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Determining Quality of Bread Wheat Cultivars Using Protein Electrophoresis and STS Markers Associated with High Molecular Weight Glutenin Subunits

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ABSTRACT: In this study, the efficiency of wheat quality classification was studied with an emphasis on modern methods of specific molecular markers for qualitative assessing and qualitative classification of 13 kinds of bread wheat cultivars. Evaluation parameters of wheat quality such as SDS-PAGE and STS-PCR were also compared using five specific primers. The results for the identification of molecular- weight subunits in SDS-PAGE analysis confirmed the results of the STS-PCR method. This method represented different subunits of the given cultivars, but the STS-PCR method showed the presence or absence of a subunit-specific based on the primers designed for the studied allele; thus, reducing the error while detecting and isolating the subunits. Of all identified subunits, the subunit composition, D1x5 + D1y10, had the largest share in improving bakery quality and the Axnull subunit had the largest share in reducing the quality of bread wheat cultivars. According to the results of both tests, it can be inferred that Tajan, Pastor, Alborz, Maroon, and Zagros genotypes were considered as the most superior genotypes, whereas Frontana was identified as a genotype lacking bakery quality. In general, using specific molecular markers can be considered as the most proper method for predicting the quality of bakery, so selecting molecular markers leads to saving time, reducing errors in result description, lowering the risks of acryl amide gel toxicity, and identifying cultivars in the corrective strategies. In fact, using these markers, which provide about 5 - 6 years of progress in the implementation of a reform program, could be a suitable alternative for other classical methods of plant reform, especially in the quality of the cultivators.

Key words: Bakery Quality, SDS- PAGET, specific marker, wheat, HMW-G

INTRODUCTION

Flour and baking quality in a cultivar is a complex trait influenced by several environmental and genetic factors. In fact, the balance between the different components such as starch, gluten, lipids, and water and their interaction determine the quality of a cultivar (Johansson et al., 1998). Well - known genes whose allelic variation affects the finished wheat product include genes controlling heavy glutenin (Glu-1), light glutenin (Glu-3), (Gli-1,2,3), the quantity of proteins (Pro1, Pro2), grain hardness (Ha, Pina-D1, Pinb-D1), and starch. Starch includes waxy proteins (GBSS-Wx-1) and amylose and amilo pectin content and other biosynthesis starch genes, lipids, and lipo- proteins, pigments and alpha and beta amylase enzymes (Morris, 1998). Among various factors, seed storage proteins are the most important factors in making a difference in the quality of wheat varieties.

One of the most significant characteristics of grain storage proteins is their role in determining the quality of flour, bread baking, and pasta in commercial wheat cultivars. Today, it is known that variation in different rates of commercial wheat is due the quality and the nutritional properties of flour, so grain storage proteins are considered as a key indicator of the value in the in-breeding programs of wheat. The association between heavy glutenin subunits and the technological properties of flour and baking in hexaploid wheat ((*Triticum aestivum* L) has been reported by several researchers (Payne, 1987, Morris, 1998). Grain storage proteins have two groups of proteins, including AGA and glutamines. Glutenins are a heterogeneous group of grain storage proteins. They are multi- chain and are classified into glutenin subunits with high molecular weight (HMW) and low molecular weight glutenin subunits (LMW).

High molecular weight are encoded by genes in three gene loci, Glu-A1, Glu-D1, and Glu-B1,which are respectively placed on the long arm of 1 D, 1 B, and 1A chromosomes in the hexaploid wheat. These genetic sites are generally entitled Glu- 1 (Payne *et al.,* 1987). Each locus contains two genes: one of them controls a subunit called X which moves slowly, and the other one controls a subunit, Y, that moves faster. Generally, in case of the agricultural wheat, the encoding genes represent the high molecular weight subunits as Glu-1, so each of them includes two identical genes called Glu-1-2 and Glu-1-1 (Devoka, 2005). Studies have recognized 3 alleles for the genetic locus Glu-A1, 6 alleles for Glu-D1 and 11 alleles for Glu-B1 locus. The molecular results showed that some genes of high molecular weight subunits are dormant.

