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COMPARING PROTEIN PATTERN AND DROUGHT TOLERANT INDICATORS AS SCREENING TECHNIQUES FOR DROUGHT TOLERANCE IN COMMON WHEAT GENOTYPES

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ABSTRACT: Drought stress is an important factor limiting crop production. Selection of resistant genotypes is a method to decrease the drought effects. In this investigation, eight wheat genotypes were assessed in two environments (irrigated and rain-fed) using randomized complete block design with three replications. Drought resistance indices were calculated using yield data in both stress and non stress conditions to identify resistant and susceptible genotypes. The analysis of variance based of proline content and yield showed genotypic differences among the wheat plants in response to the drought stress. Non-significant negative correlation was observed between seed yield in stress condition and proline content in stress condition. Proline content exhibited significant negative correlations with STI, GMP, HARM and MP. Significant correlations between proline content in stress condition and MP or TOL were also observed. The Cluster analysis assigned the genotypes into three groups with High-yielding (number 8), moderate-yielding (numbers 1 and 2) and low-yielding (numbers 7, 3, 4, 5 and 6). The SDS-PAGE analysis showed that resistant genotype (Pishgam) had lower variation in the protein bands pattern but three sensitive genotypes have most variation in the protein bands pattern.
Key words: Protein bands pattern, SDS-PAGE, Drought, Cluster analysis

INTRODUCTION

Wheat is the national staple food in forty -three countries [17]. According to the statistics of the food and agriculture organization [7], during 2008-2009 growing season 682 million tons of wheat were produced and it is estimated that up to 690 million tons will be produced in 2012- 2013 growing season. Meanwhile, more than 250 million ha of the world soil is cultivated with wheat [7]. As the world population increases, so the demand noticeably for wheat will be increased. The experts contend that the amount of the annual wheat production must be 2% higher than the annual demand. The world does not have enough potential for increasing the soil level cultivated with wheat; therefore in order to increase the wheat production, we have to increase the productivity of the fields which have been cultivated with wheat. Developing new genotypes should be for the sake of increasing the grain yield and quality, and its resistance to biotic and abiotic stresses [22]. Development of stress tolerant varieties is always a major objective of many breeding programs but success has been limited by adequate screening techniques. Drought is one of the most significant factors among abiotic stresses that limit plant performance, growth and productivity [5]. Drought stress is attributed to the condition wherein the swelling of cells and tissues is not in a perfect state; this problem may vary from a minor decrease in water potential to the plant's permanent withering. To put simply, the water shortage occurs when transpiration is more than the amount of absorbed water [1]. It is not clear whether high proteolytic activity under stress conditions is advantageous for the plant allowing reorganization of protein pattern or it leads to cell disintegration [28]. Some experimental evidence suggests that drought sensitive species and varieties have higher proteolytic activity compared to resistant ones [13, 21 and 28], however, data on relation of proteolytic activity to drought sensitivity or resistance are still quite limited.

Drought affects morphological, physiological, biochemical and molecular processes in plants resulting in growth inhibition, stomata closure with consecutive reduction of transpiration, decrease in chlorophyll content and inhibition of photosynthesis and protein changes [16 and 30]. Crop plants which can use water most efficiently and maintain acceptable yields are perspective regarding their tolerance. Drought tolerance is achieved by modulation of gene expression and accumulation of specific protective proteins and metabolites [20 and 29]. Some proteins are emerged in challenging with drought that is called induced proteins. Others that always present in tissues and affected on drought tolerance are called constitutive proteins. The major of researches on drought tolerance related proteins are focused on induced protein. In recent years, the applications of proteomic tools have become popular, and the tools are powerful methodologies for detecting and examining changes in protein composition accurately. Accumulation of specific proteins and other compounds for nutrient storage to high levels is one of the characteristic events during seed development [24]. Improvement of storage protein in seed is being given more and more attention all over the world [15]. Storage protein is a method to investigate genetic variation and to classify plant varieties [14]. It has been widely suggested that such banding patterns could be important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivar [25]. Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice [27]. Among biochemical techniques SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm [19]. The analysis of storage protein variation in wheat has proved to be a useful tool not only for diversity studies but also to optimize variation in germplasm collections [6]. Also, several selection indices (such as STI, TOL and SSI) based on the mathematical relations between the stress condition and normal condition have been suggested for determining the drought resistant genotypes. The most suitable index, based on which you select the genotypes, must be one that causes the yield improvement in both the stress and non-stress conditions [11]. In an experiment, Mohammadi et al. used principal components analysis to classify the genotypes of durum wheat into groups by their drought resistance indices [18]. There were three main groups of genotypes. The first group had much positive first component with optimal yield and drought resistance in rain-fed conditions. The second group had positive amount of first component, negative amount of second component; and was good at drought resistance. However they yielded well only in irrigation conditions because these genotypes were closer to the irrigation yield and were more selected by use of TOL index. The third group is negative in terms of both components and was considered susceptible.

