Trends in Life Sciences
An International Peer-reviewed Journal
www.sciencejournal.in

ESTIMATING THE BAKERY VALUE OF WHEAT SPECIES BY USING STS MARKER RELATED TO GLUTENIN SUBUNITS WITH HIGH MOLECULE WEIGHT

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ABSTRACT

Although eugening and better farming researchers have led to the increase of wheat production in an area to an agreeable level, the low level of quality in bakeries is still considered one of the serious weak points in most of the new species. Many organic compounds control the subunit effective proteins on the value of wheat flour quality and have changed the quality of bread to a very complicated one. Glutenin with high molecule weight are the most important types of these proteins that affect the maximum resistance and tensility of dough. In this research, by using DNA symbols based on PCR from secret coded genes of Glutenin subunits with high molecule weight, 13 cases of highly used cases in Golestan province are selected and planted in the education and research farm of Agricultural Science and Natural Resource University of Gorgan. After doing the chain reaction of polineriz and horizontal electrophoresis , the species under study were categorized in 4 different groups based on subunits with high molecule weight related to bakery quality. Species in the second group include Naaz, Shiroodi, Alborz, Golestan, Inia, Pastor and Morvarid which were subunits with higher molecule weight and therefore had higher quality. Moreover, the present research showed that determining the weakness and strength of wheat species in bakery value point of view in gene places Glu-A1, Glu-B1 and Glu-D1 are provided by using their specific primers in the shortest time. Using simple, fast and accurate method of PCR can be a suitable replacement for screening genotypes bread wheat with suitable quality.

KEY WORDS: Glutenin, PCR,DNA,STS, cluster decomposition

INTRODUCTION

For years it has been known that gloton proteins have the most important role in determining the quality of baking bread. Pine *et al* were the first to mention the individual correlation between Glutenins with high molecule weight (HMW) and the quality of wheat flour (Pine *et al* 1983). After them, many researchers confirmed and expanded this relation (Pine *et al* ,1989, Barnov *et al*, 1980). The major storing proteins of andosperm are known as prolamins that include two groups of Glutenin and gliadin. Gliadins are little monomer proteins that include subgroups of alpha, gamma and omega. These proteins include 50% of prolamins. Glutenin are formed of subunits with high and low molecule weight. Although Glutenin subunits with high molecule weight form only 10% of the total storing proteins compared with those with low molecule weight (40%) (Pine *et al*, 1984), these subunits have more effect on the quality of bakeries. Glutenins proteins cause the quality features of dough to make the final product of flour by covalent bands that are formed between sistein amino acids existing in Glutenin subunits with high and low molecule weight. HMW-GSs are coded by places of Glu-A1, Glu-B1 and Glu-D1 that are located on the long arms of 1A, 1B and 1D chromosomes that every place includes two coding genes for subunit type of X and subunit type of Y (Shouri *et al* 1992), because these two subunits (X and y) may not be presented in the Glu1 gene place. Therefore, in varieties of wheat 3 to 5 subunits are witnessed. Among Glutenin subunits with high molecule weight in the place of Glu-B1 the element of 1Bx17 is the most common subunit that is witnessed in many of the varieties of bread wheat and durum (Locart *et al*, 1993).). B1x7 is usually appeared with one of the B1y subunits i.e. B1y8 or B1y9. Although it is difficult to discriminate effect of B1x7 from these two simultaneous subunits, but both of these duplexes, 7+8 or 7+9, are located in groups intermediate to

