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Identification of AFLP Marker Associated with Stress Tolerance Index in Sardari Wheat Ecotypes

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ABSTRACT

Sardari is one of the most important landraces of common wheat (*Triticum aestivum* L.) that is mainly cultivated in drylands and mountainous area of Iran. Moreover, it shows a high level of genetic diversity. In the present research a total of 73 Sardari wheat ecotypes were evaluated for drought tolerance. Genetic diversity was analyzed using amplified fragment length polymorphism (AFLP) marker based on three pairs of primer combinations. Of the 2,431 AFLP bands detected, 1,582 (73.92%) were polymorphic. Cluster analysis divided all ecotypes into eight major groups. Ecotypes also showed genetic diversity for drought tolerance and were classified into three groups. The first group consisted of forty-two of the 73 landraces and had a low stress tolerance index (STI), ranging from 0.165 to 0.401, while the second (23 landraces) and the third group (7 landraces) had a medium and high STI ranging from 0.425 to 0.640 and 0.662 to 0.817, respectively. Discriminant analysis (DA) identified twenty-four markers selected from 218 AFLP markers that accounted for the difference between the three phenotypic groups. By using the selected markers, DA validated the phenotypic grouping, with a zero error rate. The results showed a high degree of genetic diversity between the Sardari ecotypes, suggesting that Sardari can be used as a germplasm source for wheat improvement toward releasing more desirable cultivars.

Keywords: AFLP, Discriminate analysis, Genetic diversity, Sardari wheat, STI.

INTRODUCTION

Sardari is a heterogeneous common wheat landrace that has been cultivated in dry lands and mountainous area of Iran for more than three decades. It has been selected and improved from Iranian native landraces. Sardari has the mean height of 105 cm and is semi-winter, awned spike and elliptical shape, yellow seed, resistant to cold, susceptible to smuts, resistant to rusts, good baking quality, and susceptible to lodging in wet years. Also, Sardari is resistant to shattering and its yield under desirable condition is 1.5 to 2 t ha^{-1} (Khodabandeh, 1992).

As a predominant dry land landrace in Iran, Sardari has received great attention and its genetic diversity of different set of ecotypes from different area has been previously studied. Sadeghzadeh *et al*. (2004) showed that yield, rust resistance, spikelets per spike, stem height, spikelet number, 1000 grain weight, seed protein (%), lodging resistance, and earliness traits vary in Sardari derived lines. In another investigation by Pirseyedi *et al*. (2006), Sardari derivates showed high level of genetic variation based on both

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morphological character and SSR markers and they concluded that the high levels of variation might be caused by stress conditions, or incorrect selection and single spike selection.

Molecular markers provide an excellent tool for obtaining genetic information and their use in the assessment of genetic divergence in wheat has increased in the last few years (Almanza- Pinzon *et al*., 2003; Roy *et al*., 2004; Sofalian *et al*., 2008; Naghavi *et al*., 2009). AFLP markers have proved useful for assessing large numbers of polymorphic loci (Vos *et al*., 1995). Some of the advantages of this method are reproducibility, insensitivity to DNA concentration, speed, and reproducibility of assays, without the need for primary sequence information to design primers (Mackill *et al*., 1996; Tohme *et al*., 1996; Rouppe *et al*., 1997). AFLP markers have been compared with other available marker systems in wheat genetic diversity assessment (Barrett *et al*., 1998; Bohn *et al*., 1999; Soleimani *et al*., 2002; Almanza-Pinzon *et al*., 2003). Based on these studies and DNA marker analyses in other self-pollinating crop species (Maughan *et al*., 1996; Powell *et al*., 1996; Qi *et al*., 1997; Van Toai *et al*., 1997), AFLPs offered high efficiency in terms of polymorphism rate.

A number of stress indices have been developed for evaluation of drought stress in plants. Stress tolerance index, $STI= [(Yp)\times(Ys)/(\acute{Y}_p)^2]$, which can be used to identify genotypes that produce high yield under both stress and non-stress environments (Fernandez, 1992; Saba *et al*., 2001). Previous studies showed that STI is a useful yield-based drought tolerance index to be employed in plant breeding programs for wheat (Clark *et al*., 1992; Fernandez, 1992; Hassanpanah *et al*., 1998; Tarinejad *et al*., 1998; Moghaddam *et al*., 2000). Sio-Se Mardeh *et al*. (2006) evaluated drought resistance indices under various environmental conditions in bread wheat and the results showed that, under moderate stress, *STI* was more effective in identifying high yielding cultivars under both drought-stressed and irrigated conditions.

