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Research Article

Resistance to take-all disease and diversity in glutenin subunits of different bread wheat genotypes

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Abstract: Take-all is a devastating soil-borne disease of wheat *Triticum aestivum* L. The disease is caused by the pathogenic fungus *Gaeumannomyces tritici*, a pathogen distributed worldwide in major wheat production areas that causes severe damage to wheat production. Identification of genotypes with the high nutritional value of seeds can be considered in controlling this disease and in wheat breeding programs. Variation of high molecular weight glutenin subunits (HMW-GS) at the Glu-A1, Glu-B1, and Glu-D1 loci was studied using SDS-PAGE electrophoresis in 15 genotypes of bread wheat. A positive correlation was found between 1000-seed weight and the 5 + 10 allele (r = 0.594), indicating that presence of this allele will increase 1000-seed weight. A simple corresponding analysis was conducted to show the relationship between the take-all index and the genetic diversity of genotypes and the association between the bilateral groupings of individuals based on two criteria (genetic diversity and disease response). The result of stepwise regression showed that glutenin subunit null, 7 + 8, 2*, 7 + 9, 5 + 10 have linkage with resistance to take-all disease. Findings are useful in breeding programs to improve baking quality, develop uniformity and improve heterogeneous genotypes by selecting the best genotypes.

Keywords: glutenin subunit, take-all index, chi-square test, correlation, 1000-kernel weight, stepwise regression

Introduction

Triticum aestivum L. (AABBDD) is the most widely cultivated wheat (95% of the wheat grown worldwide) and an important source of protein for human and livestock nutrition. High crop yields, adaptation to a wide range of environments, unique properties of dough, and resistance to biotic and abiotic stresses are traits that should be paid much attention to. Properties of dough in wheat are determined by the complex of seed storage proteins (Shewry, 2009). Gluten is composed of two prolamine groups, gliadins, and glutenin, a storage protein found in the endosperm of the seed. Glutenins, consist of low and high molecular weight (LMW and HMW) complex subunits and constitute about 30-40% of flour protein (Kaya

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and Akcura, 2014; Kilic *et al.,* 2017). Due to their high polymorphism storage, gliadins and glutenin are the first genetic markers for studying the diversity of bread wheat (Sobko and Poperelya, 1986; Kozub *et al*., 2017). HMW-GS (High molecular weight glutenin subunit) are encoded by two types of genes called (x: y) that are located on three loci (Glu-A1, Glu-B, and Glu-D1) placed on long arms of chromosomes that have quantitative and qualitative effects on the quality of the grain in wheat (Lawrence and Shepherd, 1981; Heyne, 1987). The flour functionality is influenced by many factors such as genotype, protein content, grain hardness, growing condition, and crop season (Kaur *et al*., 2013; Nemeth *et al*., 1994). Different genotypes with high-yielding and good end-use quality are a significant concern for wheat breeders (Huang *et al*., 2006). In breeding programs, the objective is to improve the germplasm bank's quality to develop wheat with adequate gluten strength and extensibility for baking quality (Costa *et al*., 2013). The SDS-PAGE electrophoresis method is a conventional way to separate protein components based on the relative mobilities in SDS-polyacrylamide gel (Kilic *et al*., 2017). The features of gluten strength, high yield, and disease resistance are among the essentials that should be paid much attention to in wheat breeding.

Wheat is cultivated in most areas of Iran and the world. Plant pathogens significantly reduce the yield and quality of wheat. In addition to the many biotic and abiotic stresses that attack wheat during growth, a number of store-pit fungi can also cause significant damage to wheat. *Cladosporium*, *Alternaria*, *Rhizopus*, and *Fusarium* species are among the most important store-pit fungi that make undesirable changes in the quality and appearance of wheat grains (Saberi Riseh *et al*., 2004b). Wheat take-all is a destructive root disease caused by the soil-borne ascomycete fungus *Gaeumannomyces tritici* (Hernandez-Restrepo *et al*., 2016; Bai *et al*., 2020). This pathogen infects healthy wheat roots via infectious hyphae that penetrate the cortical cells of the root and progress upward into the stem base. This process disrupts water flow, resulting in the premature death of infected plants (Kang *et al*., 2019). Generally, wheat roots with take-all disease exhibit black spots followed by gradual necrosis of vascular tissues, resulting in hindered plant growth and development (Youn-Sig and Weller, 2013). Different genotypes with stronger tillering ability and well-developed roots have greater disease resistance (Bai *et al*., 2020).

