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RESEARCH ARTICLE

Genetic Linkage Map of *Glu-D1* and Allelic Variation of HMW Glutenin Subunits in Some Iranian Bread Wheat genotypes

Amin Azadi[1*] and Saeedeh Khani Bafrouei[2]

[1]Department of Plant Breeding, Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran.

ABSTRACT: High-molecular weight (HMW) glutenin subunits are encoded by the *Glu-1* loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) on the long arms of chromosomes 1A, 1B and 1D. In the present study, we constructed genetic linkage map of *Glu-D1* and then investigated the allelic variation of HMW glutenin subunits at Glu-*A1* and *Glu-D1* gene loci in 30 Iranian genotypes using Functional markers. *Glu-D1* was located at 50.8 cM on chromosome 1D and it was tightly linked to wPt-3743 marker (>1 cM). QTL analysis using composite interval mapping detected one significant QTL for grain yield (GY) on chromosome 1D. This QTL (*QYld.abrii-1D*) was located between wPt-3743 and *Glu-D1* gene locus. Allelic variation of HMW glutenin subunits showed the most frequent alleles were the null allele at *Glu-A1* and Dx2+Dy12 alleles at Glu-D1loci. The frequency of Null alleles or 1 and 2* were 40% and 60% respectively. Only 9 genotypes included allelic combination of Dx5+ Dy10 and the rest of genotypes had Dx2+Dy12 in the *Glu-D1* locus. According to the *Nei's* genetic diversity index, alleles at *Glu-A1* locus have more dispersion in genotypes compared to *Glu-D1* locus. The cluster analysis of data based on the Simple Matching coefficient and UPGMC methods, classified the genotypes into four groups. Six genotypes including: Bezostaya, Tajan, Navid, Karaj1, Neyshabour, and Golestan had Ax2* and Dx5+ Dy10 subunits at *Glu-A1* and *Glu-D1* gene loci. Identification of genotypes with suitable allelic combinations can be used in breeding programs, especially in hybridization.

KEYWORDS: High-molecular weight (HMW) glutenin subunits; Genetic map; Allelic diversity; Bread wheat

INTRODUCTION

More than 90% of cultivated wheat in Iran is being used for baking bread. In Iran more than six million ha is under wheat cultivation either irrigated or dryland (1). It has been estimated about 19400 and 904650 tons of wheat products are wasted in bakery and consumption (2). Gluten is the seed storage protein and it can be divided into two main fractions according to their solubility in aqueous alcohols including the soluble gliadins and the insoluble glutenins (3). Glutenins subunits have been divided into high molecular weight (HMW) and low-molecular-weight (LMW) subunits held together by disulphide bonds (4,5). The high molecular weight (HMW) glutenins subunits are the major determinants of the visco-elastic properties of wheat dough (6).

In hexaploid wheat, genes encoding glutenin subunits with high molecular weight are on the long arms chromosomes of 1A, 1B and 1D. These loci together are known as Glu-1. Major studies on the relationship between the glutenin subunits and the quality of bread

*Corresponding author (✉): azadi.amin@gmail.com
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wheat were performed by using SDS-PAGE electrophoresis technique (4). In the traditional method, SDS-PAGE is used for the separation and identification of HMW glutenin subunits in wheat, which is not amenable to high throughput analysis (7).

Generally, performance of this method requires much time and work and mobility of subunits in this system does not often match with their molecular size. Also, polymorphism at protein level is not too high; On the other hand, all of the genetically variations or changes created at the DNA level do not appear in the protein level. So, the use of molecular markers is preferred.

Allelic diversity of high molecular weight glutenin subunit is done by different researchers. (7-9). in an experiment conducted by Giraldo et al., 2010 (10), to study Allelic variation in HMW glutenins in 165 Spanish wheat landraces, they identified two subunits of glutenin at Glu-A_1 gene locus in some of the varieties. In another experiment 77 accessions have been studied from the USDA-ARS germplasm collection and three different HMW-GS encoded by *Glu-A1* locus were found. Subunits 2* and null were found with a frequency of 3.9% and 93.5% respectively (11).

