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Analysis of Allelic Variation in HMW-Glu-1 Gene Blocks in Iranian Wheat Cultivars using ALP Molecular Marker

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Abstract

In order to study the allelic variation of Glu-1 gene (High molecular weight glutenin), 100 Iranian wheat cultivars including imported and domestic genotypes were analyzed using ALP-PCR technique. Four specific primer pairs were used based on the genetic loci of Glu-A1, Glu-B1, and Glu-D1 to perform the chain polymerase reactions. PCR reaction products were resolved on %2 agarose gel. Since allele "a" had the largest relative frequency (0.707), two alleles (a 344bp; b 362bp) were identified by P1-P2 primer for Glu-A1 locus. Three alleles (a 800bp; b 500bp; c 300bp) were detected for Glu-B1 locus by P5-P6 primer, and allele "b" was assumed as the highest relative frequency (0.618). Two primer pairs were applied for Glu-D1 locus. Ultimately, four alleles were identified, where allele "c" had the highest relative frequency (0.525). The observed genetic variation value for Glu-D1 locus (H=0.648) exhibits the maximal polymorphism. Using cluster analysis, the relationship between the observed polymorphism and geographical variation was investigated. The results indicated that there exists a remarkable variation in Glu-1 locus between the Iranian wheat cultivars.

Keywords: Non-functional Requirements, Square Spline Interpolation, Software Architecture Style

1. Introduction

Gluten proteins of wheat are divided into gliadins (single-chained) and glutenins (multiple-chained) categories. These two groups totally account for % 85 of all protein supply in wheat3. Glutenins of wheat grain are divided into two subgroups in terms of molecular weight: High Molecular Weight Glutenin (HMW-G) and Low Molecular Weight Glutenin (LMW-G). High molecular weight glutenins are encoded by Glu-1 genes with Glu-A1, Glu-D1, and Glu-B1 loci which are situated on the long arm of chromosomes 1A, 1B, and 1D. Glutenin, in accordance with low molecular weights, are encoded by Glu-3 genes including Glu-B3, Glu-A3, and Glu-D3 positioned as a gene block on the short arm of chromosomes 1A, 1B, and 1D[7,11]. LMW-Gs have higher allelic variation compared to HMW-Gs. However, based on the formerly conducted experiments, it was known that the baking quality level is controlled by high molecular weight gluten whereas low molecular weight glutens have no significant effect[17]. Variations in length of genes and number of cysteine are the two factors affecting the protein quality[9].

The obtained a Amplicon Length Polymorphism (ALP) between gene donor and recipient represents the simplest and fastest way of detecting polymorphism[9]. Length variation of genes can be assessed using specific primers. In addition, with the aid of the same markers, it is possible to select the genotypes containing high-quality alleles in primary growth stages, to achieve information

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concerning allelic variation, and finally, to study fabrication of fake genes in wheat genome[2,6]. Aghaei et al. observed a broad variation through studying Glu-A3 and Glu-A1 loci using SDS-PAGE method. They also realized that distribution of some alleles in wheat cultivars is greater in a particular geographical zone[1]. The objective of the present research is to investigate the genetic variation in Glu-A1, Glu-B1, and Glu-D1 loci, and to investigate their correlation with geographical variation of bread wheat cultivars planted in Iran.

2. Material and Methods

2.1 Plant Materials

In the current research, 100 wheat cultivars (Triticum aestivum L.) comprising the commercial cultivars planted in Iran (Table 1) were prepared from Moghan Agricultural Research Center.

2.2 Derivatization Procedure

Four specific primer pairs were used for the genetic loci Glu-A1, Glu-B1, and Glu-D1 in the present study. Two primer pairs were designed by means of the sequences recorded in NCBI website. The other two pairs of primers were designed by Sixin et al[15] and Sameri et al[14] (Table 2). Of the four used primer pairs, one pair was for Glu-A1, another pair was for Glu-B1, and two other pairs were for Glu-D1

2.3 DNA and PCR Extraction

DNA was extracted using Dellaporta method. Then, quantity and quality of the extracted DNA were deter-