Various methods have been used to minimize the damage of plant disease and take-all so far. Several methods for controlling plant pathogens were suggested by some researchers (Zeynadini-Riseh *et al*., 2018; Saberi Riseh *et al*., 2021c; Moradi-Pour *et al*., 2019; Jamali *et al*., 2004; Saberi Riseh *et al*., 2004a). The encapsulation method was used to control take-all disease by beneficial bacteria. This method developed a new type of formulation through bacterial and nanoparticles loaded chitosan-gellan gum microcapsules with a spray drying method. Plants treated with bacterial microcapsules had the highest fresh weight of roots and shoots. Also resulted in 75% and 90% control of take-all disease (Saberi-Riseh and Moradi-Pour, 2021).

Greenhouse and field experiments were established to assess the effect of horizontal versus vertical distribution of *G. tritici* inoculum on disease severity. This research determined that inoculum spatial location and distance greatly influenced take-all. Most infections occur within the upper 15 cm of the soil. The closer the inoculum to the seed, the earlier the pathogen will contact roots to begin infection (Kabbage and Bockus, 2002).

Using resistant genotypes is another alternative practice to manage diseases caused by fungal pathogens. The information about wheat germplasm diversity and genetic relatedness among genotypes is fundamental in genetic and plant breeding. Furthermore, the presence of genetic diversity in plant germplasm is a way to offer promising options for the deployment of resistance genes in agriculture. Genetic diversity reduces epidemic incidence due to pathogens in natural populations (Garret

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and Mundt, 1999). Identifying genotypes that have significant variability in glutenin and gliadins subunits can be useful in improving crop quality and resistance of wheat to biotic and abiotic stresses. For example, some of the loci (gliadins) in wheat and its relatives have proved to be closely linked to disease resistance genes such as leaf rust, yellow rust, powdery mildew, and Fusarium diseases (Kozub *et al*., 2017). Studies have shown that the future wheat breeding program also depends upon the availability of genetic variability to increase the gain in productivity. Hence, to achieve self-sufficiency and sustainability, there is a need to develop genotypes with diverse genetic bases (Ali *et al*., 2010).

Various algorithms have been used in studying genetic diversity in cluster analysis. Also statistical methods such as correlation can identify superior genotypes in terms of yield. Combining genotype classification methods based on glutenin subunits with UPGMA and COMPLETE methods and identifying high-performance genotypes with other statistical methods, the best genotype based on baking performance and properties can be identified (Shumate, 2020).

Due to the global importance of wheat, the high genetic diversity that exists between wheat genotypes, and the lack of sufficient information on the resistance of bread wheat varieties to take-all diseases this study was performed with two main goals: 1) to identify genotypes with high 1000-kernel weight and functional glutenin subunits; 2) to investigate the relationship between glutenin subunits and take-all disease resistance in several bread wheat genotypes.

Materials and Methods

Wheat genotypes resources

Previously about 1000 genotypes of bread wheat (*Triticum aestivum* L.) (Collected and received from different locations in Iran and other countries) were planted in the field at Vali-e-Asr University of Rafsanjan. These genotypes were maintained in the germplasm collections of Vali-e-Asr University and are available at any time to investigators for use or research. Fifteen genotypes of this germplasm were selected and used in this research (Table 1).

Table 1 The list of wheat genotypes used in this research.

Row	Genotypes	Characteristics
1	468	Winter, resistant
2	408	Winter, semi-sensitive
3	2068	Winter, resistant
4	60	Winter, resistant
5	766	Winter, resistant
6	1382	Spring, sensitive
7	1464	Winter, semi-resistant
8	1626	Winter, semi-resistant
9	765	Spring, semi-sensitive
10	912	Spring, sensitive
11	1801	Spring, semi-sensitive
12	Chinese Spring	Spring
13	Mihan	Spring
14	Pishgam	Spring-winter
15	Darab	Spring

Field cultivation and experimental design

The 15 genotypes listed in table 1 were randomly selected to measure seed storage protein content. These genotypes were planted in one row in augmenting design during the growing season 2021-2022. The trait of 1000-kernel weight was measured.

Fungus resource

T-41 isolate of *Gaeumannomyces tritici*, a highly pathogenic isolate, was selected for this research. This isolate was obtained from the mycological collection of the Vali-e-Asr University of Rafsanjan (Gholizadeh Vazvani *et al*., 2017).

Take-all index or disease incidence

These genotypes were screened for resistance and susceptibility to take-all (T-41 isolate) in the greenhouse (Gholizadeh Vazvani *et al*., 2015, 2017).

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Contamination levels based on the percentage of necrosis in the roots and crowns were scored based on a scale of 0 to 5 as follows: 0 = Roots and crowns without necrotic spots; 1 = Roots with one or more necrotic spots and crowns without symptoms; 2 = Roots with continuous necrotic spots (more than 25% and less than 50% necrosis of roots) and crowns without symptoms; 3 = More than 50% necrosis of the roots and blackened crowns; 4 = Roots approximately black with 75% blackened crowns; 5 = Blackened and dried roots and crowns. (Ownley *et al*., 2003). Disease incidence (DI) or take-all index (TAI) was calculated according to this equation: DI (TAI): $\frac{\text{Sum of scores of each pot}}{5\times\text{number of plants}}\times 100$.