The main goals of this study were to (i) map *Glu-D1* locus (ii) identify the association of *Glu-D1* with grain yield and (iii) investigate and detect the allelic diversity of HMW-GS encoding genes in some Iranian bread wheat genotypes using allele specific markers.

MATERIALS AND METHODS

DNA Extraction and Marker Analysis

Thirty Iranian wheat varieties (Table 1) and a population of 186 F_9 recombinant inbred lines (RIL) derived from a cross between Superhead#2 and Roshan were genotyped for the present of *Glu-D1* gene locus. Leaf tissue of each genotype was used for DNA extraction according to Azadi *et al.* (2015) method. PCR primers were designed based on the previously published reports (12). Amplification was performed with a thermal cycler (Applied Biosystems, CA) using initial denaturation (one cycle) at 94°C for 5 min, followed by 38 cycles of 40 s at 94°C, 45 s for annealing (temperature depending on the primer annealing conditions: 56°C for *Glu-A1*) and 1.30 min at 72°C for extension. An additional final extension of the PCR products was performed for 5 min at 72°C. The PCR products were separated by electrophoresis in 2% Agarose and visualized by gel red staining. DArT analysis of the RIL population was done in Australia and supported by Agricultural Biotechnology Research Institute of Iran (ABRII).

Table 1. HMW glutenin Subunit compositions for 30 Iranian wheat cultivars

code	Cultivar	Glu-A1	Glu-D1	
1	SuperHead	N-1	Dx5	Dy10
2	Golestan	2*	Dx5	Dy10
3	Sistan	2*	Dx2	Dy12
4	Khazar1	N-1	Dx2	Dy12
5	iniya	N-1	Dx2	Dy12
6	Navid	2*	Dx5	Dy10
7	Mahdavi	2*	Dx2	Dy12
8	Darab2	N-1	Dx2	Dy12
9	Dez	2*	Dx2	Dy12
10	Karaj1	2*	Dx5	Dy10
11	Zare	N-1	Dx5	Dy10
12	Alamout	2*	Dx2	Dy12
13	Neyshabour	2*	Dx5	Dy10
14	Sardari	2*	Dx2	Dy12
15	Shiraz	N-1	Dx2	Dy12
16	Bezostaya	2*	Dx5	Dy10
17	Tajan	2*	Dx5	Dy10
18	Baharan	2*	Dx2	Dy12
19	Bahar	N-1	Dx2	Dy12
20	Karaj2	N-1	Dx5	Dy10
21	Rasoul	N-1	Dx5	Dy10
22	Orum	N-1	---	----
23	Roshan	N-1	Dx2	Dy12
24	Azadi	2*	Dx2	Dy12
25	Shahriyar	2*	Dx2	Dy12
26	Hirmand	2*	Dx2	Dy12
27	Moghan1	N-1	Dx2	Dy12
28	Alborz	2*	Dx2	Dy12
29	Tous	2*	Dx2	Dy12
30	Aflak	2*	----	----

Data analysis for thirty Iranian wheat varieties

Banding profiles were scored according to their banding size. Different methods and algorithms were used for constructing similarity matrices and dendrograms. The efficiency of clustering algorithms and their goodness of fit were determined based on the cophenetic correlation coefficient using NTSYS-pc version 2.11 software (13). Finally, the dendrogram was constructed using the unweighted pair-group method with centroid (UPGMC)

clustering method based on simple matching coefficient using SPSS software. The genetic diversity index of Nei (14) was calculated by POPGENE software version 1.32(15).