The current gene type, y, was never expressed at the locus Glu-A1. Sometimes, both x subunits were dormant so that no band genes were observed in the electrophoresis gel leading to a null allele. In case of Glu-B1, both genes are often expressed: Glu-B1 subunits are lighter than Glu-A1 subunits, but in Glu-D1 in which both genes are also expressed the D1x type is heavier than the B1x type, whereas D1y is lighter than B1y (Bushakand Rasper, 1996). To investigate the high molecular weight glutenin subunits in each genotype, it is possible to analyze the protein products or the DNA. This analysis of the protein product in these subunits, which requires using biochemical markers (proteins and enzymes) and the molecular markers (DNA), is a new tool for fast and accurate evaluation of the genetic supplies of the plants. Today, these indices are used different research fields ground, such as cultivar identification, philogenetic assessment, genome homology, and genetic structure of germoplasm (Lafiandra *et al.,* 1993).

Among protein markers, grain storage proteins with a high level of wheat genetic diversity have found vast uses in evaluating wheat genetics. creating a quality assessment method regarding the scores for each of the alleles in the above- mentioned gene sites provides the opportunity to determine the qualitative value of each cultivar is based on the allelic composition of this subunit. This scoring method which is based on the effect of each alleles/ allelic combinations on the height of the SDS sedimentation volume, which is a good indicator of the quality and power of gluten was in isogonics lines. Due to the additive effects of alleles, it is possible to calculate and use the scores for better assess of the quality score of a genotype (Payne, 1987). During the previous years in Iran, using grain storage protein is proposed and used as a genetic quality parameter of flour, bread, and pasta in commercial wheat cultivars (Haghparast *et al.,* 2009).

But today morphological and protein markers are rarely used in breeding programs and stock classification of genetic wheat classification because of their less polymorphism and more time- consuming than DNA markers. In fact, DNA markers usually reveal the coding regions, the non-coding variants, and the marginal genome sequences. On the other hand, the DNA markers which are based on polymerized chain reaction (PCR) provide facilities for basic and applied bio- molecular studies. With no doubt, the invention and introduction of PCR has left its most important effect on development of DNA markers. PCR is a rapid method for in vitro replication of the DNA fragment(s) (Naqavi *et al.,* 2004). Availability of the specific primers related to high molecular weight gluten subunits makes the researchers to determine the status of each genotype in terms of alleles related to subunit groups, regardless of phenotype evaluations.

Olson et al. (1989) introduced the STS markers in human genome. They found that it was possible to use DNA ranges of single copy markers which have a known status on the chromosomes as useful markers for providing important genetic or physical maps of genes. In fact, STS is a short and unique range (usually less than 500 bp) in genome that can be replicated by PCR. This study followed two objectives: quality evaluation of some of the most important bread wheat cultivar on the basis of banding patterns of high molecule weight of glutenin protein and STS molecular markers aassociated with it and comparison of the performance and the results of the two protein electrophoresis methods and DNA in evaluating the quality of wheat grain so that desirable results for identifying the most optimal genotypes in terms of baking properties resulted in the shortest possible time. Selecting parents with high quality scores in the hybridization and breeding programs would probably lead to results with optimally results. With multiple intersections between them and the qualitative but ordinary high yielding varieties attempt to transfer the desired genes to high yielding varieties. Thus, despite their high yielding, bakery quality properties are developed.

MATERIALS AND METHODS

The study, conducted at the University of Agricultural Sciences and Natural resources of Gorgan in 2012, was consisted of two parts: PCR electrophoresis products subunits with primers linked with HMW-G subunits and Electrophoresis protein of HMW-G subunits available in 13-grain bread wheat cultivars (Table 1). At first, DNA was extracted from the genotypes studied by Doyle and Doyle (1987) using 2-3 leaf plants. To powder the texture, about 0.2 g fresh leaves of plants was used in liquid nitrogen.

Quality and safety of DNA was determined using agarose gel, and samples with clear band and no elongation were prepared for all genotypes. A spectrophotometer (Nano Photometer IMPLEN) was also used to measure DNA concentrations in the samples. Due to the concentration of DNA in each sample, several samples with the same concentration were prepared for performing PCR. in this study 5 primers were used (Table 1). PCR responses were also performed with specific primers based on thermal cycles (Table 2). The volume of each Polymerase chain reaction was 25 micro liters (Table 3). In order to load the first PCR samples, the samples were first loaded with buffer (consisting of 400 macro liter double-distilled water, 700 macro liter glycerol, some blue Brumophenol), mixed (4 macro liter of sample, 2 macro liter of buffer), and they were injected onto 1.5% agarose gel. Moreover, to electrophoresis the samples, the constant current of 75 A was applied for 45 minutes. Each obtained gel was transferred to the imaging device (document gel) and the images were used for analysis (Fig.1 and 5). In the second part of the study, to evaluate the protein products, heavy glutenin was extracted from three embryo- free seeds of each cultivar.