Extraction of high molecular weight glutenin subunit (HMW-GS)

The gluten variation was analyzed with some change using SDS-PAGE (Damania *et al*., 1983; Ali *et al*., 2010). For protein extraction, a single seed was ground to a fine powder. Total 400 µl sample buffer was added to a 0.01 gr (10 mg) seed powder and mixed thoroughly by vortex in an Eppendorf tube. The extraction buffer contained the following final concentration: 0.5M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol, Coomassie brilliant blue, and 5% 2-mercaptoethanol, and Eppendorf tubes kept 5 min in boiling water (100 $^{\circ}$C) and centrifuged at 13000 rpm for 10 min.

SDS-PAGE method

Sample proteins were loaded in each well to monitor the movement of the protein in the gel. Seed protein was analyzed using 10% polyacrylamide gel. SDS-PAGE of total seed protein was carried out in a discontinuous buffer system following the method of Laemmli (Laemmli, 1970). The gels were stained with Coomassie brilliant blue and destained until the background became transparent. Table 2, shows the glutenin subunit in control genotypes.

Statistical analysis

Identification of the subunits were performed based on catalogue described by Payne *et al*. (1981). Glutenin subunit data were scored as presence (1) or absence (0) of Glu-A1, Glu-B1, and Glu-D1 alleles. The genetic similarity and dissimilarity coefficient among the wheat genotypes was calculated using a simple matching coefficient, and clustering analysis was done with the NTSYS-pc 2.20 software. Simple correspondence analysis in MINITAB16 software was used to investigate the relationship between glutenin subunit and disease incidence (take-all index) using χ^2 statistics. The correlation and stepwise regression were performed by MINITAB16.

Result

Differences in high molecular weight glutenin subunits and resistance to take-all disease

Glutenin subunits of the 'Pishgam, Darab, Mihan, and Chinese Spring' were compared to other wheat genotypes (11 genotypes) with identical alleles encoded at Glu-A1, Glu-B1, and Glu-D1 loci by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Proteins banding pattern (HMW-GS) of different wheat varieties is presented in table 3. The highest genotypic scores were observed in genotypes 1801, 1464, 765, 60, 912, 765, and 1382. The baking quality of the genotype depends on the genotypic score glutenin subunit. HMW-GS 5 + 10 increases the bakery properties of the genotype. These genotypes were 766 and 60, with genotypic values of 9 and 10, respectively (Table 3).

Table 4, shows the frequency of glutenin subunit alleles in different wheat genotypes. According to this table, most genotypes (63.63%) had allele 2* on chromosome A1 and allele 5 + 10 on chromosome D1. The allele 1 (on chromosome A1) had the lowest frequency (9.09%). Genotypes with a lower take-all index have null alleles 1, 7 + 8, and 2 + 12. This issue is under investigation. Cluster analysis of the banding pattern was done based on the simple matching coefficient, and the

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complete linkage method result is shown in Fig. 1. The wheat genotypes analyzed in this study were classified into two groups. Group 1 had three genotypes (468, 2068, and 1626), and the other genotypes were placed in group 2.

Table 2 HMW-GS banding pattern in control wheat genotypes.

Genotypes	Glu-A1	Glu-B1	Glu-D1	Allelic score	Genotypic score
Chinese Spring	null	7 + 8	2 + 12	1 + 3 + 2	6
Darab	2*	17 + 18	2 + 12	3 + 3 + 2	8
Pishgam	2*	7 + 9	5 + 10	3 + 2 + 4	9
Mihan	1	7 + 9	5 + 10	3 + 2 + 4	9

subunit 2* having the greater electrophoretic mobility.

Table 3 HMW-GS banding pattern of different wheat varieties and their baking quality scores.