Table 1. The studied genotypes with ALP markers

No.	Cultivar	No.	Cultivar	No.	Cultivar	No.	Cultivar
1	Karaj-1	26	Gascogne	51	Bayat	76	Cross of Shahi
2	Karaj-2	27	Soisson	52	Falat	77	Maroon
3	Karaj-3	28	Shahriar	53	Heirmand	78	Kavir
4	Azadi	29	Tous	54	Darab-2	79	Hamoon
5	Ghods	30	Pishgam	55	Atrak	80	Bam
6	Mahdavi	31	C-84-8	56	Chamran	81	Akbari
7	Niknejad	32	Oroom	57	Star	82	Sistan
8	Cross of Bulani	33	Zaree	58	Dez	83	Arg
9	Bulani	34	Inia	59	Vee/Nac	84	Yavarous
10	Kalk Afghani	35	Khazar-1	60	Line A	85	Karkheh
11	Sayonz	36	Mughan-1	61	Aflak	86	Aria
12	Shiraz	37	Mughan-2	62	Baaz	87	Dena
13	Parsi	38	Mughan-3	63	Shahpasand	88	Behrang
14	Sivand	39	Golestan	64	Omid	89	UN-11
15	M-85-7	40	Alborz	65	Roshan	90	Kohdasht
16	WS-82-9	41	Kaveh	66	Tabassi	91	Ohadi
17	WS-85-10	42	Rassoul	67	Sholleh	92	Dehdasht
18	DN-11	43	Tajan	68	Sorkhtokhm	93	Pato
19	Bezostaya	44	Shiroudi	69	Adl	94	Rasad
20	Navid	45	Darya	70	Bayat	95	Hama-4
21	Alamout	46	Arta	71	Sardari	96	Ch
22	Alvand	47	Morvarid	72	Azar-2	97	Homa
23	Zarin	48	N-85-5	73	Zagross	98	Seimareh
24	MV-17	49	Arvand	74	Sabalan	99	Saji
25	Gaspard	50	Chenab	75	Sp.Bc of Roshan	100	Norstar

Table 2. The name and sequence of used primers and annealing temperature in this study

Primer	Sequence ('5→'3)	Genes and Alleles	Annealing Temperature
P1 (F)	CGAGACAATATGAGCAGCAAG	Ax2; Ax1, Ax-null	59
P2 (R)	CTGCCATGGAGAAGTTGGA		
P3 (F)	AACCCCAACAACACCAAC	Dx5	60
P4 (R)	AAACCTGCTGCGGACAAG		
P5 (F)	CATCATCACCCACAACACC	Bx7	60
P6 (R)	TGTTGCCCTTGTCCTGTTT		
P7 (F)	ATGGCTAAGCGGCTGGTC	Dy10	58
P8 (R)	CCCTTGTTGCCCTTTTCCT		

mined using photometry and electrophorese on 2% agarose gel. Polymerase Chain Reaction (PCR) occurred in a 25µl volume containing 0.1µl polymerase Taq enzyme (Cinnagen) (1U), 5µl PCR buffer (10X), 50ng of genomic DNA, 0.7µl of MgCl2, 5µl of dNTP, and 1µM of each of forward and reverse primers (Cinnagen); the reaction volume was finally increased to 25 µl using distilled water. Heating cycles of PCR reaction were applied as follows: one primary denaturation stage at 94oC for 5 minutes, 30 cycles at 94 oC for 1 minute, 58 oC – 60 oC for 1 minute (based using photometry and electrophorese on 2% agarose gel. Polymerase Chain Reaction (PCR) occurred in a 25µl volume containing 0.1µl polymerase Taq enzyme (Cinnagen) (1U), 5µl PCR buffer (10X), 50ng of genomic DNA, 0.7µl of MgCl2, 5µl of dNTP, and 1µM of each of forward and reverse primers (Cinnagen); the reaction volume was finally increased to 25 µl using distilled water. Heating cycles of PCR reaction were applied as follows: one primary denaturation stage at 94oC for 5 minutes, 30 cycles at 94 oC for 1 minute, 58 oC – 60 oC for 1 minute (based on primer annealing temperature in Table 2), 72 oC for 1 minute as the extension temperature, and final extension temperature of 72 oC for 8 minutes.