Table 1: Name, sequence, and properties of the used primers.

source	Allele or gene position	sequence3')⟶(5'	**primer**
NCBI	A1x2; A1x1, A1x-null	CGAGACAATATGAGCAGCAAG	P1 (F)
		CTGCCATGGAGAAGTTGGA	P2 (R)
Ahmad. M (2000)	D1y10, D1y12	GTTGGCCGGTCGGCTGCCATG	P3 (F)
		TGGAGAAGTTGGATAGTACC	P4 (R)
Ahmad. M (2000)	D1x2, D1x5	GCCTAGCAACCTTCACAATC	P5 (F)
		GAAACCTGCTGCGGACAAG	P6 (R)
Ahmad. M (2000)	B1x7	ATGGCTAAGCGCCTGGTCCT	P7 (F)
		TGCCTGGTCGACAATGCGTCGCTG	P8 (R)

Table 2: Properties of PCR thermal cycles for the used primers.

allele	primers	Thermal cycles of Polymerase chain reaction	**Product size**
A1x1	P_1+P_2	1x(94 5′) 40x(94 1′, 59 1′, 72 1′) 1x(72 10′)	Bp363
A1x2	P_1+P_2	1x(94 5′) 40x(94 1′, 59 1′, 72 1′) 1x(72 10′)	Bp344
D1y10	P_3+P_4	1x(94 5′) 45x(94 1′, 63 1′, 72 1′) 1x(72 10′)	Bp576
D1y12	P_3+P_4	1x(94 5′) 45x(94 1′, 63 1′, 72 1′) 1x(72 10′)	Bp612
D1x5	P_5+P_6	1x(94° 5) 45x(94° 1, 63° 1, 72° 1) 1x(72° 10)	Bp450
B1x7	P_7+P_8	1x(94 5′) 45x(94 1′, 61 1′, 72 3′) 1x(72 10′)	Bp2373

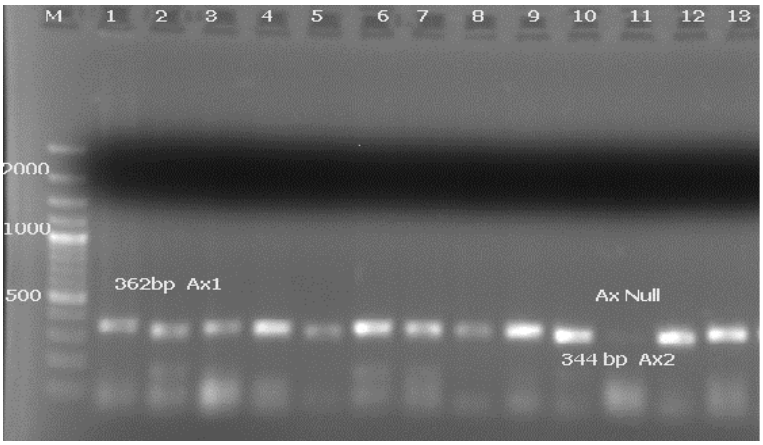

Fig. 1. Results of primers p1 and p2.

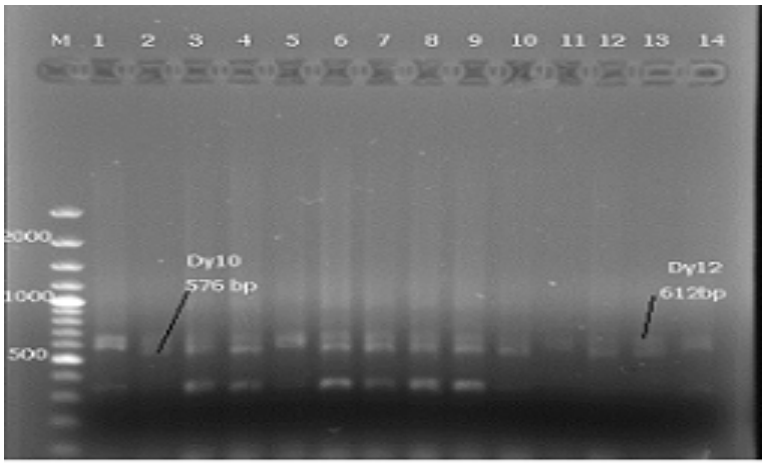

Fig. 2. Results of primers p3 and p4.