Despite the fact that the response of protein composition to environmental factors in mature wheat grain results from changes in protein deposition during plant development, very few studies has examined the effects of water stress on protein profiling of [23]. The aim of the present research is comparing the results of protein pattern using seed storage protein polymorphism and drought tolerant indices in eight genotypes to evaluate the potential of SDS-PAGE technique to assess drought-resistant genotypes.

MATERIALS AND METHODS

Plant material and experimental design

Eight genotypes of bread wheat (*Triticum aestivum* L.) listed in Table 1 were received from Dryland Agriculture Research Sub-Institute (Sararood Station). They were assessed using a randomized complete block design with three replications under two water regimes (irrigated and rain-fed) during 2008–2009 growing season in the experimental field of the college of Agriculture, Razi University of Kermanshah, Iran (34°19′N, 47°03′E, 1322 m above sea level, Koppen climate classification; CS3). Mean precipitation in 2008–2009 growing season was 455 mm (figure 1).

Table 1. List and pedigree of 30 wheat genotypes used in this study

Genotype	Genotype pedigree & name	Important character (s)
1	F103-L-1-12//PONY/OPATA	Short awn
2	OR F1.158/FDL//BLO/3/SH14414/CROW/4/C ICWH99381-0AP-0AP-OMAR-6MAR	High straw yield
3	PYN/BAU//VORONA/HD2402	Awn-less, short peduncle
4	KATILA-13	High thousand seed weight
5	SARDARI-HD35/5/DMN//SUT/AG(ES86-7)/3/ ICWH99-0552-0AP-0AP-OMAR-3MAR	Drought resistance
6	STAR/SHUHA-4	Dwarfness
7	KATILA-1	Short spike
8	Pishgam (Bkt/Zhong)	Dwarfness, high potential yield, drought resistance

The soil of experimental field was clay loam with pH 7.1. Sowing was done by hand in plots with four rows 2 m in length and 20 cm apart. The seeding rate was 400 seeds per m^2 for all plots. At the rain–fed experiment, water stress was imposed after anthesis. Non stressed plots were irrigated three times after anthesis. All cultural practices were carried out as recommended for wheat production.

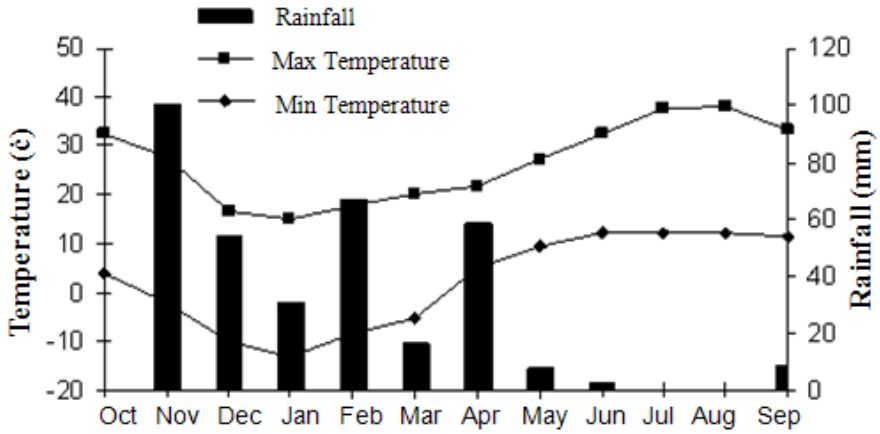

Figure 1: precipitation rate, maximum and minimum temperature of the studied region