good for bread quality (Ahmad, 2000). Najafian and AbdMeishani(1374) through studying the relation between Glutenins with high molecule weight with the bakery quality of planted wheat in Iran observed that in gene place of Glu-A1 the subunits of 1 an d2 and in the gene place of Glu-D1 the subunit of 5+10 have more value in the bakery quality than other subunits in these gene places. The subunit of A×2 has more intense coordination with the quality of bakery compared with A×1. Glutenin subunits with low molecule weight (LMW-GS)are coded by Glu-3 including (Glu-A1, Glu-B1 and Glu-D1) that are in the form of gene blocks and are located on the short arm of 1A,1B,1D chromosomes (Mesi *et al*, 1998, Jule *et al*, 2004). Metakowiski *et al* (1990), in a study on 28 species of Australian wheat, determined that not only Glutenins with high molecule weight have an effect on the quality, but also Glutenins with low molecule weight which are located on the short arm of chromosomes of group one have strong coordination with the quality of dough. It ws clear that Glutenins with low molecule weight have significant effect on the resstance and traction of dough (Gupta *et al*, 1991). Gupta *et al* (1995) have reported that the dough obtained from the genotypes 5+10 and 17+18 have more resistance than those with subunits of 2+12 and 14+15. During the same research it was found that the dough obtained through the genotypes with units 7+9 have less resistance compared with that of 7+8 (Lafindra *et al* ,1993). by providing newcomposition inbreed lines from the firelo variety, showed that there was no significant difference between in the height of sediment between the subunits of 5+12 and 2+12, while there was a significant difference between the subunits of 5+12 and 5+10. Apparently the subunit 10 is the major factor for this. The purpose of doing this study is to analyze the relation between Glutenin subunits with high molecule weight and with bakery value through multi variable statistics to determine the degree of wheat genotypes in quality through Glutenin subunits with high molecule weight.

MATERIALS AND METHODS

13 cultivars of bread wheat (*Triticum aestivum* L.) were planted in the research farm of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran in 2012 (Table 1). For DNA extraction about 0.2 g fresh leaf tissue of young seedlings were grinded in liquid nitrogen as the method described by Doyle and Doyle (1987). Purity and quality of extracted DNA was tested by electrophoresis the samples of all genotypes on agarose gel. Samples with equal and appropriate concentration of DNA were prepared for PCR by measuring the concentration by spectrophotometer (NanoPhotometer, IMPLEN). As shown in table 2 to 4, five pairs of primers were used for PCR and reactions were performed in accordance with specific condition and thermal cycles of each primer and the volume of each reaction was adjusted 25 µl (Table 3).

For loading the PCR products on %1.5 agarose gel, 4 µl of the sample was mixed with 2 µl of loading buffer (including 400 µl distilled water, 700 µl liter glycerol, and blue Brumo phenol). The obtained gels were transferred to the special photography device (Dakumnte gel) and the images were used for analysis (Figures 1 and 5). In order to classify genotypes based on STS markers, the dendrogram was drawn using NTSYSpc-2/02e software. The coefficients of simple, Dice and Jaccard similarity coefficient were calculated and the dendrogram was chosen based on a higher similarity percentage.

Table (1) The name and sequences of primers used to amplify HMW glutenin subunits for bread wheat genotypes

source	gene place	Sequence(5′…..3′)	primer
	Ax2; Ax1, Ax-null	CGAGACAATATGAGCAGCAAG CTGCCATGGAGAAGTTGGA	P1 (F) P2 (R)
Ahmad. M (2000)	Dy10, Dy12	GTTGGCCGGTCGGCTGCCATG TGGAGAAGTTGGATAGTACC	P3 (F) P4 (R)
Ahmad. M (2000)	Bx7	ATGGCTAAGCGCCTGGTCCT TGCCTGGTCGACAATGCGTCGCTG	P5 (F) P6 (R)

Table (2) The thermal cycle profiles of used primers in PCR and the alleles amplify by each pairs of primer