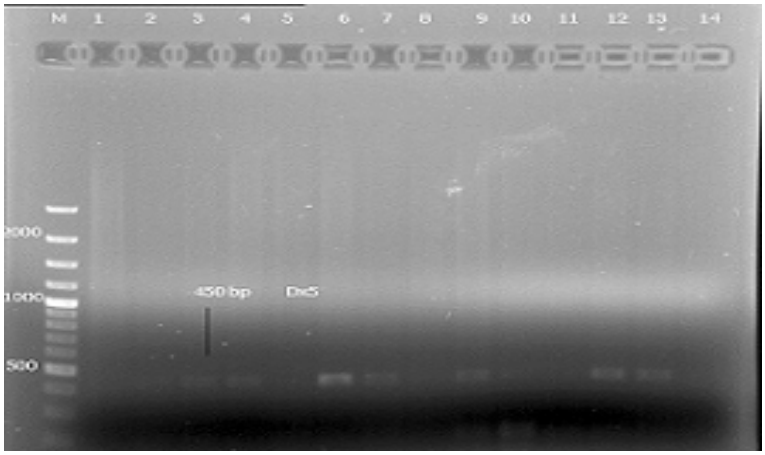

Fig. 3. Results of primers p5 and p6.

To extract glutenins with Dimethylformamide- DMF, the remaining sediment were suspended in a milliliter of PH = 6.8 0.125M Tris-HCL buffer containing SDS% 10 has been suspended After centrifugation, glutens using extraction buffer (volume ratio 1: 5 includes a buffer PH = 6.8 0.125M Tris-HCL, SDS% 2.75, glycerol 10%, DTT% 2 and gamma pyronine) for one hour at 60°C water bath. The heavy glutamine subunits was used with SDS-PAGE method on 10% gel (C = 1.28) (Ravesh pin *et al.*, 1983) and was analyzed and separated electrophoretically. Based on the naming method introduced by Payne and Lawrence (1983), glutenin subunits of heavy genotype (HMW) and the quality score of each line was determined based on the sum of the scores of two loci.

To evaluate the purity of a sample, two grains were randomly selected and were used separately by extracting protein (Bushuk and Zilman, 1978.)

RESULTS AND DISCUSSION

A. Genome A

The pairs of primers p1 and p2 are found in the subunit x of the genome A (Table 1). Alleles 1 and 2 of this subunit increase high quality of bakery, so all genotypes including alleles can form the band. Figures lacking these two bands have null allele in this gene locus; of course, this allele is associated with poor bakery quality. Cultivars of Tajan, Helmand, Atrak, Zagros, and the Caspian have A1x2 alleles as well as the subunit Frontana A1xNull, but they do not produce any band, and just represent A1x1 subunit. As seen in the picture of gel electrophoresis of this primer, the sizes of the amplified fragment for the A1x2 and A1x1 subunits are 344 bp and 362bp, respectively (Table 3).

Table 3: Used density in PCR.

Final density in 100 micro liter	Rate (Micro liter)	Reactors
One time	10	(10x)PCR buffer
Mm 0.2	2	DESOXY nucleotides (10 mM)
mM 4	3	Magnesium Chloride(50 Mm)
unit 2.5	0.5	Polymerase
nano gram 250	1	(5recombinant TaqU)DNA
MM 10	5	(NANOGRAM/ LITER 100) DNA
-	78.5	primers
		Distilled water

B. Genome B

The pairs of primers p7 and p8 produce a fragment with a length of 2373 bp of Glu-B1genome. This locus contains 11 sets of alleles. The coding subunits of X and y are always expressed together by Glu-B1 locus. The alleles B1x17 + B1y18 and B1x7 + B1y8 or B1x7 + B1y9 at this locus improve the properties of dough and bread baking. The size of the produced part in electrophoresis gel represents the desired banding pattern for the subunit no. 7 in the gene locus. The results of 13 bread wheat genotypes which had this subunit are shown in Table 3 (Fig. 4). This primer was designed based on the Glu-B1 alleles' sequence of the subunit no. 7 (Ahmad, 2000).