Following the PCR reaction and electrophorese of the reaction products, 0/1-matrix was constructed in Excel software based on presence and absence of possible bands and the genetic distance matrix was evaluated by means of NTSYS 2.0 software based on Nei coefficient. The results were represented as cluster diagrams using UPGMA method and Jaccard coefficient. The cluster analysis approach was chosen based on cophenetic correlation coefficient and smallness of chain state of the dendrogram. Discriminant function analysis was used to select the cutting point of dendrogram.

2.4 Calculation of Genetic Variation

Nei index was taken for evaluation of genetic variation in Glu-1 locus. In this formula, if pi represents the relative frequency of i-th allele in a locus within the under-analysis population, the genetic variation in the respective position will be equal:

$$\varepsilon = 1 - \sum pi^2$$

To calculate the mean genetic variation (H) as the averages of εs in all genetic loci, the following formula was proposed by Nei (1973):

$$H = \frac{N}{N-1} \times \frac{\sum_j (1 - \sum_i P_{ij}^2)}{N_j}$$

Where, N is number of varieties, Pij2 is relative frequency of i-th allele, and Nj is number of genetic loci.

3. Result and Discussion

In Glu-A1 locus, two alleles "a" (Ax1, Ax-null) and "b" (Ax2) with lengths of 362bp and 344bp were amplified by (P1-P2) primer pair (Figure 1). Allele "a" with relative frequency of 0.707 was assumed as the largest relative frequency. Sixin15 reported a length of 344bp in the presence of allele Ax2 and a length of 362bp in presence of both Ax1 and Ax-null alleles. In their study on protein subunits of Glu-A1 locus in Zanjan's indigenous

wheat biomasses, Mohammadi et al. observed that % 84 and %16 of samples have allele Ax-null and allele Ax2, respectively, while allele Ax1 was not detected in any of the samples12. The genetic variation for this genetic locus was calculated H = 0.414

In Glu-A1 locus, both Ax1 and Ax2 alleles positively affect the quality of the wheat-derived dough whereas Ax-null allele has a negative effect on dough quality10. Using (P1-P2) primer, no polymorphism was observed in alleles (Ax2) and (Ax1, Ax-null) and application of such primers was limited by identification of allele type. In addition, due to monomorphic structure of this primer, a challenge arises between Ax1 subunit and Ax-null allele in differentiation of populations for selecting Ax-null allele in presence of Ax1 subunit10. Fortunately, there exists a nucleotide replacement between A and G base pairs in this zone identified as a SNP which requires to be analyzed in subsequent studies. Three alleles were identified for Glu-B1 locus by (P5-P6) (Figure 2), allele "b" (Bx7) with an approximate length of 500 bp and a relative frequency of 0.618 was the most abundant case whereas allele "c" with an approximate length of 300 bp and a relative frequency of 0.109 exhibited the least abundance. The calculated genetic variation for this genetic locus equaled H = 0.532. Application of P5-P6 primer enables identification of allelic combination (Bx7+By) that can be detected by SDS-PAGE technique identifying the combi-

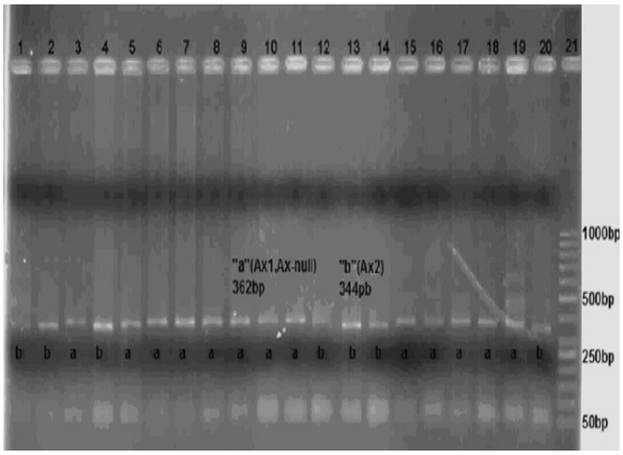

Figure 1. Length variation High Molecular Weight (HMW) glutenin genes using of p1-p2 primer.