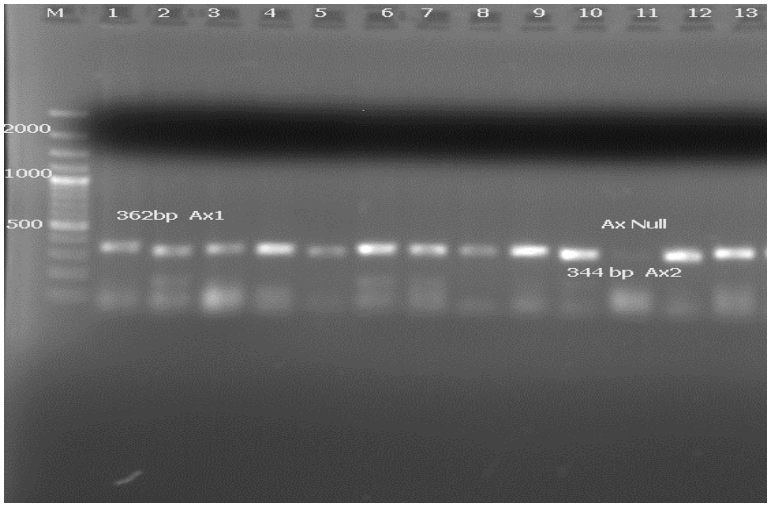
Product size	Thermal circles of polimeraz chain reaction	primer	Allele
344bp	1x(94 5′) 40x(94 1′, 59 1′, 72 1′) 1x(72 10′)	P_1+P_2	Ax2
362bp	1x(94 5′) 40x(94 1′, 59 1′, 72 1′) 1x(72 10′)	P_1+P_2	Ax1
576bp	1x(94 5′) 45x(94 1′, 63 1′, 72 1′) 1x(72 10′)	P_3+P_4	Dy10
612bp	1x(94 5′) 45x(94 1′, 63 1′, 72 1′) 1x(72 10′)	P_3+P_4	Dy12
2373bp	1x(94 5′) 45x(94 1′, 66 1′, 72 3′) 1x(72 10′)	P_5+P_6	Bx7

Table 3. concentration used in the PCR

Final Concentration	Volume	**Concentration**
10XPCR buffer	10µl	**1X**
10Mm Dntp mix	2µl	**0/2mM**
50Mm $Mgcl_2$	3 µl	**4mM**
Taq DNA polymeras	0/5 µl	**2/5 unit/100µl reaction**
Template DNA	1 µg	**250ng**
Primers	5 µl	**10mM**
Autoclaved distilled water up to	**78/5**	-

RESULTS

Pair primers multiply of P1 and P2 of the correlated genome areas with subunit of X from the genome A. therefore, genotypes whose PCR product has the band for this primer is in a desirable level based on the quality.