Discriminant analysis (DA) has been used to combine molecular marker data with phenotypic performance of genotypes to identify meaningful markers (Capdevielle, 2001; Zhang *et al*., 2005). This method was first used to identify RAPD markers associated with disease resistance in rice (Capdevielle *et al*., 2000). Then, it was extended to other marker types such as SSR markers (Zhang *et al*., 2005), and AFLP markers (Capdevielle, 2001; McCharo *et al*., 2005; Miano *et al*., 2008). The idea of applying DA in this particular context is to identify molecular markers significantly associated with a classification of plant material into groups of extreme performance based on an agronomical trait such as *STI*. These markers could be used for genotypic classification, i.e. to allocate new individuals to a predefined (STI) group.

The present research is the first study of the genetic diversity among a large set of Sardari ecotypes based on AFLP analysis and drought tolerance. The objectives of the study were to examine the genetic diversity and phylogenetic relationship among Sardari ecotypes and to use this information for developing strategies in breeding programs.

MATERIALS AND METHODS

Experimental Material

Seventy three Sardari wheat ecotypes were used. Seeds were provided by the Dryland Agriculture Research Institute, Sanandaj, Iran. All ecotypes were collected from Maraghe, Kurdistan and Zanjan in Northwest Iran. Of these 73 ecotypes, 41 ecotypes belonged to Maraghe, 23 ecotypes to Kurdistan and the remaining nine wheat ecotypes belonged to Zanjan.

The experiment was conducted at Ghamloo, in Kurdistan Province (northwest of Iran) from November 2005 to July 2006. Ghamloo (1,850 m above sea level, 35°23′ N, 47°14′E) has an annual average rainfall

of 350 mm. The soil texture was clay-loam (37% clay, 27% silt and 36% sand) with 0.62% organic matter and a pH of 7.5. Available P and K were 14 and 320 ppm, respectively. The experimental design was split plot arrangement of treatments within a randomized complete block design with three replications. Water regimes, i.e. irrigated and non irrigated (rainfed), were allocated to the main plots and Sardari wheat ecotypes to the subplots. The irrigated plots were watered at planting, tillering, jointing, flowering and grain filling stages. Non irrigated plots received no water other than rainfall. Each plot consisted of four 5 m rows, spaced 20 cm apart with seed density of 300 seeds m^{-2}. STIs were calculated using the following relationship (Fernandez, 1992): $STI= [(Y_p \times Y_s)/(\acute{Y}_p)^2]$

Where, Y_s is the yield of ecotype under stress, Y_p the yield of ecotype under irrigated condition, and $\acute{Y}_p$ is the mean yields of all ecotypes under irrigated condition.

For DNA extraction, seeds were germinated in pots and young leaves at 6-7-leaf stage were harvested and stored at -80˚C until DNA extraction.

AFLP Analysis

Leaf tissue was sampled from ten plants per ecotype before flowering and was frozen and lyophilized. All samples per ecotype were ground together. DNA was isolated using the Dellaporta method (Dellaporta *et al*., 1983). The concentration of the extracted DNA was checked in 1% agarose gel by comparing with a *Hind* III marker (Fermentas, Canada Inc.) followed by ethidium bromide staining.

AFLP analysis was done according to Vos *et al*. (1995). Genomic DNA, 300 ng, was digested with 5U each *Mse*I and *Eco*RI restriction enzymes (BIORON, Germany). The double-digested DNA fragments were ligated to 5 pmol *Eco*RI and 50 pmol *Mse*I adaptors (Table 1). The adaptor-legated DNA was subjected to pre-amplifications with *Mse*I and *Eco*RI primers. (Bio-RAD Thermal cycler) and the concentration of the amplified DNA was checked in 1.5% agarose gels. Selective amplifications were performed with the primers containing three additional bases in the 3'-end. Three-primer combinations used for selective amplifications are reported in Table 1. Among the primers, only those three primer combination produced strong and clear bands. Amplified products were mixed with equal volume of formamide loading buffer, denatured at 95˚C for 5 minutes, and resolved on 6% denaturing polyacrylamide

Table 1. *Eco*RI- and *Mse*I-selective nucleotide combinations used for AFLP analysis. Sequences of the adaptors and primers used in the pre-amplification indicated at the bottom rows.