Drought tolerance indicators

Grain yield (g m^2) in stress (Ys) and non-stress (Yp) environmant were measured by harvesting each plot at crop maturity. Stress tolerance index (STI) was calculated using the following formula: STI = [(Yp) (Ys) /$(Y_{p-})^2$] [8]. Tolerance index was calculated using the following formula: TOL = [(Yp-Ys)] [12]. Mean productivity (MP) was calculated using the following formula: MP =[(Yp + Ys) / 2]. Stress susceptibility index was calculated using the following formula: SSI = [1- (Ys) /(Yp)] / SI; SI is the stress intensity and calculated as: SI = [1- (Ys) /(Yp)], [9]. Yield stability index was calculated using the following formula: [(Ys) /(Yp)] [4]. Yield index was calculated using the following formula: [(Ys) / (mean Ys)], [10]. Geometric mean productivity was calculated using the following formula: [√(Yp)× (Ys)] and Harmonic mean was calculated using the following formula: Harm = [2(Yp× Ys)/ (Yp+ Ys)] [2] where Yp and Ys are the yield of a given genotype in a normal and stress environment, respectively, and Yp is the mean yield for all genotypes in normal condition.

Proline Concentration (PC)

The PC was determined according to the method of Bates [3]. Plant material (leafs) (0.1 g) was grinded after anthesis stage with 10 ml of 3% sulfosalicylic acid. The homogenate was filtered and 1 ml of glacial acetic acid and 1 ml acid ninhydrin reagent were added to 1 ml of filtrate. Then the mixture was shaken by hand and incubated in boiling water bath for 1 h. After that, it was transferred to ice bath and warmed to room temperature. 2 ml Toluene was added to the mixture and the upper toluene layer was measured at 520 nm using UV spectrophotometer.

Grain protein and Electrophoresis

A single seed was grounded and 10 mg (0.01g) out of this seed flour was taken. 400µl of the protein extraction buffer of 10% glycerol, 5% β-mercaptoethanol, 5 M urea and 0.0001% bromo-phenol blue was added and mixed well by vortexing. The crude homogenates were then centrifuged with 13000 rpm for 20 min. The supernatant were collected and used for protein profiling. Protein concentration was determined by absorbance at 595 nm [3]. A standard curve was prepared with bovine serum albumin. Supernatant was mixed (4:1) with cracking solution (10 ml containing 1g SDS,0.01g bromo-phenol blue, 2 ml β-mercaptoethanol, 1.5ml 0.5M tris, pH 6.8, 5g sucrose and 6.5 ml water) on vortex mixer and heated in a boiling water bath for five minutes to denature the proteins. Protein profiling of samples was performed using SDS- polyacryl amide gel electrophoresis as described by Laemmli (1970). Equal quantities of proteins (150 µg) from each sample along with protein molecular weight marker were loaded into 10% gels. Electrophoresis was performed at constant voltage (100 volts). Gels were stained in coomassie blue G-250 for 45 min. Then gel fixed in solution containing 10% Acetic acid and 40% Ethanol overnight, with constant agitation on a shaker.

Statistical analysis

Analysis of variance appropriate to RCBD was carried out using SAS (version 9.1). Cluster analysis was conducted using Ward method based on distance matrix obtained by SPSS (version 16.0) software.

RESULTS AND DISCUSSION

The analysis of variance was indicated significant difference among different wheat genotypes and between normal and stress levels in seed yield and proline content. Interaction between genotype and stress was significant ($p<0.05$) for proline content but it was not significant for seed yield. Mean comparison of genotypes for seed yield and proline content indicated that highest amount of seed mean yield in normal and stress condition produced in Pishgam (genotype number 8 in table 1) with 10.1 t ha^{-1} and 3.86 t ha^{-1}, respectively. Genotype 3 had the highest and genotype 6 had the lowest value of proline among different genotypes in normal condition. In stress condition, genotype 5 had the highest and genotype 8 had the lowest value of proline. According to results, genotype 8 had the maximum seed yield and low proline content in drought stress condition. These results indicated that genotype with minimum proline content in stress condition had high and stable yield. Proline content of genotype 8 in normal and stress condition was 1.68 (µmol/g dry wt) and 6.03, respectively. In general, stress condition cause to increase proline value in plant tissues [26]. Proline accumulation is believed to play adaptive roles in plant stress tolerance. In this research, drought stress condition did not affect proline content of genotype 8 as same as the other genotypes (table 2 and 3). It can be concluded the drought tolerant and susceptible genotypes do not respond similarly to drought stress condition and tolerant genotypes increase their proline content lower than susceptible genotypes