Data analysis for mapping population

UMN19, UMN25 and UMN26 markers (12) were assessed for the existence of polymorphism between parents and only UMN25 and UMN26 markers showed polymorphism between parents. Then parents and 186 RILs were genotyped with gene-based markers for the *Glu–D1* locus according to the method of Liu *et al.* (2008). The genetic linkage map of chromosome 1D was constructed previously by Azadi *et al.* (2015) using only DArT markers. In the present study, the new genetic map of chromosome 1D with *Glu–D1* locus was constructed and QTL analysis was performed using Map Manager QTX (Manly et al. 2001) and QTL Cartographer v2.5 (Wang et al. 2012a, b), respectively according to Azadi *et al.* (2015). The setting of parameters in Map Manager QTX and QTL Cartographer were according to Azadi *et al.* (2015). QTL nomenclature was specified according to the catalogue of gene symbols for wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm). ABRII was used as the laboratory designator in QTL nomenclature. Grain yield was measured previously by Azadi *et al.* (2015). Finally, to identify association of *Glu-D1* with grain yield, single-marker analysis using the linear regression method was performed.

RESULTS

In the present study, first, the presence of glutenin subunits with high molecular weight at the Glu-A1 and *Glu-D1* loci were investigated in thirty wheat varieties by molecular markers and then, the genetic linkage map of chromosome 1D was constructed.

Two alleles were located at *Glu-A1* (Ax null, Ax1 and Ax2*) locus and four alleles were located at *Glu-D1* (Dy12+Dx2 and Dy10+Dx5) locus. As mentioned earlier, UMN19 primer amplified two different bands. The variety with subunit Ax1 or the null allele and Ax2* were expected to amplify the 362-bp and 344-bp DNA fragments respectively. So, all genotypes had at least one of these alleles. Null alleles or 1 and 2* at *Glu-A1* gene locus had 40% and 60% frequencies respectively.

At *Glu-D1* locus, agarose gel electrophoresis of PCR products indicated UMN25 marker amplify the 281bp (Dx5) and 299bp (Dx2) DNA fragments respectively, and UMN26 marker amplify the 397bp for Dy10 subunit, and 415bp for Dy12 subunit (Fig. 1).

Nei's gene diversity index (14) was used to calculate the variation at gene loci. The gene diversity for each locus revealed that UMN-19 primer was more capable of identifying polymorphic alleles than the other markers (Table 2). Cluster analysis was performed using SPSS software (Fig. 2).

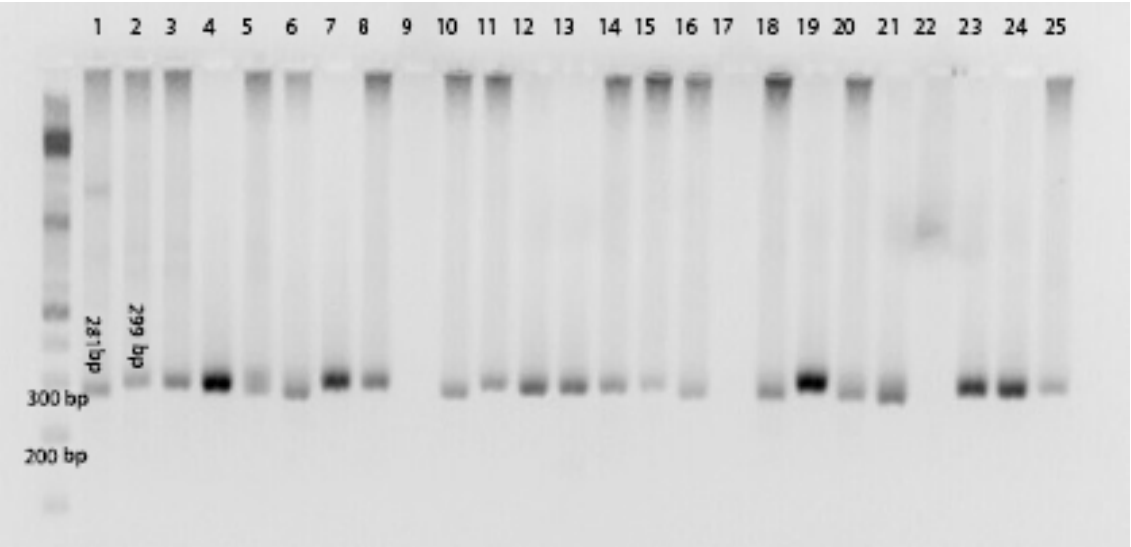

Fig 1. Agarose gel electrophoresis image for the *Glu-D1* locus for the first 25 Iranian wheat cultivars according to Table 1. From left to right: ladder mix and then 25 wheat cultivars.