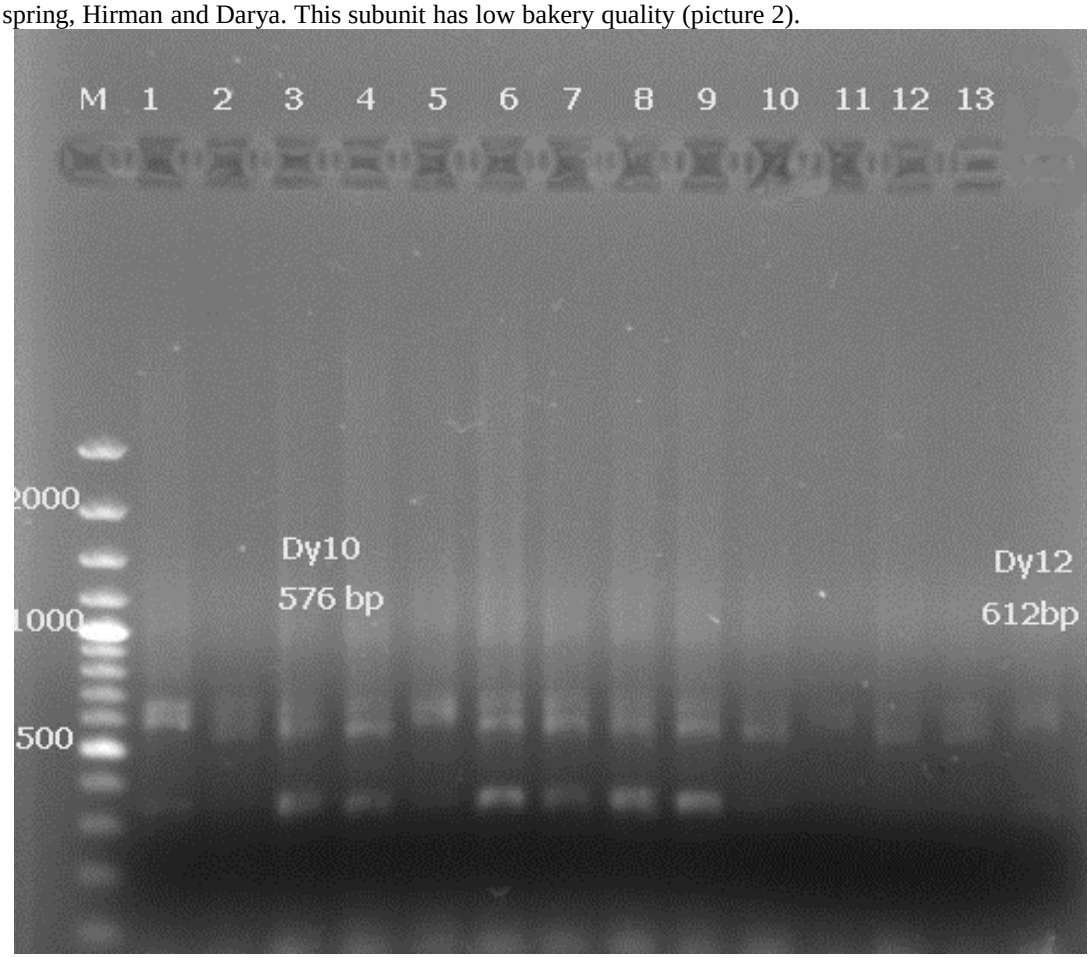


Picture (1). The image of electrophoresis gel of wheat genotypes for P1 and P2 primers

M- Marker 1- Chinese spring 2-Tejen 3-Naz 4-Alborz 5-Hirmand 6-Shiroodi 7-Golestan 8- Inia 9-Pastor 10-Arta 11- Darya 12-Moghan 3 13-Morvarid

As it is seen in picture (1) the genotypes Tajan, Hirmand, Arta and Moqan 3 had alleles Ax2. And the genotype of Darya did not produce any bands that were a show of Axl subunit. And the other species showed the subunit of Ax2. Genotypes had one of the two bands belonging to the subunit X(Ax1 and Ax2), therefore had good conditions according to bakery quality.

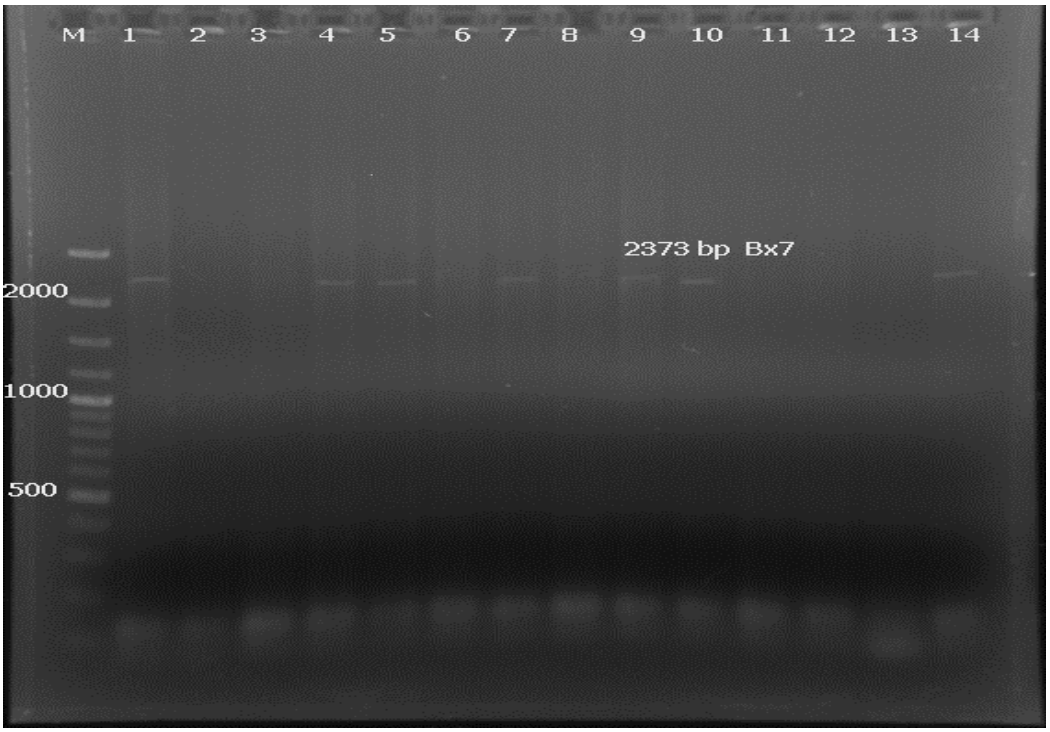
As it is seen in the picture of electrophoresis gel of this primer, the size of the multiplied piece 344 bp for the subunit of A2 and 362 bp for the subunit of A1 (picture 1). By using the pair primer of p3 and p4 species with subunits of 1Dy10 produced 576 bp that include: Tajan,Naz, Alborz,Shiroodi, Golestan, Inia, Pastor, Arta, Moghan 3 and Morvarid. Therfore, these species were in good situation based on the quality of bakery and the species without them with subunit of 1Dy12 produced a 612bp band that includes: Chinese spring, Hirman and Darya. This subunit has low bakery quality (picture 2).