Genotype	Property	Glu-A1	Glu-B1	Glu-D1	Allelic score	Genotypic score
Chinese Spring	Control for glutenin subunit	null	7 + 8	2 + 12	1 + 3 + 2	6
Darab	Control for glutenin subunit	2*	17 + 18	2 + 12	3 + 3 + 2	8
Pishgam	Control for glutenin subunit	2*	7 + 9	5 + 10	3 + 2 + 4	9
Mihan	Control for glutenin subunit	1	7 + 9	5 + 10	3 + 2 + 4	9
766	Resistance (DI = 3%)	2*	7 + 9	5 + 10	3 + 2 + 4	9
468	Resistance (DI = 3%)	null	7 + 8	2 + 12	1 + 3 + 2	6
912	Susceptible (DI = 63%)	2*	13 + 16	5 + 10	3 + 3 + 4	10
60	Resistance (DI = 3%)	2*	7 + 8	5 + 10	3 + 3 + 4	10
765	Moderately susceptible (DI = 40%)	2*	13 + 16	5 + 10	3 + 3 + 4	10
2068	Resistance (DI = 3%)	null	7 + 8	2 + 12	1 + 3 + 2	6
408	Moderately susceptible (DI = 46%)	2*	7 + 9	2 + 12	3 + 2 + 2	7
1382	Susceptible (DI = 63%)	1	7 + 9	5 + 10	3 + 2 + 4	9
1464	Moderately susceptible (DI = 33%)	2*	7 + 8	5 + 10	3 + 3 + 4	10
1626	Resistance (DI = 13%)	null	7 + 8	2 + 12	1 + 3 + 2	6
1801	Moderately susceptible (DI = 33%)	2*	13 + 16	5 + 10	3 + 3 + 4	10

subunit 2* having the greater electrophoretic mobility.

Table 4 Frequency of protein glutenin alleles in different wheat genotypes.

Entries	Chromosome							
	Glu-A1			Glu-B1			Glu-D1	
	Null	2*	1	7 + 8	7 + 9	13 + 16	2 + 12	5 + 10
Number of genotypes	3	7	1	5	3	3	4	7
Mean of take-all index (%)	9.66	31.57	63	6.75	37.33	45.33	16.25	34
Frequency (%)	27.27	63.63	9.09	45.45	27.27	27.27	36.36	63.63

subunit 2* having the greater electrophoretic mobility.

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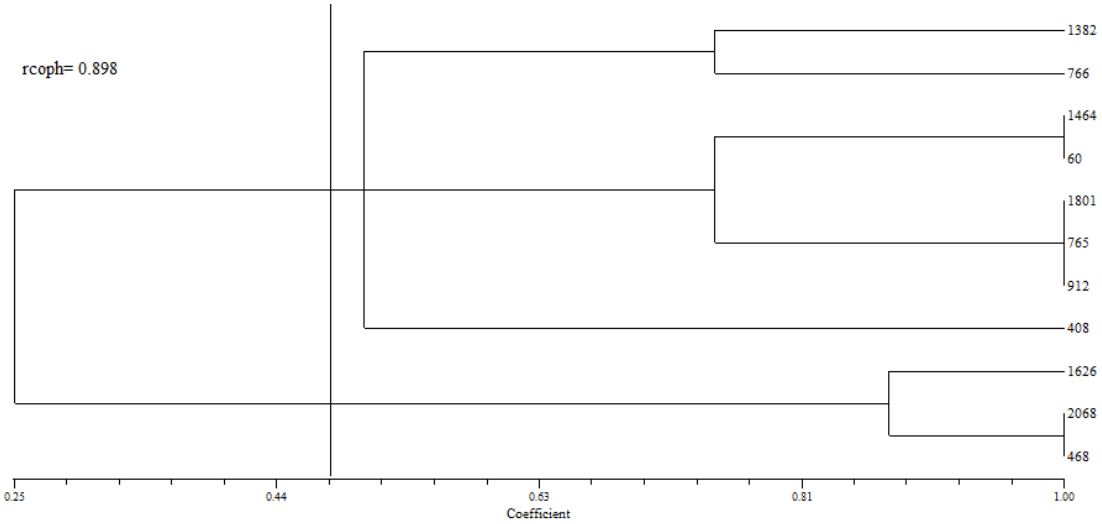

Figure 1 Dendrogram of cluster analysis based on simple matching similarity coefficient and complete method in 11 genotypes.

To show the relationship between disease response and genetic diversity of genotypes derived from high molecular weight glutenin subunits, the association between the bilateral grouping of individuals (based on two criteria: genetic diversity in cluster analysis and disease response), a simple corresponding analysis was conducted (MINITAB software) using contingency table and chi-square test (Table 5). Genotypes were divided into two groups based on the average take-all index. This trait was measured by authors previously (Gholizadeh Vazvani *et al*., 2015; 2017). The genotypes 912, 1801, 765, 1464, 408, and 1382 with mean of take-all index 46.33, were placed in the semi-resistant to semi-susceptible groups, and genotypes 468, 2068, 1626, 766, and 60 were placed in the resistance group. The results showed that the p-value of the table is less than $p < 0.05$, the null hypothesis was rejected, and the alternate hypothesis was accepted. So, there is a significant relationship at the level of 0.05 between these two groups, and they aren't independent. Therefore, glutenin subunits in cluster analysis are most likely related to the disease response of genotypes and explain the most variability in disease incidence.