1-Azadi 2-Ghods 3- Shahriar 4-Mahdavi 5-Pishgam 6 - C-84-8 7-Oroom 8-Zaree 9-Inia 10-Khazar-1 11-Mughan-1 12-WS08209 13-Bezostaya 14-Navid 15-Alborz 16-Kaveh 17-Rassoul 18-Tajan 19-Shiroudi 20-Alamout 21-Ladder.

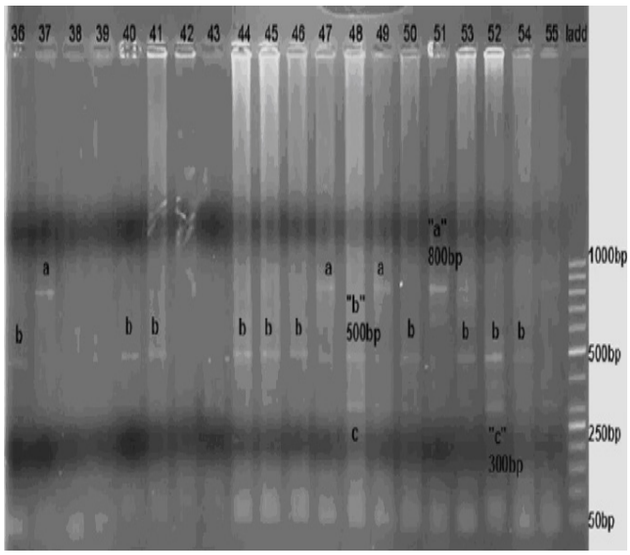

Figure 2. Length variation High Molecular Weight (HMW) glutenin genes using of p5-p6 primer.

36-Mughan-1 37-Mughan-3 38-Golestan 39-Alborz 40-Kaveh 41-Rassoul 42-Tajan 43-Shiroudi 44-Darya 45-Arta 46-Morvarid 47 - N-85-5 48-Arvand 49-Chenab 50-Bayat 51-Falat 52-Heirmand 53-Darab-2 54-Atrak

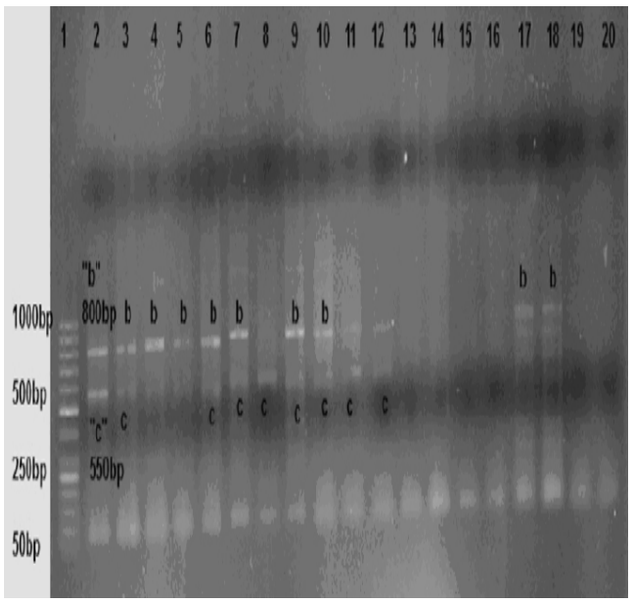

Figure 3. Length variation in High Molecular Weight (HMW) glutenin genes using of p3-p4 primer.

1-Ladder 2-Karaj-1 3-Karaj-2 4-Karaj-3 5-Azadi 6-Ghods 7-Mahdavi 8-Niknejad 9-Parsi 10-Sivand 11-M-85-7 12 - WS-82-9 13-WS-85-10 14-DN-11 15-Bezostaya 16-Navid 17-Alamout 18-Alvand 19-Zarin 20-MV-17.