S/N	Primer combination		Polymorphic bands			*PIC* [a] (SD) [b]	*MI* [c]
	*Eco*RI-	*Mse*I-	Total bands	Number	%		
1	CAC	CAT	776	694	89/43	0/28 (0/16)	194.32
2	GCC	GCG	1252	523	41/77	0/15 (0/19)	78.45
3	CGG	GGA	403	365	90/57	0/25 (0/15)	91.25
Mean			810/3	527.33	73/92	0/23 (0/17)	364.02
Total			2431	1582	221/77		
*Eco*RI adaptor	Ligation		*5′ - CTC GTA GAC TGC GTA CC -3′* *3′ - CAT CTG ACG CAT GGT TAA-5′*				
*Mse*I adaptor			*5′ -GAC GAT GAG TCC TGA G-3′* *3′ -TAC TCA GGA CTC AT-5′*				
Primers *Eco*RI *Mse*I	Pri-amplification		*5′-GACTGCGTACCAATTC -3′* *5′- GATGAGTCCTGAGTAA -3′*				

[a] Mean PIC value observed for AFLPs of the particular PC; [b] Standard deviation, [c] Marker index.

gels [acrylamide-bis-acrylamide (29:1)]. AFLP bands were visualized by silver staining protocol (Bassam *et al*., 1991).

Statistical Analysis

Although many bands were detected, only strong and clear bands were scored (Figure 1). Distinction of bands was performed using Cross Checker software (Buntjer, 1999) and also manually. AFLP fragments were read from the gels, and data were entered into a matrix of observations and scored as present (1) or absent (0) for each marker. The data were transformed to a matrix of similarity coefficients using the Jaccard (Johns *et al*., 1997), Dice (Nei and Li, 1973), and simple matching (Rohlf, 1997) methods. All matrices were compared using the matrix comparison function of NTSYS. Since the three similarity matrices were highly similar (R^2> 0.97), only the Jaccard coefficient matrix was used for further calculations. The similarities between ecotypes were displayed in dendrograms using the UPGMA clustering algorithm. Statistical calculations were done using NTSYSpc 2.02 (Rohlf, 1997). Discriminate function analysis and PCA were performed by SAS Ver 8.2 software to complement the cluster analysis method for the confirmation of results. To determine heterozygosity, Popgene Ver1.31 software (Yeh *et al*., 1999) and to determine Shannon's Information Index, MVSP Ver 3.13 software (Kovach, 2003) was used.