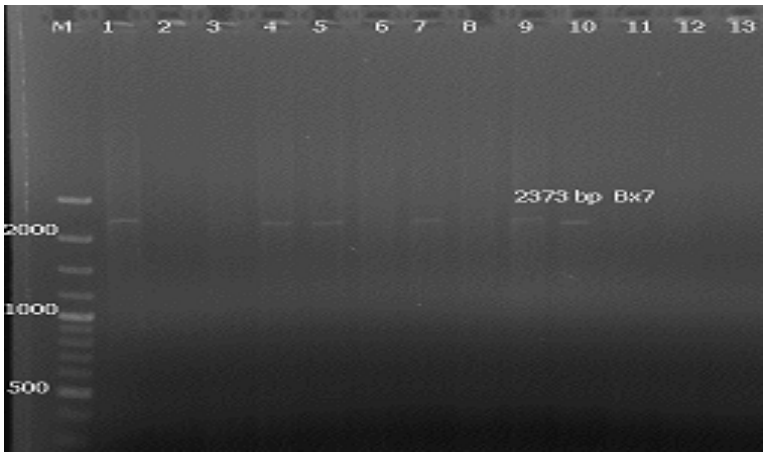

Fig. 4. Results of primers p6 and p7.

Fig. 5. Separation of glutamine subunits with high molecule weight in some wheat bread genotypes using SDS-PAGE.

C. Genome D

Given the importance of this gene in the genome of wheat bread, two primers were designed in which the primers p3 and p4 reproduce the position of Glu-D1 (Table 1). It produces the subunit D1y10 of processing 576 bp pairs, but the figures lack it and in turn, they produce the subunit D1y10of a band with 612 bp pairs. Smith *et al.*, 1994, designed these primers based on alleles 10 and 12 of the Glu-D1 gene (Table 1).

Ahmad (2000) identified the amplified fragment with a length of 612 bp I presence of the allele D1y12 for Chinese spring primers and by using these primers. The results of this study for the Chinese Spring were consistent with his results (Table 3). The primers p5 and p6 (Anderson *et. al.* 1989) which designed Glu-D1 gene based on the subunit D1x5 were associated with high bakery quality (Table 1).

While the alleles D1x2, D1x3 or D1x4 are associated with poor bread quality (Kocourková *et al.* 2008) and the glutenin alleles with high molecular weight subunits which include D1x5 + D1y10 at Glu-1D locus improves the dough baking properties. Based on the sequence of the amplified part in polymerase chain reaction, the length of this fragment was 450 bp. As seen in Fig. 3, only 9 out of 13 samples made bands on electrophoresis gel, so the cultivars equipped with the alleles D1x2 was related to bad quality of bakery and no fragment was reproduced during the polymerase chain reaction (Table 3). No polymorphism was amplified for the fragment size, so the length of the reproduced fragment matched the expected fragment length. The results of Ahmad's research were studied for this primer, proving the presence of the 450 bp band for three genotypes: classic, Hartouj and Nipava of five studied genotypes. Various studies showed that the subunits 10+5, 2, 1, and 7 had a positive effect on the bakery quality (Fatehi *et al.,* 2007). The frequency of these three alleles in cultivars was more interesting than the other two alleles, indicating that glutenin subunits with high molecular weight were used for the genomes A, B, and D. It showed that the highest frequency of the alleles for the genome D belonged to 5+10 allele composite, so this combination had a direct relationship with the high bakery quality of wheat bread cultivars. For genome A A1x1 subunits and the subunits A1x2 the highest allele frequency has improved the quality of the bread as well as for its abundance B1x7 subunit is the 15/46 percent. According to studies and statistics that at least two Heavy subunits are associated with high quality are of good quality. For the genome A the subunits A1x1 and A1x2 had respectively the highest allele frequency and improved the bakery quality. In case of the subunit, its frequency B1x7 was 46.15%, and based on the studies done, the cultivars with at least two high quality heavy subunits had an optimal quality. So, based on the results mentioned in Table 4, the cultivars of Shirudi, Atrak, Falat, Niknejad, and Maroon were recognized as the cultivars the highest quality. The cultivars spring Hines. Alborz, Zagros, and Pasteur had high quality, whereas the cultivars Hirmand AND Khazar had medium quality, and the cultivars Frontana had no quality.