Picture (2). The image of electroforz gel of wheat genotypes for P3 and P4 primers
M-marker 1-chinese spring 2- Tajan 3-Naz 4-Alborz 5- Hirmand 6-Shiroodi
7-Golestan 8- Inia 9- Pastor 10- Arta 11- Darya 12-Moghan 3 12-Morvarid

Pair primers of P5, P6 produces a piece with the length of 2373 pairs from the genom Glu-B1. The size of the produced piece in the electrophose gel shows well that the mentioned band pattern for the subunit of 7 in this gene place. Picture number 3 shows bread wheat genotypes. Species with band respectively include Chinese spring, Alborz, Hirmand, Golestan, Inia, Pastor and Morvarid. The presence of this specific product the gene place of 1Bx7 in the product PCR provides the possibility of identifying species with average to good potential in this position.

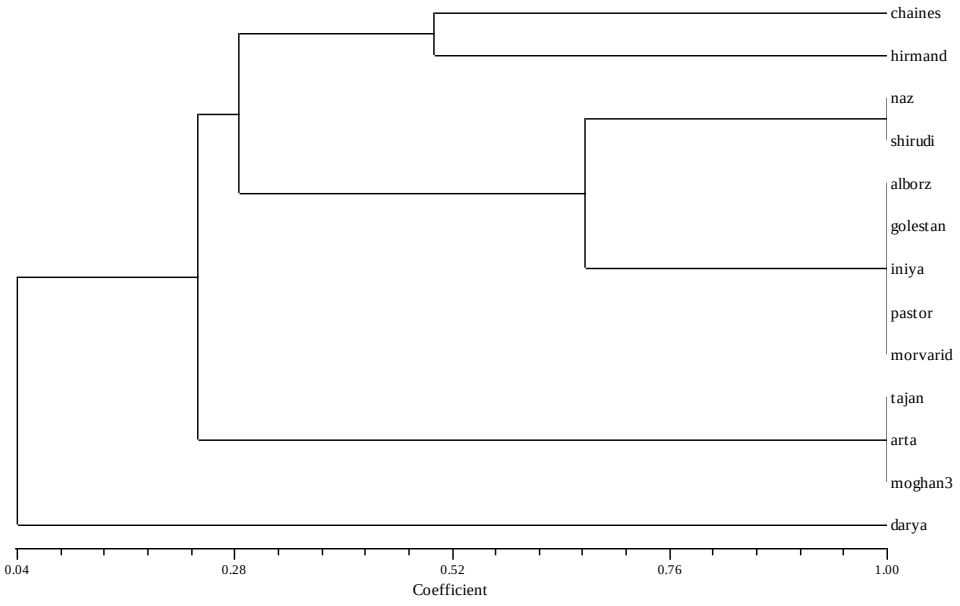
In this study, to categorize genotypes based on STS, dendrogram primers were drawn by using the NTSYS software. The cofentic coefficient and mantel test were estimated for every method. Finally, it was clear that Jakard ratio with the amount of 0.95 compared with the ratio of the simple compatibility method with the amount of 0.90 and the Dice method with the amount of 0.87 had the highest level of coefficient of Cofentic. Therefore, the rest of the analysis was done by the method of Jakard.