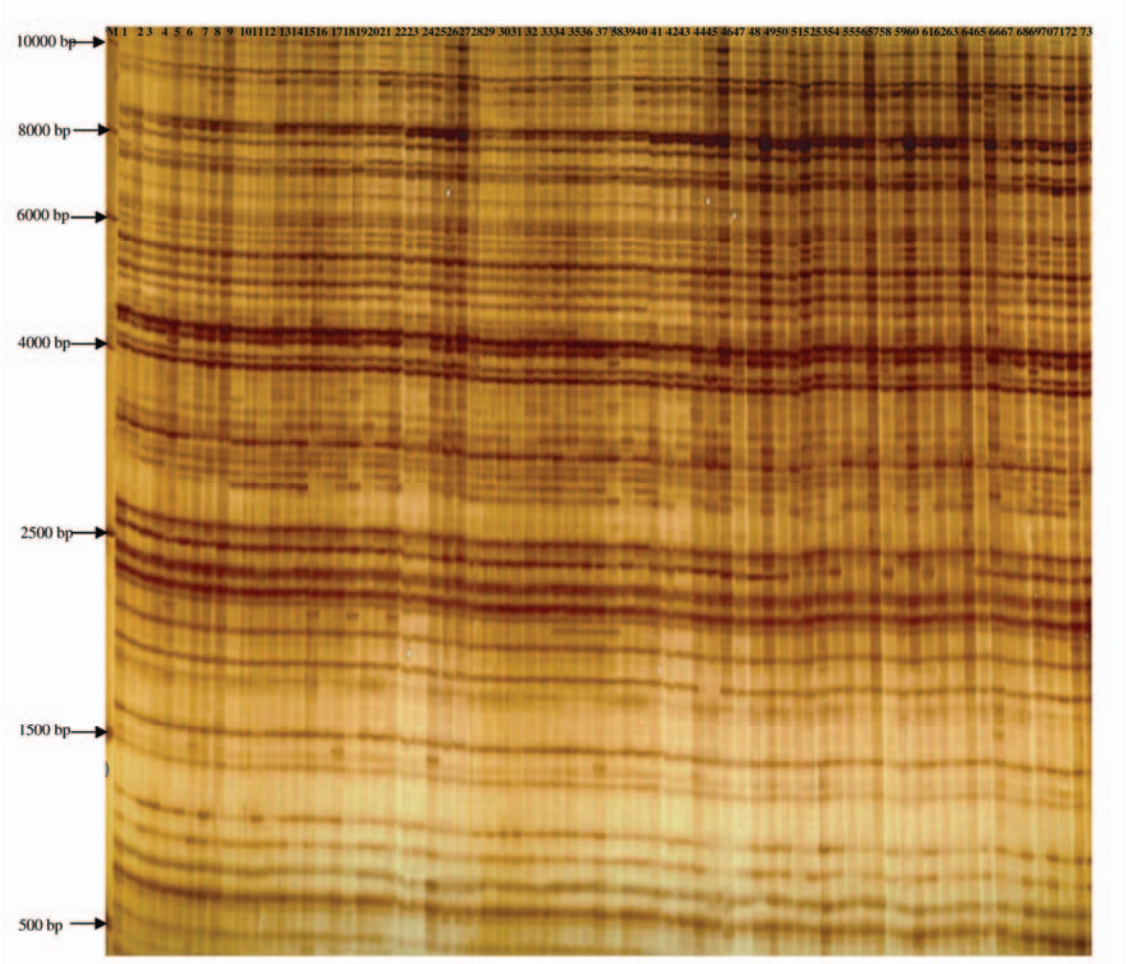

Figure 1. AFLP polyacrylamide gel profile of 73 Sardari wheat ecotypes using primer combination *EcoRI-GCC* and *MseI-GCG*. M is lane of 1 kb molecular ladder.

Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. Variability at each locus was measured by the PIC index (Anderson *et al*., 1993). Also, the Marker index (MI) was calculated for each AFLP primer combination as $MI = PIC \times nb$, where *PIC* is the mean *PIC* value, *n* the number of bands, and b is the proportion of polymorphic bands (Powell *et al*., 1996).

Phenotypic grouping of the ecotypes was done by cluster analysis via application of the NTSYSpc 2.02. Due to the numerous markers generated, it was necessary to use a variable reduction technique to select the most discriminating markers. STEPDISC procedure (SAS, 2001) was used to select the most informative markers from the original set of markers. The forward selection option in STEPDISC was applied to select markers to be included in the classification model. A significance level of 0.1 was imposed to choose the most discriminating markers (SAS, 1990). The level of significance was based on a study by Costanza and Afifi (1979). Wilk's lambda was used as the criterion to determine the classification efficiency with the entry of each marker. The selected markers were then used with the DA, DISCRIM option (SAS, 2001), to develop and validate a phenotypic group prediction model and to predict group membership of the test genotypes. The performance of the discriminant criterion was evaluated by posterior probability error rate and group specific error count estimates during cross-validation.

RESULTS AND DISCUSSIONS

Six different primers (Table 1) were used in three combinations to generate AFLP fingerprints. A total of 2431 AFLP bands were identified, of which 1582 were polymorphic with clear and reliable reading. The mean percentage of polymorphism was 73.92%. The *PIC* mean in Sardari wheat ecotypes was 0.23±0.17, but *E-CAC/M-CAT* primer combination showed higher value compared to the mean (Table 1). The *PIC* values were very close for the primer combinations used so that the higher *MI* values were detected for combinations presenting the higher number of polymorphic bands. *MI* in *E-CAC/M-CAT* primer combination was the highest (194.32) (Table 1). The combination producing the highest number of polymorphic bands (694) was *M-GCG/E-GCC* while the combination producing the lowest number of polymorphic bands (365) was *M-GGA/E-CGG*.

Pirseyedi *et al*. (2006) reported that *PIC* values among 35 Saradari cultivars ranged from 0.107 to 0.829 with an average of 0.447 by SSR markers. Thus, the level of microsatellite polymorphism and the number of allele per locus in Sardari landraces is much lower than the other crops. One possible reason presented by them was that the materials used were all from a particular area in dry lands of the country; therefore, they had a relatively narrow genetic base (Pirseyedi *et al*., 2006).

In our investigation, there was a high level of genetic diversity between the Sardari ecotypes. Probably, cultivation of native Sardari masses over many years and combination of genotypes screened by natural selection to be compatible with their stressful environmental condition in different areas caused such genetic diversity. Sadeghzadeh *et al*. (2004) reported that residual impurity in initial purified lines, cross-fertilization (although limited), genetic mutation, mechanical mixture, exchange among fields and single spike selection can have important role in development of genetic diversity of these Sardari ecotypes. This diversity can be justified by the yield stability and compatibility of Sardari with diversified environmental conditions in cold dryland regions.

AFLP analysis was clearly demonstrated as a powerful means of DNA profiling in wheat, with substantial polymorphisms between 73 ecotypes. The low number of monomorphic bands obtained from all

primer combinations showed that AFLP analysis has a high potential for detecting the genetic variability present in these wheat genotypes. A similar result was reported by Corbellini *et al*. (2002) who analyzed 40 wheat genotypes from Central and Southern Europe by testing five AFLP primer combinations. They obtained an average of 40 polymorphic bands per primer combination and a total of 200 polymorphic bands. Slightly lower levels of polymorphism have also been detected in wheat, such as the 59% reported by Almanza-Pizon *et al*. (2003) and the 47% reported by Roy *et al*. (2004).