Table 4: Results of used primers.

row	name								
1	Chines spring	D1y12	-	D1x2	-	-	-	A1x1	B1x7
2	tajan	-	D1y10	-	D1x5	-	A1x2	-	-
3	hirmand	D1y12	-	D1x2	-	-	A1x2	-	-
4	shirudi	-	D1y10	-	D1x5	-	-	A1x1	B1x7
5	pastor	-	D1y10	-	D1x5	-	-	A1x1	-
6	atrak	-	D1y10	-	D1x5	-	A1x2	-	B1x7
7	falat	-	D1y10	-	D1x5	-	-	A1x1	B1x7
8	alborz	-	D1y10	-	D1x5	-	-	A1x1	-
9	zagros	-	D1y10	-	D1x5	-	A1x2	-	-
10	niknejhad	-	D1y10	-	D1x5	-	A1x2	-	B1x7
11	marun	-	D1y10	-	D1x5	-	-	A1x1	B1x7
12	khazar	D1y12	-	D1x2	-	-	A1x2	-	-
13	forontana	D1y12	-	D1x2	-	A1xnull	-	-	-
	%allele frequency	30/76	69/23	30/76	69/23	7/69	46/15	46/15	46/15

D. SDS- PAGE

Table 5 shows the results of electrophoresis gel, Sodium dodecyl acryl amide sodium , in terms of type and frequency of alleles with high molecular weight for the studied cultivars. These results are in accordance with the results of the molecular tests. Based on the trilogy scoring (out of 10), the cultivars with a score of 8 have a good quality. The two Chinese spring genotypes as well as Frontana with a score of 6 and 7, respectively, had lower bakery quality among the studied genotypes. Other varieties owned desirable bakery quality: the genotypes of Tajan, Pastor, Alborz, Zagros, maroon with a score of 10 had the highest bread quality. MacRitchie et al. (1990) demonstrated that genomic score justifies a significant proportion (50-70%) of the variation in grain quality of wheat genotypes.

Table 5: Glutamine subunits and genome score for 13 bread wheat genotype using SDS-PAGE.

row	name	D	A	B	Genome score	Total score
1	Chines spring	2+12	1	7+8	2+3+2	7
2	tajan	5+10	2	13+16	4+3+3	10
3	hirmand	2+12	2	17+18	2+3+3	8
4	shirudi	5+10	1	7+9	4+3+2	9
5	pastor	5+10	1	17+18	4+3+3	10
6	atrak	5+10	2	7+9	4+3+2	9
7	falat	5+10	1	7+9	4+3+2	9
8	alborz	5+10	1	17+18	4+3+3	10
9	zagros	5+10	2	17+18	4+3+3	10
10	niknejhad	5+10	2	7+9	4+3+2	9
11	marun	5+10	1	7+8	4+3+3	10
12	khazar	2+12	2	13+16	2+3+3	8
13	forontana	2+12	N	13+16	2+1+3	6

DISCUSSION

The results of identification of subunits with molecular weight which were obtained in the SDS-PAGE analysis included and confirmed the results of the STS-PCR method. This method represents different available subunits in the studied cultivars, so it can reduce the inevitable error while identifying and classifying the subunits. Among the selected subunits, the combined subunit D1x5+ D1y10 had the highest role in improving bakery quality, whereas the Axnull subunit had the highest role in reducing this quality in terms of wheat cultivars.

According to the results of both tests, it was inferred that the genotypes of Tajan, Pastor, Alborz, Zagros, and Maroon were known as the superior genotypes, whereas Frontana was identified as a genotype with no bakery value. However, the results of this study proves the efficiency of molecular markers on the protein markers, since SDS- PAGE method was one of the most widely used methods used in the laboratories to identify allele forms, associated with good or weak bakery quality. Replacement of glutenin subunits with high molecular weight is not always performed with their actual molecular weight (Schwarz *et al.*, 2004).

This, in turn, is a problem for modifiers in selecting parents in modifying programs, yet it can be avoided by using molecular markers in describing the results that is obtained with regard to advancements in DNA technology and its selection with the aid of markers, so the problem is resolved the new solutions are provided for identifying suitable genotypes. PCR methods along with specific primers are gene- like the one alleles and new useful tools used to identify genotypes in specific cases (Ahmad, 2000). In addition, it is another problem found in the SDS-PAGE method along with acrylamide gel toxicity, and high risk of its excessive application. The fact is that most mutations are not shown in this method.

Another advantage of this choice is that it helps molecular markers reduce time required for breeding programs. Using these markers, it would save 5-6 years time for such programs and would reduce the risk of losing genotypes in breeding programs. In other words, using molecular markers is a faster, more accurate and less costly way than classical methods in modifying plants, especially in terms of cultivar quality.

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