Table 2. Nei's (1973) gene diversity for *Glu-D1* and *Glu-A1* loci.

Locus	Allele	Relative Frequency	Genetic diversity per locus
Glu-A1	a/c	40%	46%
	b	60%	
Glu-D1	a	33%	30%
	d	66%	

Table 3. QTL detected for grain yield in the SuperHead#2×Roshan mapping population. Significance at the 0.01% level are indicated by ****.

Trait	QTL	Marker Interval	Position (CM)	LOD
Grain yield	*QYld.abrii-1D*	wPt-3743**** - *Glu-D1****	48.3	5.8

The results showed that grouping based on simple matching coefficient, had cophenetic coefficient of 0.91. In SPSS, the distance measure has been rescaled from 0 to 25 with 0 representing no distance and 25 rating the greatest distance. The cluster analysis showed that cultivars were divided in four groups with relatively highcoefficient of similarity. Group one, includes Ax2* and Dx2+Dy12 at Glu-A1 and *Glu-D1* loci, respectively. The second Group includes Ax null or Ax1 and Dx2+Dy12 at Glu-A1 and *Glu-D1* loci, respectively. The third group includes Ax null or Ax1 and Dx5+Dy10 at the Glu-A1 and *Glu-D1* loci, respectively and the fourth group includes Ax2* and Dx5+Dy10 at Glu-A1 and *Glu-D1* loci, respectively. This group (the fourth group) is the most desirable group for the good bread making quality because includes desirable alleles. The genetic linkage map of chromosome 1D contains 13 DArT markers and *Glu-D1* gene locus (Fig. 3). *Glu-D1* was located at 50.8 cM on chromosome 1D and it was tightly linked to wPt-3743 marker (>1 cM). QTL analysis using composite interval mapping detected one significant QTL for the trait of grain yield on chromosome 1D. This QTL (*QYld.abrii-1D*) was located between wPt-3743 and *Glu-D1* gene locus (Fig. 3 and 4). The significantly associated markers with *QYld.abrii-1D* are indicated by asterisks in Table 3. As shown in this table, wPt-3743 and *Glu-D1* gene locus were tightly linked to *QYld.abrii-1D* with P≤0.0001. (Fig. 4).

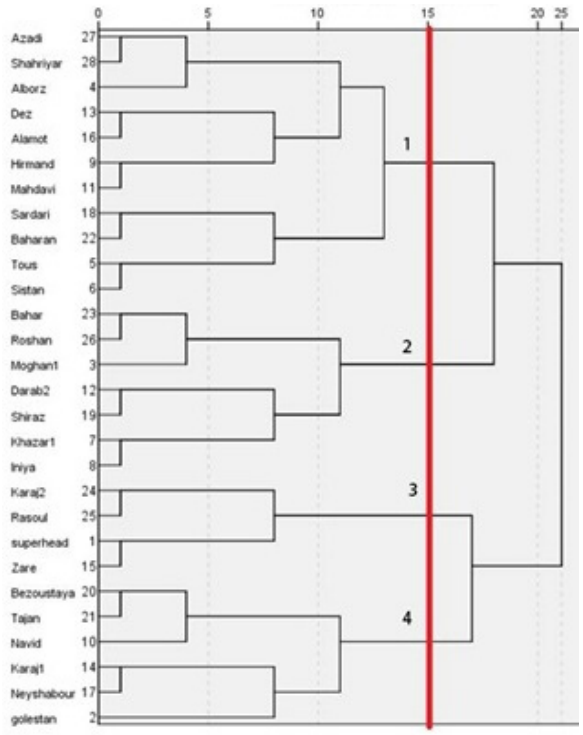

Fig 2. Cluster analysis using the unweighted pair-group method with centroid (UPGMC) based on simple matching coefficient