Table 5 The result of simple corresponding analysis by chi-square test based on two grouping criteria.

Groups	Glutenin subunit	
	1	2
Resistance	3	0
	1.964	1.636
Semi-resistant to semi-susceptible	2	6
	0.736	0.614

$\chi 2$ total = $4.95^{*} > \chi^2$ table = 3.84, df = 1, p-value = 0.026.

In this study, we combined glutenin subunits to learn whether the presence of glutenin alleles simultaneously plays a role in developing resistance to take-all disease (Table 6).

Stepwise regression of glutenin subunit markers was performed to identify suitable alleles interacting with the take-all index. Stepwise regression was performed on all glutenin subunits in table 6 using the MINITAB16 to determine the HMW-GS associated with disease resistance. Three glutenin subunits accounting for 68.50% of the phenotypic variation for the take-all index were detected (Table 7).

Relationship between 1000-kernel weight and glutenin subunits

In some cases, the correlation between 1000-kernel weight and glutenin subunits was significant (Table 8). A positive correlation was found between 1000-seed weight and 5 + 10

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allele (r = 0.594), indicating that presence of this allele will increase 1000-seed weight. Negative correlations were found between 1000-seed weight and null and 2 + 12 alleles (-0.635 and -0.594, respectively). The presence of these alleles reduces the weight of 1000 seeds. Accordingly, a good result has been obtained from the correlation between 1000-seed weight and the null, 5 + 10, 2 + 12, and 7 + 8 alleles.

Table 9, shows the frequency of protein glutenin alleles in different wheat genotypes. According to this table, most of the genotypes (63.63%) had allele 2* on chromosome A1 and 63.63% of the genotypes had allele 5 + 10 on chromosome D1. Genotypes with lower 1000-seed weight have null alleles, 1, 7 + 8, and 2 + 12. Genotypes with higher 1000-seed weight have 2*, 1, 13 + 16, and 5 + 10 alleles.

Table 6 The composition of glutenin subunits and number of wheat genotypes in each composition.

Glutenin subunit	Code	Number of genotypes	Glutenin subunit	Code	Number of genotypes
Null	G1	3	1, 5 + 10	G25	2
2*	G2	4	7 + 8, 2 + 12	G26	1
1	G3	4	7 + 8, 5 + 10	G27	2
7 + 8	G4	7	7 + 9, 2 + 12	G28	0
7 + 9	G5	3	7 + 9, 5 + 10	G29	3
13 + 16	G6	3	Null, 7 + 8, 2 + 12	G30	2
2 + 12	G7	1	Null, 7 + 8, 5 + 10	G31	0
5 + 10	G8	7	Null, 7 + 9, 2 + 12	G32	0
Null, 7 + 8	G9	2	Null, 7 + 9, 5 + 10	G33	0
Null, 7 + 9	G10	0	Null, 13 + 16, 2 + 12	G34	0
Nll + 13 + 16	G11	0	Null, 13 + 16, 5 + 10	G35	0
2*, 7+8	G12	2	2*, 7 + 8, 2 + 12	G36	0
2*, 7+9	G13	2	2*, 7 + 8, 5 + 10	G37	2
2*, 13+16	G14	3	2*, 7 + 9, 2 + 12	G38	1
1, 7 + 8	G15	0	2*, 7 + 9, 5 + 10	G39	1
1, 7 + 9	G16	1	2*, 13 + 16, 2 + 12	G40	0
1, 13 + 16	G17	0	2*, 13 + 16, 5 + 10	G41	3
Null, 2 + 12	G18	2	1, 7 + 8, 2 + 12	G42	0
Null, 5 + 10	G19	0	1, 7 + 8, 5 + 10	G43	0
2*, 2 + 12	G20	1	1, 7 + 9, 2 + 12	G44	0
2*, 5 + 10	G21	6	1, 7 + 9, 5 + 10	G45	0
1, 2 + 12	G22	0	1, 13 + 16, 2 + 12	G46	0
13 + 6, 2 + 12	G23	1	1, 13 + 16, 2 + 12	G47	0
13 + 16, 5 + 10	G24	2			

subunit 2* having the greater electrophoretic mobility.

Table 7 Stepwise multiple regression analysis between take-all index as a dependent variable and composition of glutenin subunits as independent variables.

Step	Allele	Constant	Glu1	Glu39	Glu12	R^2 (%)	R^2 (adj) (%)
1	Null (Glu1)	0.3550	-0.292			32.62	25.13
2	2*, 7 + 9, 5 + 10 (Glu39)	0.4014	-0.338	-0.37		53.83	42.28
3	2*, 7 + 8 (Glu12)	0.4900	-0.437**	-0.46*	-0.31*	77.95	68.50

Take-all index = -0.437Glu1 –0.46Glu39 -0-31Glu12

*and**: significant at 0.05 and 0.01, receptivity.