Correlation among drought tolerance indicators, seed yield and proline content was tested. There were statistically significant correlations between most of the drought tolerance indicators and seed yield in stress and normal condition. Statistically significant negative correlation between proline content in stress condition and seed yield (normal or stress condition) was seen (r= -0.82). Non-significant negative correlation was observed between seed yield in stress condition and proline content in stress condition. Proline content exhibited significant negative correlations with STI, GMP, HARM and MP (r= -0.76, -0.75, -0.71 and -0.76, respectively). Significant correlations between proline content in stress condition and MP or TOL were also observed (r= -0.76 and -0.85, respectively). According to these results, it can be concluded genotypes with high proline content are susceptible to drought stress. Maybe, these susceptible genotypes increase proline content to obtain high tolerance in stress conditions.

Table 2: Yield under stress and non stress conditions along with drought stress indices

Genotype	Seed yield (ton/ha)		PC (µmol/g dry wt)		Stress indicators							
	N	S	N	S	STI	GMP	SSI	MP	TOL	HARM	YSI	YI
1	8.17	3.82	2.92	7.99	0.62	5.59	1.02	5.99	4.35	5.2	0.47	1.13
2	7.51	3.66	3.04	7.24	0.55	5.25	0.98	5.59	3.85	4.93	0.49	1.08
3	5.89	3.05	**5.55**	7.62	0.36	4.24	0.92	4.47	2.84	4.02	**0.52**	0.9
4	6.6	2.67	4.10	7.97	0.35	4.2	1.14	4.63	3.93	3.8	0.4	0.79
5	7.28	2.78	3.00	**8.33**	0.4	4.5	**1.18**	5.03	4.5	4.03	0.38	0.82
6	7.83	3.07	1.54	7.29	0.48	4.9	1.16	5.45	4.77	4.41	0.39	0.91
7	6.15	2.85	3.77	8.08	0.35	4.19	1.03	4.5	3.31	3.9	0.46	0.84
8	**10.1**	**3.86**	1.68	6.03	**0.78**	**6.25**	**1.18**	**6.99**	**6.27**	**5.58**	0.38	**1.14**
LSD	1.88	1.88	0.43	0.43	-	-	-	-	-	-	-	-
Average	7.45	3.22	3.2	7.57	0.49	4.89	1.08	5.33	4.23	4.48	0.44	0.95

Abbreviations: PC— proline concentration; STI—stress tolerance index; GMP— Geometric mean productivity; SSI— Stress susceptibility index; MP— Mean productivity; TOL— Tolerance index; HARM — Harmonic mean; YSI — Yield stability index; YI — Yield index; N (Normal stress); S (Stress).

Table 3: Correlation Analysis of 8 wheat Genotypes based drought indicators and seed yield data and proline concentration.

	yp	ys	STI	GMP	SSI	MP	TOL	HARM	YSI	YI	pcns
yp	1										
ys	.74*	1									
STI	.95**	.91**	1								
GMP	.95**	.92**	.99**	1							
SSI	.53 ns	-.17 ns	.24 ns	.23 ns	1						
MP	.98**	.85**	.99**	.99**	.36 ns	1					
TOL	.95**	.49ns	.80*	.79*	.76*	.87**	1				
HARM	.88**	.97**	.98**	.98**	.08 ns	.96**	.695	1			
YSI	-.53 ns	.17 ns	-.24 ns	-.23 ns	-1**	-.36	-.763*	-.08 ns	1		
YI	.74*	1**	.91**	.92**	-.16 ns	.85**	.49 ns	.97**	.16 ns	1	
pcns	-.82*	-.45 ns	-.68 ns	-.69 ns	-.68 ns	-.76*	-.85**	-.61 ns	.68 ns	-.45 ns	1
Pcs	-.75*	-.64 ns	-.76*	-.75*	-.21 ns	-.76*	-.67 ns	-.71*	.21 ns	-.64 ns	.51 ns

Abbreviations: pcns — proline concentration (non stress), pcs — proline concentration (stress)

Cluster analysis

The clustering pattern of the wheat genotypes based on drought indicators, seed yield and proline concentration data was studied by Ward method and depicted in Figure 2. The analysis assigned the genotypes into three groups. Group 1 included one genotype (Pishgam) characterized by high seed yield, STI, GMP, MP, TOL, HARM and YI. In the second cluster, two genotypes (numbers 1 and 2) grouped together which were moderate-yielding in normal and stress conditions. The third cluster included genotypes 7, 3, 4, 5 and 6 with the low-yielding in normal and stress condition. Three groups of genotypes had lower STI, GMP, MP, HARM and YI.