DISCUSSION

Allelic variation of HMW glutenin subunits in thirty Iranian wheat varieties

At Glu-A1 locus, both Ax1 and Ax2* subunits have a positive impact on the quality of bread wheat and the null allele has a negative impact on the quality of bread. The high frequency of allele 2*, which has high-quality value, is considered as a positive rating for genotypes and these genotypes are desirable for hybridizations. Abounding in the number of alleles detected at each of the loci, cause high allelic diversity among the cultivars. In Australian wheat, the frequency of Ax1 and Ax2* alleles and the null allele are 47.1, 45.4 and 6.7%, respectively (16). At this locus, Ax1 and Ax2* subunits that are closely related to higher baking quality, are present in approximately all US and Canada varieties, 98% of the Argentina varieties, 91% of Russian and 75% Yugoslavia (16-18). In Iranian bread wheat, the frequency of null allele at *Glu-A1* was 46.4% (20). The prevalence of the null allele *Glu-A1c* at the *Glu-A1* locus of 95 Iranian wheats was believed to be due to the

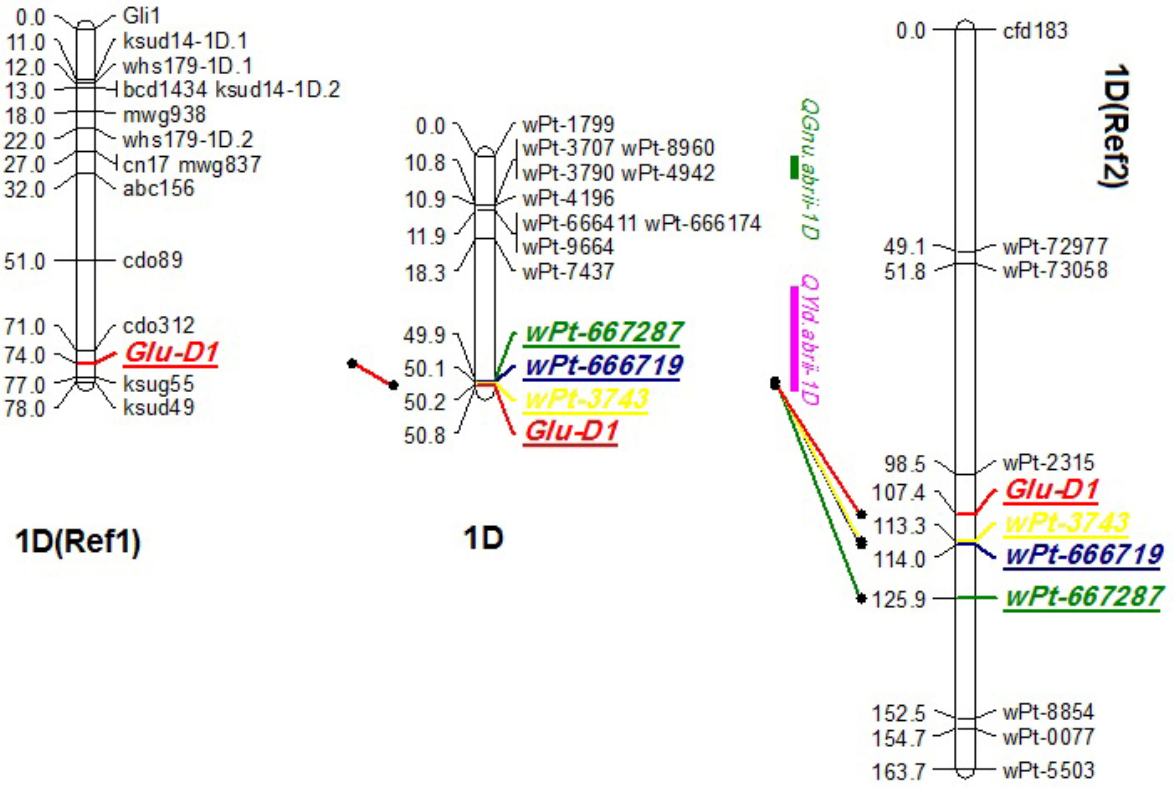

Fig 3. Genetic linkage map of chromosome 1D and comparative maps for *Glu-D1*. Ref1 and 2 are GrainGenes database and Deng et al. (2013) respectively. QYld QTL for grain yield (Yld).