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According to the genotypes distance of various groups, parents in two different extremes may be identified and used in the crossing projects to generate further variability to select and realize the plant improvement. So, genotypes specific band(s) that were identified may be exploited for hybrid identification in a breeding program and also could be used for the breeder's needs. Moreover it seems that seed protein SDS-PAGE could be used as a rapid, easy, inexpensive, and reliable method for routine identification of *Triticum aestivum* genotypes in breeding programs.

According to genotypes distance, parents in two different extremes may be identified and used in the crossing projects and hybridization to generate further variability for selecting and realizing the plant improvement. Table 10, showed simple matching's similarity matrix. The similarity coefficient ranged from 0.250 (least similarity and the highest distance) to 1 (highest similarity and minimal distance).

Genotypes with a specific band(s) must be identified and screened in terms of other characteristics such as resistance to biotic and abiotic stresses and be used in wheat breeding programs to identify superior genotype. Genotypes with the least or the most distance can be used as candidate genotypes for heterosis in breeding programs. Pishgam, 1382, 912, 60, and 765 genotypes with similarity coefficients of 0.750 and high 1000-seed weight and 2*, 5 + 10, and 1 glutenin subunits can be used in cross-breeding and hybridization programs to produce valuable heterosis or heterobiosis genotypes.

Cluster analysis of banding pattern of studding species based on simple matching coefficient and COMPLETE method resulted in 2 groups (Fig. 2). The results showed a very good correlation between the dendrogram and the similarity matrix (r-coph = 0.909). First group encompassed genotypes 1626, 2068, 468, and Chinese Spring; and second group included 1464, 60, 1801, 765, 912, 408, Mihan, Darab, and Pishgam. Group 2, with a mean of 1000-seed weight 39.40 g is the best. These genotypes can be used as candidates for breeding purposes in wheat breeding programs.

Table 8 The correlation of glutenin subunit with 1000-kernel weight.

Trait/Allele	W1000	Null	7 + 8	2 + 12	2*	7 + 9	13 + 16	1	5 + 10
W1000	1								
Null	-0.635**	1							
7 + 8	-0.187	0.533*	1						
2 + 12	-0.594**	0.739***	0.289	1					
2*	0.404	-0.739***	-0.289	-0.444	1				
7 + 9	0.259	-0.426	-0.500	-0.289	0.001	1			
13 + 16	0.316	-0.302	-0.354	-0.408	0.408	-0.354	1		
1	0.245	-0.237	-0.277	-0.320	-0.480	0.555*	-0.196	1	
5 + 10	0.594**	-0.739***	-0.289	-0.999***	0.444	0.289	0.408	0.320	1

*, **, and ***: significant at 0.05, 0.01, and 0.001, receptivity.
subunit 2* having the greater electrophoretic mobility.

Table 9 Frequency of HMW-glutenin alleles in different wheat genotypes.

Entries	Chromosome							
	Glu-A1			Glu-B1			Glu-D1	
	null	2*	1	7 + 8	7 + 9	13 + 16	5 + 10	2 + 12
Number of genotype	4	9	2	5	5	4	10	5
Frequency (%)	27.27	63.63	9.09	36.36	27.27	27.27	63.63	36.36
Genotypes	1626, 2068, 468, and Chinese Spring	1464, 1801, 408, 60, 765, 766, 912, Darab, and Pishgam	1382 and Mihan	1464, 2068, 468, and Chinese Spring	1382, 408, 766, Mihan, and Pishgam	1801, 765, 912, and Darab	1801, 765, 912, 1382, 766, 1464, 60, Darab, Pishgam, and Mihan	408, 2068, 468, and Chinese Spring
1000-kernel weight	31.58	39.10	40.07	35.87	39.3	39.68	39.6	32.71

subunit 2* having the greater electrophoretic mobility.

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Table 10 Simple matching's similarity matrix based on HMW-GS banding pattern.

Genotype	1382	Pishgam	765	912	60	468	Darab	1801	Mihan	1464	408	2068	Chinese Spring	766	1626
1382	1														
Pishgam	0.750	1													
765	0.500	0.750	1												
912	0.500	0.750	1	1											
60	0.500	0.750	0.750	0.750	1										
468	0.250	0.250	0.250	0.250	0.500	1									
Darab	0.500	0.750	1	1	0.750	0.250	1								
1801	0.500	0.750	1	1	0.750	0.250	1	1							
Mihan	1	0.750	0.500	0.500	0.500	0.250	0.500	0.500	1						
1464	0.500	0.750	0.750	0.750	1	0.500	0.750	0.750	0.500	1					
408	0.500	0.750	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	1				
2068	0.250	0.250	0.250	0.250	0.500	1	0.250	0.250	0.250	0.500	0.500	1			
Chinese Spring	0.250	0.250	0.250	0.250	0.500	1	0.250	0.250	0.250	0.500	0.500	1	1		
766	0.750	1	0.750	0.750	0.750	0.250	0.750	0.750	0.750	0.750	0.750	0.250	0.250	1	
1626	0.375	0.375	0.375	0.375	0.375	0.875	0.375	0.375	0.375	0.375	0.625	0.875	0.875	0.375	1