The 73 ecotypes were grouped into 8 clusters. The similarity coefficient value of Jaccard between the 73 Sardari wheat ecotypes ranged from 0.50 to 0.89. The genetic similarity estimated with AFLP markers showed that the most similar ecotypes were 16 and 17 and the most different were 23 and 24. Most ecotypes were located in Cluster 2 (38.35 percent), and Cluster 4 (36.98 percent). Also, the least numbers were located in cluster 5, 6, 8 (each 2.73 percent) (Figure 2).

Discriminant analysis was used to determine the distance between the clusters and the degree of grouping. The result showed that the fifth and eighth cluster had the most distances (1.1580) followed by the first and the eighth cluster (1.05287). The second and third clusters had the least distance (0.20031) and there was no grouping mistake.

The AFLP data were also used for conducting PCA to further study the genetic diversity among the 73 wheat ecotypes. Eigen vector of about forty-one Eigen value was bigger than the vertical vector. These forty-one components of PCA accounted for 90.38% of the total variation. The whole diversity was explained by the first seventy

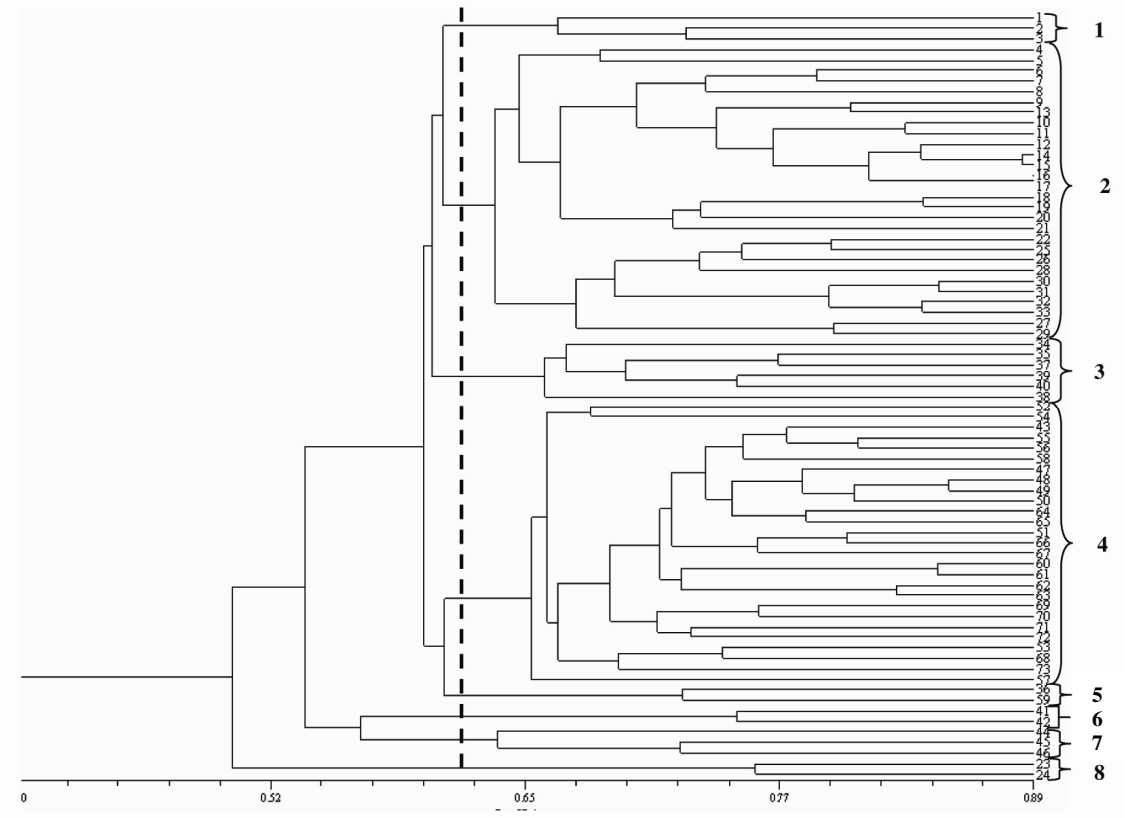

Figure 2. Dendrogram generated for 73 Sardari wheat ecotypes using UPGMA cluster analysis based on Jaccard´s Similarity estimates for AFLP data. Brackets subjectively identify grouping of genetically similar accessions.

components. Among them, the first two components accounted for most of the variance (18%). This clustering pattern of ecotypes obtained on the basis of PCA largely resembled the clustering of genotypes in the dendrograms obtained through UPGMA analysis (Figures 3 and 4).