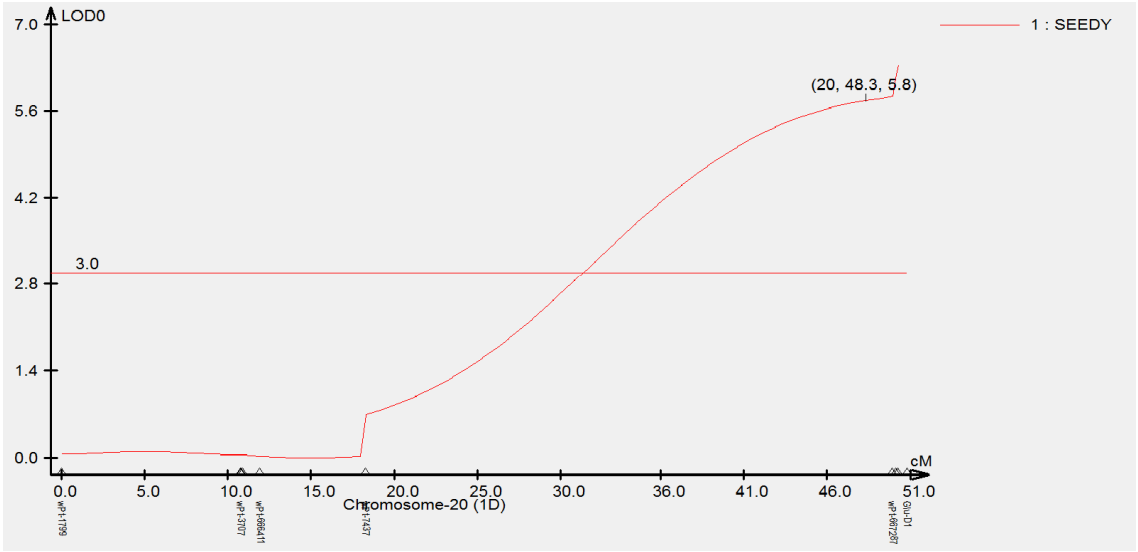

Fig 4. *QYld.abrii-1D* plot with QTL Cartographer software. The numbers on plot from left to right are the chromosome number, the cM location along the chromosome and the LOD score

Iranian baking requirement for medium-elastic dough rather than strong gluten that results in less extensible dough (20).

The combination of Dx5 and Dy10 is associated with strong dough and good bread making quality and the allelic pair Dx2 + Dy12 has negative effects on bread making quality (12).

At *Glu-D1* locus, only 9 genotypes (30%) had Dx5 + Dy10 allelic composition and the rest of the genotypes (70%) had Dx2 + Dy12 allelic combination. Previous

works on wheat has revealed that the frequency of Dx2 + Dy12 and Dx5 + Dy10 allelic combinations were 61 and 31%, respectively (20).

A number of studies on the relationship between glutenin components and the quality of bread were performed by using SDS-PAGE electrophoresis system. Overall, performance of this method is time consuming and hard work, and mobility efficiency of subunits in the system does not always match with its molecular size that lead problems in identifying allelic combinations.