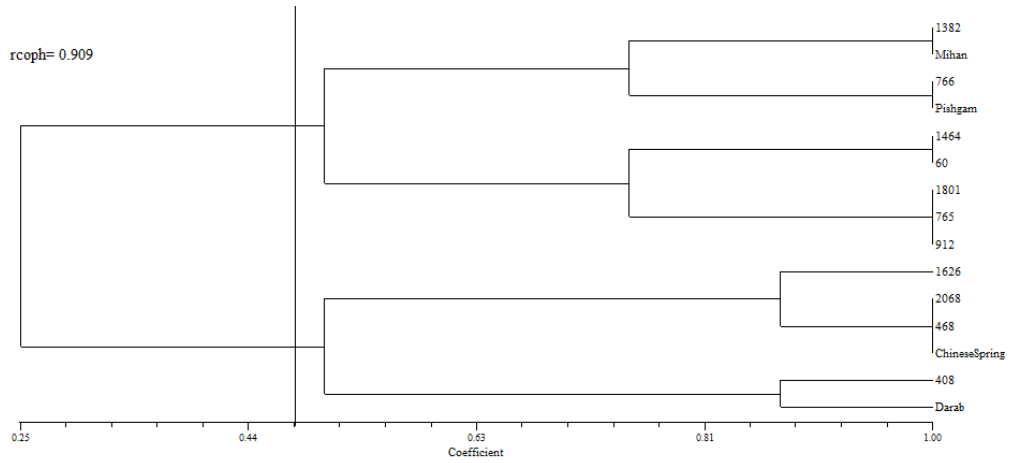

Figure 2 Dendrogram of cluster analysis based on simple matching similarity coefficient and complete method in 15 genotypes.

Discussion

Information regarding the extent and nature of genetic diversity within a crop species is essential for an effective breeding program. Protein electrophoresis is a useful method for describing the genetic structure of crop germplasm (Ciaffi *et al*., 1993; Sofalian and Valizadeh, 2009).

Based on the results, it is concluded that evaluation of genetic diversity and identification of wheat varieties by SDS-PAGE is important, and useful for molecular weight analysis of wheat seed storage proteins (Kakaei and Kahrizi, 2011). Various studies have shown that gluten subunits (glutenins and gliadinins) are strongly linked to plant disease resistance genes. Among these 11 genotypes some showed low susceptibility to the pathogen (DI = 3%, and resistance to take-all) were observed, and at the same time, had subunits

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of 5 + 10 (the main factor in the quality of wheat flour). These genotypes were 766 and 60, with genotypic values of 9 and 10, respectively. These genotypes can be used in additional wheat breeding programs and further studies. Genotypes with a specific band(s) must be identified and screened in terms of other characteristics, such as resistance to biotic and abiotic stresses and be used in wheat breeding programs to identify superior genotypes. In this study, we randomly selected several previously screened genotypes for resistance to take-all disease.

This research showed that glutenin subunits in cluster analysis are most likely related to the take-all disease response of genotypes and explain the most variability in disease incidence. In research, a non-random association between alleles of disease resistance genes (*Lr34*, *Yr18*, *Pm38*, *Sr57*, *Bdv1*, and *TDF_076_2D*) and storage protein alleles was revealed (Kozub *et al*., 2017).

The results of previous studies have shown that in the resistant group, the enzyme content of PAL, PPO, and total protein content increased simultaneously 12 days after inoculation. The activity of these two enzymes and the total protein content and genes expressed in this pathway may lead to the synthesis of several antifungal metabolites (Saberi Riseh *et al.*, 2021a).

Glutenin subunit null and 7 + 8 have linkage with resistance to take-all disease. Also, the combination of 2*, 7 + 9, and 5 + 10 alleles are related to resistance to take-all disease.

Research by stepwise regression determined that the subunits of 5 + 10, 17 + 18, and 7 + 8 accounted for 31.4% of the variation in SDS sedimentation (Maleki *et al*., 2008). In other research based on the results of stepwise regression analysis, subunits 1, 13 + 16 and 5 + 10 subunits had a positive effect on wheat grain protein content (Ghoreishi *et al*., 2014). The higher the value of plant seeds in terms of nutritional content (such as protein), the more resistant it is to disease.