Sardari ecotypes showed diversity for drought tolerance based on *STI* (Table 2). By using cluster analysis (Figure5), the 73 ecotypes were divided into three groups. The first group consisting of 42 ecotypes with low

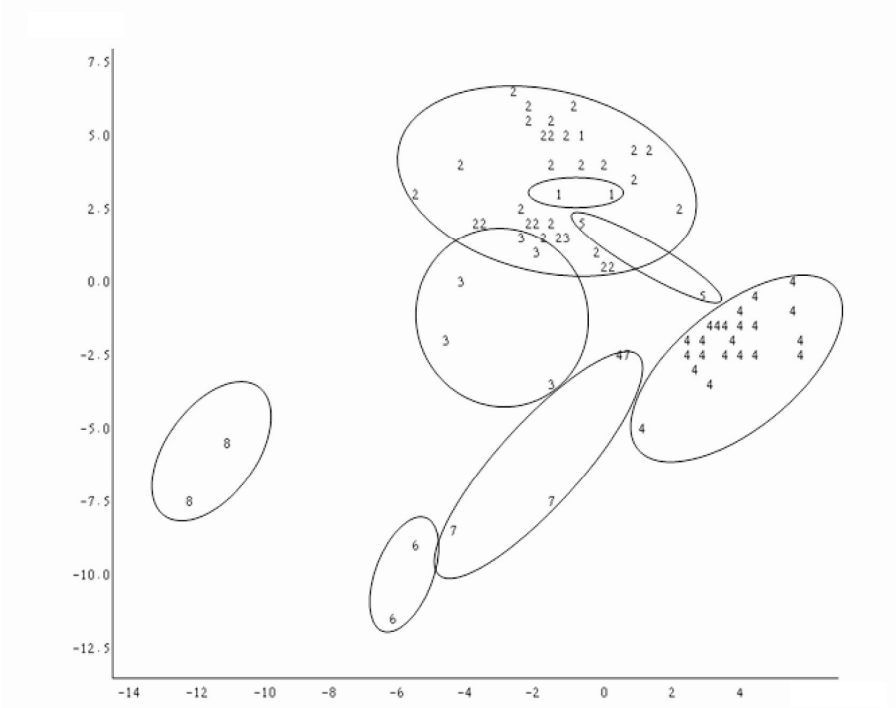

Figure 3. The distribution vector of group revealed by PCA for the first and second component based on AFLP data. Circles identify grouping of genetically similar accessions**.**

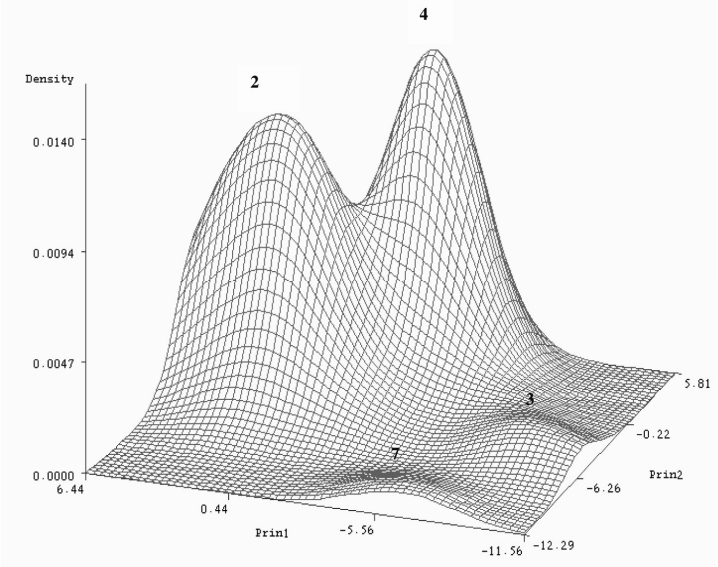

Figure 4. The density vector of group revealed by PCA and as a result of the first and second component based on AFLP data. Numbers show the clusters with high density.

Table 2. No. and STI of Sardari wheat ecotypes obtained from Agricultural Research Institute, Sanandaj, Iran.