Genetic Linkage map of Glu-D1

In the present study, *Glu-D1* gene locus is associated with the DArT marker wPt-3743. In a study conducted by Deng et al. (2013), *Glu-D1* mapped next to wPt-3743 (25). As shown in Fig 3, the *Glu-D1* gene locus on chromosome 1D was significantly associated with *QYld.abrii-1D*. This has not been reported previously. Student's *T*-tests showed that the effect of *Glu-D1* alleles on yield was significant for RILs population. That means significant differences was observed between *Glu-D1*a and *Glu-D1*d for grain yield and this trait was significantly affected by *Glu-D1*. *Glu-D1d* was associated with higher grain yield than *Glu-D1*a. Using gene specific DNA markers help to avoid the possible errors in naming pattern of protein bands in SDS-PAGE system and also it can be done at seedling stage and do not need any seeds for determining the protein compounds. Therefore, the markers can be used to identify genotypes with specific alleles and used to evaluate the quality of wheat varieties.

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نقشه ژنتیکی *glu-D1* و تنوع آللی زیرواحدهای گلوتنین HMW در برخی از ژنوتیپ‌های گندم نان ایرانی

امین آزادی [*1]، سعیده خانی بفروئی [2]

[1] گروه اصلاح نباتات واحد یادگار امام خمینی (ره)، شهر ری، دانشگاه آزاد اسلامی، تهران، ایران
[2] گروه اصلاح نباتات، واحد علوم و تحقیقات دانشگاه آزاد اسلامی، تهران، ایران

*نویسنده مسئول: azadi.amin@gmail.com

چکیده

زیرواحدهای گلوتنین با وزن مولکولی بالا که توسط مکان ژنی *Glu-1* (*Glu-A1*،*Glu-B1*, *Glu-D1*) کد می شوند بر روی بازوی بزرگ کروموزوم های ۱A ، ۱B و ۱D قرار دارند. در این مطالعه ابتدا نقشه ژنتیکی *Glu-D1* رسم شده و سپس تنوع آللی زیرواحدهای سنگین گلوتنین در مکانهای ژنی *Glu-A1* و *Glu-D1* در ۳۰ رقم گندم نان با استفاده از نشانگرهای UMN19 ، UMN25 و UMN26 مورد بررسی قرار گرفت. مکان ژنی *Glu-D1* در موقعیت ۵۰/۸ سانتی مورگان و بر روی کروموزوم شماره ۱D قرار داشت و دارای لینکاژ بسیار محکم با نشانگر wPt-3743 بود. تجزیه QTL با استفاده از روش مکان یابی فاصله ای مرکب منجر به شناسایی یک QTL بر روی کروموزوم ۱D گردید. این QTL (*QYld.abrii-1D*)بین نشانگر wPt-3743 و ژن *Glu-D1* قرار داشت و اثر آللهای ژن *Glu-D1* بر عملکرد دانه معنی دار بود. فراوانترین آلل مورد بررسی در مکان ژنی *Glu-A1* ، آلل نول و در مکان ژنی *Glu-D1* ، آلل Dx2+Dy12 بود. آلل نول یا ۱ و *۲ بترتیب دارای فراوانی ۴۰٪ و ۶۰٪ بودند. تنها ۹ ژنوتیپ دارای ترکیب آلل Dx5+ Dy10 بودند. و بقیه ژنوتیپ ها دارای ترکیب آللی Dx2+Dy12 در مکان ژنی *Glu-D1* بودند. مطابق شاخص تنوع ژنتیکی نی ، آللهای مکان ژنی *Glu-A1* دارای پراکندگی بیشتری در ژنوتیپ ها نسبت به مکان ژنی *Glu-D1* بودند. تجزیه کلاستر داده ها بر اساس ضریب تطابق ساده و روش UPGMC ، ژنوتیپ ها را به چهار گروه تقسیم کرد. شش ژنوتیپ شامل بزوستایا، تجن، نوید، کرج۱، نیشابور و گلستان دارای زیر واحدهای *Ax2 و Dx5+ Dy10 در مکان های ژنی *Glu-A1* و *Glu-D1* بودند. شناسایی ژنوتیپهای با ترکیب آللی مناسب می تواند برای برنامه های اصلاحی بخصوص در هیبریداسیون مفید باشد.

کلمات کلیدی: زیرواحدهای گلوتنین با وزن مولکولی بالا، نقشه ژنتیکی، تنوع ژنتیکی، گندم نان