nation merely as an allele. It is also noteworthy that allele Bx7 is effective in enhancing the elasticity of the wheat dough8. This primer was designed by using the sequences recorded in NCBI website under ID number X13927.3 and is proposed as a new distinctive marker for Bx7 subunit. The respective marker can be used for identification of Bx7 alleles in other wheat genotypes. Additionally, its structure can be investigated in future researches. The Glu-D1 locus was studied using two primer pairs (P3-P4 and P7-P8). Four alleles were totally identified (Figure 3), where allele "b" with an approximate length of 800 bp and a frequency of 0.525 was the most abundant case while allele "c" with approximate length of 550 bp and frequency of 0.137 was considered as the least abundance. Primer P3-P4 only generated a single allele "a" (Dx5) with an approximate length of 850 bp in a number of cultivars. This primer was designed using the sequences recorded in NCBI website under ID number X12928.5 and is introduced as a new distinctive marker for the subunit Dx5. The P7-P8 primer identified three alleles (b, c, and d). This primer was also designed using the sequences recorded in NCBI website under ID number AB281268.1. Payne proved that bread quality is consistently related to presence of (x5+y10) subunits13. Values of genetic variation, which were based on Nei index, were respectively equal to 0.414, 0.532, and 0.648 (Table 3) and the average genetic variation (H) was computed as 0.536 for the genetic blocks of Glu-1. Through a research on the genetic variation of glutenins of several native wheat biomasses of Zanjan Region, the values of genetic variation for Glu-A1, Glu-B1, and Glu-D1 respectively equaled 0.278, 0.8, and 0.586 and the average genetic variation (H) was reported 0.84414. As observed, the genetic variation pattern in Glu-1 loci is Glu-B1>Glu-D1>Glu-A1. Genetic variation of Glu-A1 is less than other two blocks; this finding is in accordance with other results[12]

Table 3. Frequency of HMW-G genes and genetic variation in studied genotypes

Locus	Primer	Allele	Relative Frequency	Genetic Variation	Average Genetic Variation (H)
Glu-A1	p1-p2	a	0.707	0.414	0.5366
		b	0.293		
		a	0.273		
Glu-B1	p5-p6	b	0.618	0.532	
		c	0.109		
	p3-p4	a	0.187		
Glu-D1		b	0.525	0.648	
	p7-p8	c	0.137		
		d	0.15		

To perform cluster analysis and to plot the tree diagram, cophenetic correlation coefficient and Mantle's test were computed using Jaccard and Dice methods. It was finally revealed that Jacquard coefficient with a value of 0.89 had a higher cophenetic correlation coefficient value compared to Dice coefficient (0.87). Thus, the tree diagram was plotted based on Jaccard coefficient. According to cluster analysis diagram, the genotypes can be divided into 12 subgroups at a similarity coefficient of 0.63. The established grouping in the cluster analysis indicate that the cultivars planted in a region are genetically similar in terms of Glu-1 gene.

Although occurrence of insufficient allelic variation is one of the disadvantages of ALP marker, a good allelic variation was seen in the current study using only four specific primer pairs. Through application of specific markers in waxy genetic loci of Iranian rice cultivars, Ghareyazie et al. did not observe any contrast in replicable fragments4. In another test conducted by Ghareyazie et al., using 15 specific primer pairs for proliferation of corresponding loci aimed at categorizing 35 Iranian rice cultivars, only 6 pairs of primers were capable of identifying ALP out of the respective rice cultivars5. Absence of sufficient allelic variation in a number of different specific loci in rice, and conversely, presence of adequate diversity in bread wheat suggest an ability to develop further variation of ALP marker in a way that the respective variation can be applied in categorization of cultivars. In addition, correlations can be made between presence and absence of certain bands with qualitative characteristics of bread. Consequently, occurrence of sufficient variation in bread wheat as an allohexaploid plant and absence of such variation for diploid plants (e.g. rice) could imply further profitability of application of this marker in polyploidy plants such as bread wheat[16].