No.	Ecotypes No.	*STI*	*STI* group	No.	Ecotypes No.	*STI*	*STI* group
1	22	0.165	Low	38	14	0.370	Low
2	28	0.191	Low	39	34	0.370	Low
3	58	0.197	Low	40	48	0.376	Low
4	26	0.199	Low	41	49	0.377	Low
5	53	0.222	Low	42	72	0.401	Low
6	46	0.225	Low	43	12	0.425	Medium
7	55	0.228	Low	44	70	0.426	Medium
8	51	0.235	Low	45	32	0.438	Medium
9	19	0.260	Low	46	71	0.441	Medium
10	27	0.260	Low	47	60	0.459	Medium
11	44	0.268	Low	48	64	0.463	Medium
12	18	0.268	Low	49	36	0.466	Medium
13	10	0.270	Low	50	45	0.472	Medium
14	21	0.272	Low	51	7	0.478	Medium
15	5	0.274	Low	52	11	0.479	Medium
16	3	0.287	Low	53	37	0.503	Medium
17	41	0.297	Low	54	52	0.514	Medium
18	50	0.297	Low	55	38	0.530	Medium
19	40	0.306	Low	56	16	0.534	Medium
20	17	0.314	Low	57	29	0.543	Medium
21	1	0.315	Low	58	66	0.548	Medium
22	6	0.325	Low	59	54	0.586	Medium
23	15	0.327	Low	60	8	0.596	Medium
24	25	0.337	Low	61	69	0.597	Medium
25	35	0.338	Low	62	67	0.598	Medium
26	39	0.344	Low	63	42	0.604	Medium
27	2	0.346	Low	64	56	0.606	Medium
28	59	0.346	Low	65	57	0.632	Medium
29	73	0.347	Low	66	43	0.640	Medium
30	47	0.350	Low	67	23	0.662	High
31	24	0.353	Low	68	63	0.665	High
32	33	0.353	Low	69	65	0.720	High
33	4	0.356	Low	70	62	0.734	High
34	61	0.358	Low	71	68	0.757	High
35	20	0.360	Low	72	31	0.786	High
36	9	0.366	Low	73	30	0.817	High
37	13	0.368	Low				

STI (0.165 to 0.401), the second group were 42 ecotypes with Medium *STI* (0.425 to 0.640), and the third group had 15 ecotypes with high *STI* (0.662 to 0.817). Based on the discriminant function analysis for *STI*, ecotype 72 was transported to the first group and ecotypes 23 and 63 were transported to the third group. A training sample consisting of 68 low *STI* and 15 high *STI* ecotypes from the original 73 was used for the development of a phenotypic group prediction model.

STEPDISC analysis with the stepwise selection option was used to reduce the number of polymorphic markers generated by the three primer combinations. STEPDISC was further used to reduce the number of markers and to form classification models of up to 24 markers

