 and 7+9 showed polymorphism at Glu-B1 locus.

The genotypes *viz.*, UP 2003, UP 2425, WH 542, UP 2450, UP 2338, HUW 234, K 9545, K 9107, PBW 343, PBW 373 and K 9607 were similar for subunit 5+10 at *Glu-D1* locus. The high Mr weight glutenin subunits 2+12 were present in UP 262, HD 2285, PBW 154, PBW 226, Raj 3765, UP 2382, HD 2329, Sonalika, UP 2418 and UP 2441. This indicated that the subunits 5+10 and 2+12 showed high polymorphism at *Glu-D1* locus (Fig. 1).

On the basis of grouping (Table 3), the genotypes UP 262, HD 2285, PBW 154, PBW 226and Raj 3765 were shown to have similar banding pattern for high Mr weight subunits of glutenin 2* for *Glu-A1* locus, 7+8 for *Glu-B1* and 2+12 for *Glu-D1* locus. The genotypes UP 2338 and Raj 3765 had similar banding pattern

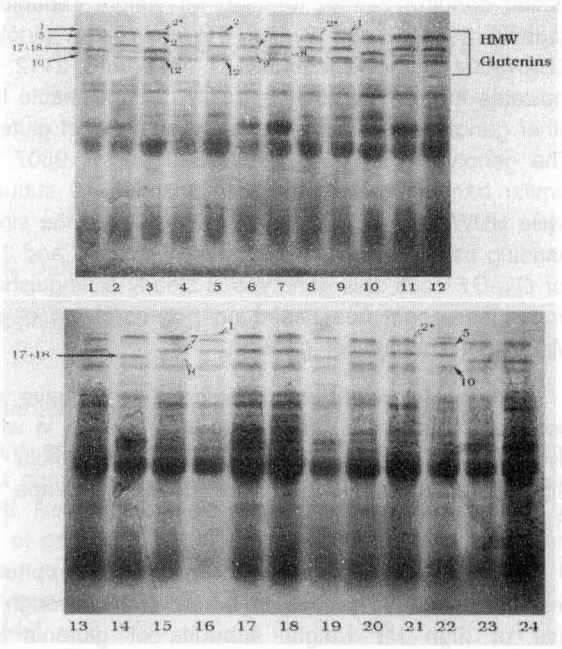

Fig. 1. High molecular weight (HMW)-glutenin subunits of 24 bread wheat genotypes

Table 3. Classification of genotypes based on typical High Molecular Weight (HMW) glutenin subunits composition

Glu-D1	Glu-A1	Glu-B1	Genotypes	Frequency	%
5+10	2*	7+9	UP 2425 WH 542 UP 2450 UP2398 P2418	5	20.83
	1	17+18	UP 2003	1	4.16
		7+9	PBW 343 PBW 373 K 9607	3	12.50
	2*	17+18	HUW 234 K9545 K9107	3	12.50
2+12	2*	7+8	UP 262 HD 2285 PBW 154 PBW 226 Raj 3765	5	20.83
		7+9	UP 2382 HD 2329 Sonalika	3	12.50
	null	17+18	UP 24411	1	4.16
	1	7+9	CPAN 3004	1	4.16
	2*	17+18	UP 2338 Raj 3077	2	8.33

for 2*, 17+18 and 2+12 glutenin subunits, while UP 2425, WH 542, UP 2450, UP 2398 and UP 2418 had similarity for 2*, 7+9 and 5+10 glutenin subunits. The genotypes UP 2382, HD 2329 and Sonalika showed

similar banding pattern for high Mr weight subunits of glutenin 2*, 7+9 and 2+12. However, the genotype CPAN3004 had the subunits 1, 7+9 and 2+12. It indicates that CPAN 3004 is easily distinguishable from other genotypes for high Mr weight subunits of glutenin. The genotype PBW 343, PBW 373 and K 9607 had similar banding pattern for 1, 7+9 and 5+10 subunits, while HUW 234, K 9545 and 9107 exhibited the similar banding pattern for subunit 2*, 17+18 and B1 and 2+12 for *Glu-D1* locus, this genotype is clearly distinguishable from other genotypes based on polymorphism of high Mr weight subunits of glutenin.

High Mr weight subunits of glutenin have also been used as a tool for varietal identification in wheat by Payne *et al.* [3], Lawrence [9], Sreeramulu and Singh [10], Hewstone and Hinrichsen [11], Vapa and Radovic [12], Bakhella and Branlard [13].

It is, therefore, concluded that the electrophoretic resolution of seed protein in bread wheat through the use of high Mr weight subunits of glutenin was remarkably successful in cultivar identification. For further resolution of cultivar identification through seed protein electrophoresis screening for additional storage protein loci namely (*Gli-1* and *Gli-2*), low Mr weight subunits of (glutenin (Glu-3)) and triticin (*Tri-1*) would be helpful.

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