In research, proteins with gliadin domains (gamma and alpha gliadins) and LMW glutenins are ascribed to defense-related functions. This highlights the possible involvement of the gliadin domain in plant immunity and biotic stress mechanisms (Zhang *et al*., 2018).

Information regarding the extent and nature of genetic diversity within a crop species is essential for an effective breeding program. Protein electrophoresis is a useful method for describing the genetic structure of crop germplasm (Ciaffi *et al*., 1993; Sofalian and Valizadeh, 2009).

Based on the results, it is concluded that evaluation of genetic diversity and identification of wheat varieties by SDS-PAGE is important, and useful for molecular weight analysis of wheat seed storage proteins (Kakaei and Kahrizi, 2011). The result of correlations showed that the presence of allele 5 + 10 increases 1000-seed weight.

Costa *et al*. (2013) reported a positive correlation between the Glu-1 quality score and the 1000-seed weight (r = 0.510). Alleles 1 and 2* (on Glu-A1) have been discovered to have a better effect on baking quality when compared to a null allele. The 5 + 10 alleles of the Glu-D1 have been correlated with higher dough strength, while the 2 + 12 alleles have been correlated with low baking quality (Gianibelli *et al*., 2001; He *et al*., 2005). Mihan with 1000-seed weight 37.5 gr and having glutenin subunits 5 + 10, 2*, and 1 of the genotypes have high bakery quality. Therefore, 1000-seed weight can be an important trait in breeding programs. The HMW subunits play a major role in determining the functional properties of flour and dough (Kilic *et al*., 2017). Alleles 1 and 2* (on Glu-A1) have been discovered to have a better effect on baking quality when compared to a null allele. The 5 + 10 alleles of the Glu-D1 have been correlated with higher dough strength, while the 2 + 12 alleles have been associated with low baking quality (Gianibelli *et al*., 2001; He *et al*., 2005).

Conclusion

Breeding high-yielding wheat varieties with disease resistance has become one of the most important tasks for breeders. Better knowledge of the defense mechanisms and genetic engineering provides a practical approach to improving wheat resistance to the disease during

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breeding. Wheat breeders can exploit variation in glutenin structural subunits to introduce new varieties with enhanced baking quality. This study showed that these genotypes have valuable biodiversity for baking quality breeding, to increase quality, quantity, yield, and resistance to take-all disease. Since glutenin subunits are also linked to rust resistance genes, these genotypes can be further studied in breeding programs and introduction of high-quality, disease-resistant wheat cultivars.

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مقاومت به بیماری پاخوره گندم نان و تنوع در زیرواحدهای گلوتنین در ژنوتیپ‌های مختلف گندم نان

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چکیده: بیماری پاخوره، یک بیماری ویرانگر در گندم *Triticum aestivum* L. است. این بیماری توسط قارچ خاکزی *Gaeumannomyces tritici* ایجاد می‌شود که در سراسر جهان که گندم کشت می‌گردد باعث خسارت‌های قابل‌توجهی می‌گردد. شناسایی ارقامی که ارزش تغذیه‌ای بالایی دارند می‌تواند در کنترل این بیماری و در برنامه‌های اصلاحی گندم مورد توجه قرار گیرد. تنوع زیرواحدهای گلوتنین با وزن مولکولی بالا (HMW-GS) در جایگاه‌های Glu-A1، Glu-B1 و Glu-D1 با استفاده از الکتروفورز SDS-PAGE در ۱۵ ژنوتیپ گندم نان مورد مطالعه قرار گرفت. هم‌بستگی مثبتی بین وزن هزار دانه و آلل ۱۰ + ۵ (r=۰/۵۹۴) مشاهده شد که نشان می‌دهد وجود این آلل باعث افزایش وزن هزار دانه می‌گردد. به‌منظور نشان دادن ارتباط بین تنوع ژنتیکی و شاخص بیماری پاخوره تجزیه ارتباط ساده‌ای با استفاده از آزمون کای دو انجام شد. نتایج نشان داد که ارتباط معنی‌داری بین این دو گروه وجود دارد. نتایج رگرسیون گام‌به‌گام نشان داد که زیرواحدهای null، ۷+۸، *۲، ۷+۹ و ۱۰+۵ با مقاومت به بیماری پاخوره ارتباط دارند. نتایج به‌دست‌آمده در این مطالعه در برنامه‌های اصلاحی برای بهبود کیفیت نانوایی و بهبود ارقام از طریق انتخاب بهترین ژنوتیپ‌ها مفید خواهد بود.

واژگان کلیدی: زیرواحد گلوتنین، شاخص بیماری پاخوره، آزمون کای اسکوئر، هم‌بستگی، وزن ۱۰۰۰ دانه، رگرسیون گام‌به‌گام

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