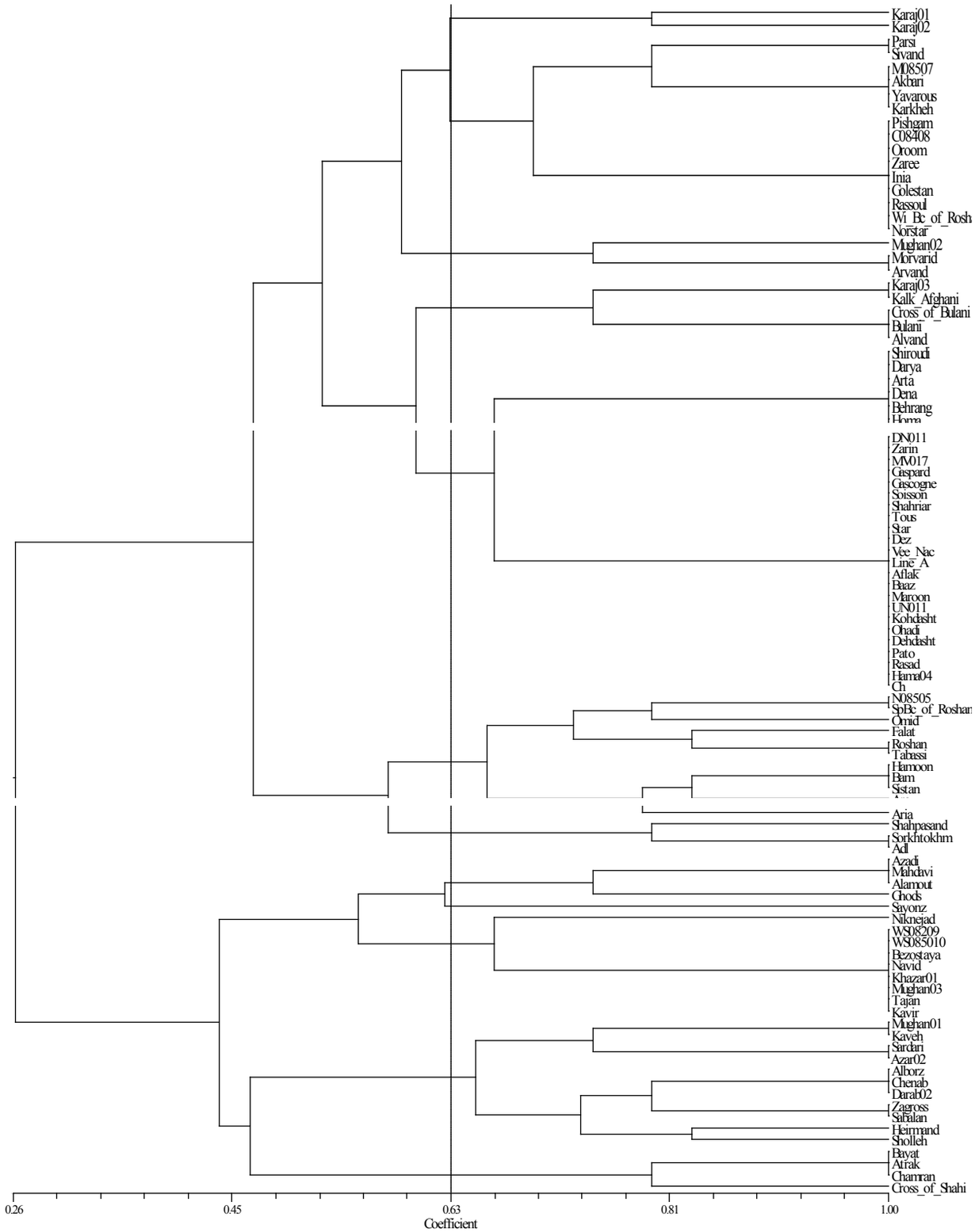

Figure 4. The dendrogram of genotypes on the base of UPGMA (Up Weighted Pair Groups Mathematical Arithmetic) and Jaccard's similarity coefficient.

4. Conclusion

The results of the current research point to the fact that there exists a relatively suitable variation in Glu-1 loci at nucleotide level among the wheat cultivars planted in Iran. This variation can benefit from a valuable source of allelic diversity of HMW-GS genes in quality improvement of wheat flour. Taking into account the significance of HMW-GS genes, this variation can be used for production of desirable varieties. In addition, it was observed that ALP marker is effectively capable of allelic identification in the respective genetic locus.

5. Acknowledgement

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6. Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

7. Conflict of Interest

The authors declare no conflict of interest.

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