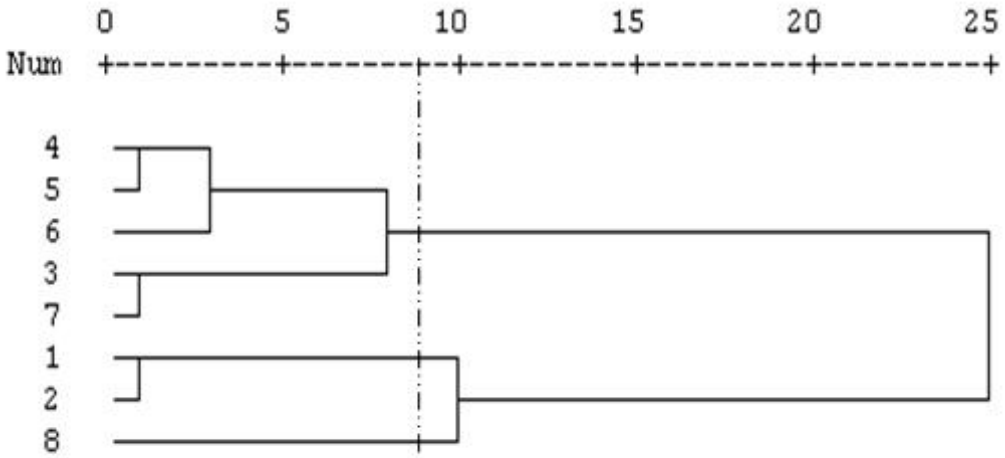

Figure 2: Ward dendrogram of 8 wheat Genotypes based drought indicators and seed yield data and proline concentration.

SDS-PAGE

The seed storage protein patterns for 8 genotypes of wheat under drought stress and normal condition by SDS-PAGE are shown in Figure 2. In total, 35bands (since below 14kDa until over 78kDa molecular weight band) per genotypes were detected in polyacrylamide gel. The SDS-PAGE results revealed drought stress has affected on the seed protein banding patterns and concentrations. Some genotypes possessed some bands which were absent in other genotypes. Comparing protein banding patterns indicated that band number 1 (locus 1 Fig 1) was not exist in genotypes 2, 4, 5 and 7. All of the genotypes had locus 1 except genotype 1. Genotypes 1, 6 and 8 had locus 4 and all of the genotypes had locus 5 except genotype 2. Just genotype 2 had locus 6 but all genotypes had locus 10 except genotype 2. Locus 17 was not observed in genotype 3.

In locus 18, all genotypes had this locus except genotype 1. Genotypes 4 and 5 had locus 26 but in the other genotypes this locus was not detected. Locus 28 and locus 29 did not detected in genotype 7 and genotypes 6 and 8, respectively. In the other loci, there are no differences among genotypes. According to these results, polymorphism was observed in two variable regions i.e., high and medium molecular weight.

Protein concentration in some genotypes had minimum change in normal and stress condition but some of them had high level of changing (genotypes 3, 6 and 7). In stress condition, the expression level of high molecular weight proteins (loci 1, 2, 5 and 6) was decreased but protein expression in loci 10 and 11 was increased. Also, in drought stress, wheat grain protein in genotypes 6 and 7 significantly increased compared to control.