Picture (3)electrophorz gel of bread wheat genotypes numbers 1-13 for the primers P5, P6
M-marker 1-chinese spring 2- Tajan 3-Naz 4-Alborz 5- Hirmand 6-Shiroodi
7-Golestan 8- Inia 9- Pastor 10- Arta 11- Darya 12-Moghan 3 12-Morvarid

Based on the cluster analysis graph in the similarity coefficient 0.34 genotypes can be divided into 4 categories. The resulted Dendrogram is shown below. Categorization of the genotypes is as follows:

The first group includes two species of Chinese spring and Hirmand that include two shared subu



nits

of Dy12,Bx7 in Hirmand. The presence of the subunit Dy12 is related to the weakness of bakery quality, but the presence of the other two subunits makes improvement in bakery quality. Considering the presence of Ax2 subunit in the Hirmand, its quality is higher than Chinese spring. The second group includes the biggest one and includes 7 varieties which is divided into two subgroups. The first subgroup includes the two varieties of Naz and Shiroodi with subunits of Dy10 and Ax1. In the second group the 5 varieties of Alborz, Golestan, inia, Pastor and Morvarid all of which include the subunits of Dy10, Ax1 and Bx7. these subunits are related to the high quality of bakeries, and the presence of all these three subunits in these varieties shows the high quality of them. In the third group the three varieties of Tajan, Arta and Moghan 3 were included all of which have the subunits of Ax2 and Dy10. In the fourth group only the Darya variety is located. Considering the categorzation done Darya genotype had the Axnull subunit and the other genotypes lacked this subunit. Lack of subunits related to the quality of bakery include Dy10, Ax1, Ax2 and 1Bx7 and the presence of alleles AxNull shows the weakness of bakery for this variety.

CONCLUSION

This research showed that determining the strength and weakness of wheat species in bakery value in gene places of Glu-A1, Glu-B1 and Glu-D3 using their specific primers in the shortest time. The presence of specific products of gene places of 1Bx7 in the product of PCR makes it possible to varieties with average and good potentials in this position. Considering that every gene place reduces two different subunits based on weight. These subunits are called x and y and are closely related and are usually mentioned in pairs (x+y)(Zoniga *et al* 2004)and in the gene position of Glu A1, the y subunit is off. Good bakery quality has direct positive relation with the existence of A1x2 and x5+y10 Glu-D1. Also, m and n shows correlation with the low volume of sediment ratio and the low quality of baking. Selection with the help of uni marker of alleles Dy10(high quality of bakery) or Dy12 (low quality of bakery) is not useful for choosing the genotype for the quality of baker, and choosing by the help of the two alleles of Dx2+Dy12 or Dx5+Dy10 is required for wheat modification. The SDS-PAGE is one of the most common methods in determining alleles compositions for ranking quality of wheat species, but the capacity of subunits movement in this system is not always compatible with the molecule size to produce problems in identifying alleles compositions of parental lines in modification programs. Recent advancement in the DNA marker technology and selecting through using markers has provided new ways to determine suitable genotypes. PCR methods with special primers are new and useful tools to identify genotypes in special cases not only for the special genes but also for allele almost similar to a gene (Ahmad ,2000). The results of this research can delete possible errors in naming band patterns of SDS-PAGE system protein and makes it possible to quickly identify the quality potentials of species in bakery value for positions of Glu-D1. Glu-B1 and Glu-A1 in modification programs. Confirmed markers of DNA for bakery value can be obtained in the stage of seedling and it is not a requirement to obtain seeds from plants and then determine their protein components. And due to this fast observation time is saved and both the quality and quantity of species are maintained in researches.

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