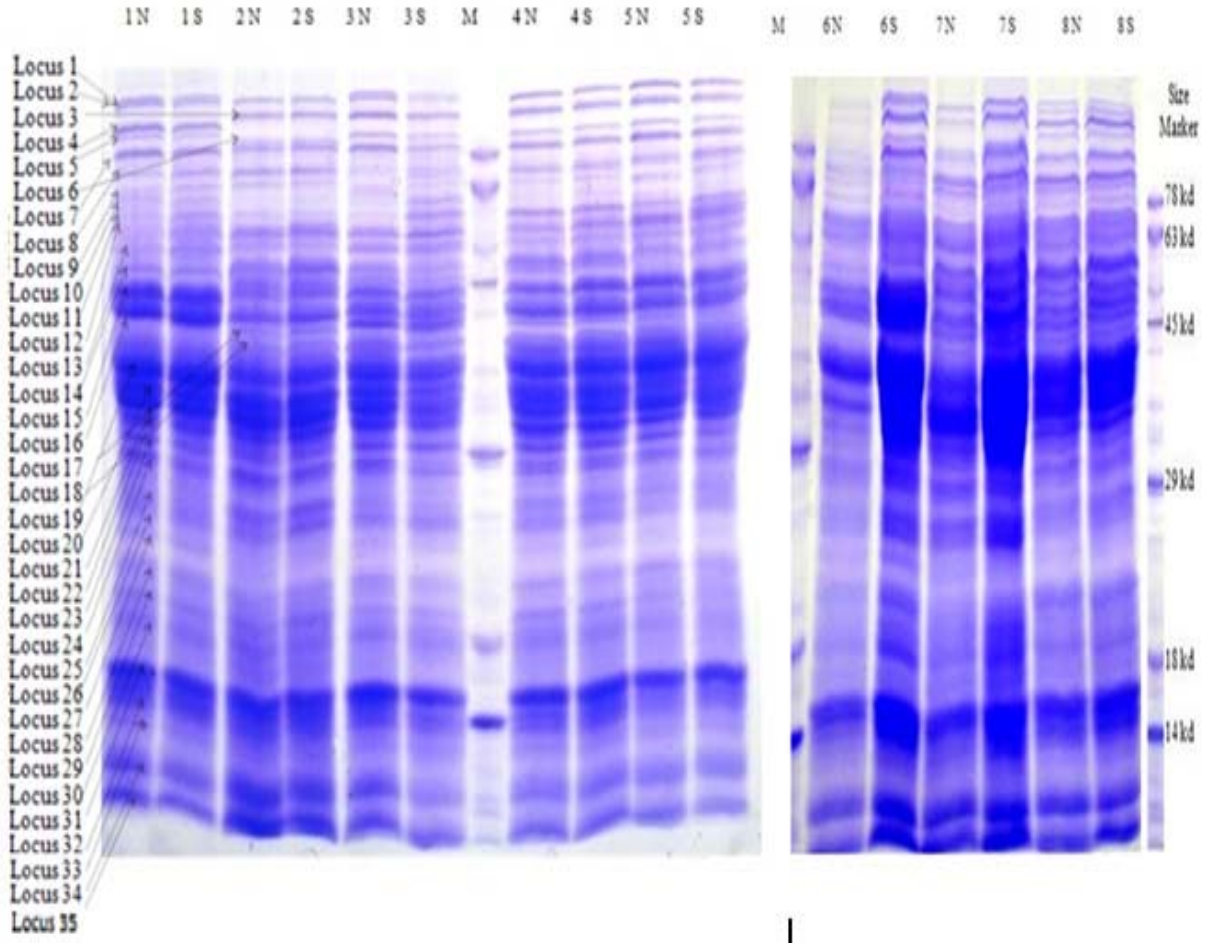

Figure 2: Seed protein banding patterns in 8 genotypes wheat by SDS-PAGE method

CONCLUSION

Drought stress increases proline content in a number of plant species, but the increasing of proline content was different in each genotype. Proline content of genotype pishgam in normal and stress conditions was 1.68 and 6.03, respectively. That shows included lower Proline content for this type content of all the most resistant genotype was detected. Genotype 3 with lower harvest in normal condition (5.89 ton /ha), had most Proline content in the normal condition (5.55 ton /ha). Although genotype 5 has a lower harvest in the stress condition (2.78 ton/hectare) but included most Proline content. The results of this assay showed negative relationship with Tatar and Gerrek result in 2008 that drought stress can increase the proline content and genotypes that have a higher Proline content in normal and stress condition had been lower Proline content (Susceptible genotypes increased proline accumulation immediately after drought stress condition (an osmotic shock).

The investigation result obtained from SDS-PAGE assay for resistant genotype (Pishgam) showed this genotype had lower variation in the protein bands pattern and sensitive genotypes had most variation in the protein bands pattern.

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