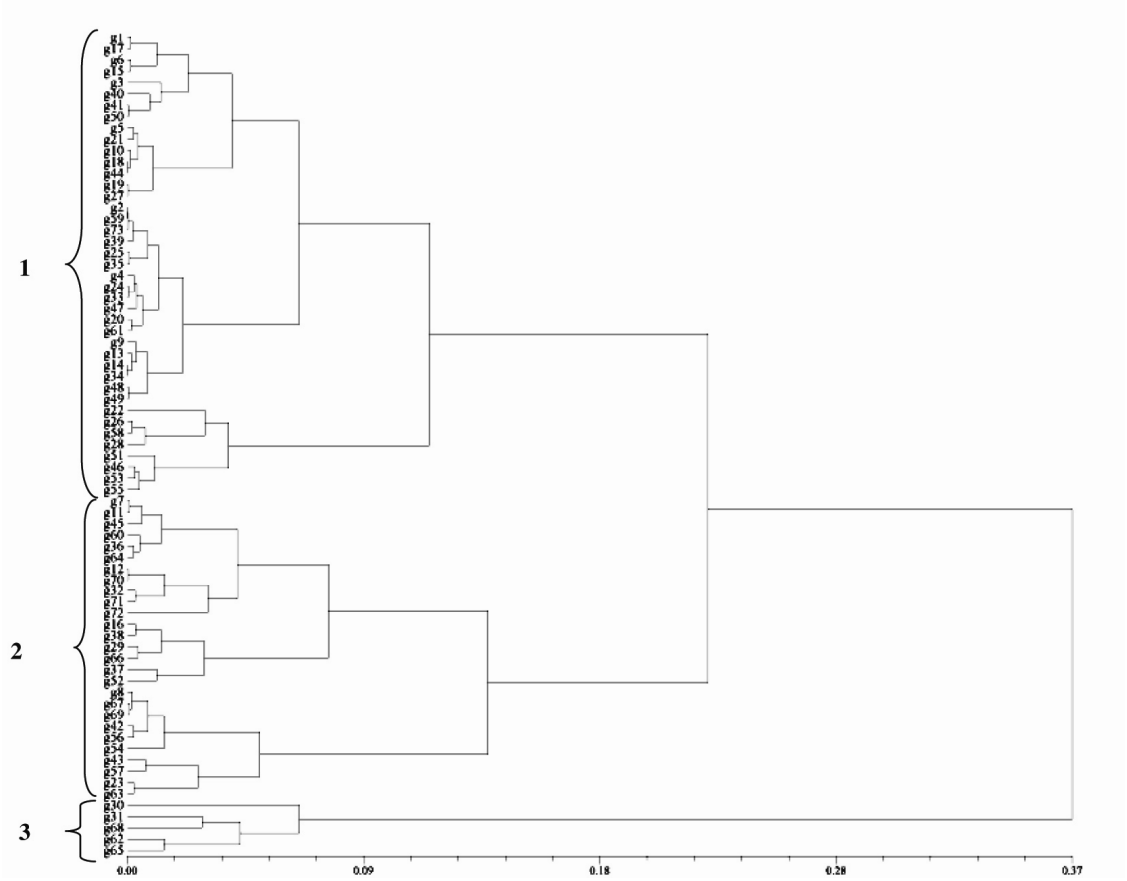

Figure 5. Dendrogram using the *DIST* coefficient and single-link clustering method between high, medium and low STI Sardari wheat ecotypes groups. Upper cluster consists of low *STI* (1), middle cluster consists of medium *STI* (2) and lower cluster consists of high *STI* ecotypes (3).

(Table 3). During evaluation by cross-validation, no more than 24 markers were required to achieve a 100% correct classification. As the number of predictor markers decreased, an increase in misclassification arose, e.g. five markers misclassified 17 clones out of 73 (Table 4). The error rates were calculated using the misclassified ecotype. The total error rate is the mean of the group error rates. The significant result by applying fewer markers, suggests that the STEPDISC procedure is useful in selecting a critical subset of markers. Concentrating on the selected markers could reduce the resources needed in investigating trait marker relationships without compromising the information gained.

The aim of our study was to identify a combination of molecular markers that could be assigned to different *STIs* for the cultivars, and to verify the predictive power of the selected markers or model. Application of DA to a molecular marker data set enables one to determine which markers can discriminate between groups and, then, use the information to predict group membership. Several successful QTL analyses have been conducted to identify loci associated with drought tolerance (Dashti *et al*., 2007; Kordenaeej *et al*., 2008). Ciuca *et al*. (2009) presented preliminary results of the association of several SSR markers with membrane stability after water stress in a set of doubled haploid (DH) lines derived from a cross between two wheat cultivars. Results showed that SSR markers wmc9, wmc596, wmc603 and barc108 were weakly, but significantly, associated with cell membrane

Table 3. AFLP predictor markers in Sardari wheat ecotypes as selected by the STEPDISC procedure.

Marker [a]	Entry step	Wilk's lambda	Pr< Lambda
PC_1-8	1	0.82954545	0.0014
PC_3-165	2	0.70176890	<.0001
PC_1-85	3	0.58131605	<.0001
PC_3-216	4	0.49553153	<.0001
PC_2-69	5	0.41285092	<.0001
PC_3-172	6	0.41285092	<.0001
PC_1-38	7	0.30366823	<.0001
PC_3-169	8	0.26043251	<.0001
PC_3-192	9	0.23421738	<.0001
PC_3-210	10	0.20822530	<.0001
PC_2-147	11	0.17955567	<.0001
PC_1-60	12	0.16140206	<.0001
PC_2-160	13	0.14476052	<.0001
PC_1-77	14	0.12610657	<.0001
PC_2-92	15	0.11397167	<.0001
PC_2-97	16	0.10339237	<.0001
PC_3-207	17	0.08727860	<.0001
PC_2-101	18	0.07528012	<.0001
PC_1-28	19	0.06762493	<.0001
PC_1-82	20	0.06058330	<.0001
PC_2-93	21	0.05411930	<.0001
PC_1-17	22	0.04813311	<.0001
PC_2-88	23	0.04225301	<.0001
PC_1-48	24	0.03696983	<.0001

[a] PC1, PC2 and PC3 are Primer combination *E+CAC/M+CAT, E+GCC/M+GCG* and *E+CGG/M+GGA*, respectively. The numbers beside the primer combination indicate the molecular weight of the marker that they were arranged from heavy to light.

Table 4. Rate of correct classification of 73 ecotypes of Sardari wheat after cross-validation using nearest neighbor in discriminant analysis.

Number of predictor markers	Error type	Low *STI* group error rate	Medium *STI* group error rate	High *STI* group error rate	Total
5	PPER [a]	0.2273 (10) [c]	0.2273 (5)	0.2857 (2)	0.2468
	APER [b]	0.2380	0.2173	0.2857	0.2328
8	[1]PPER	0.2273 (10)	0.0455 (1)	0.0000	0.0909
	APER	0.2380	0.0434	0.0000	0.1506
11	PPER	0.1136 (5)	0.0455 (1)	0.0000	0.0530
	APER	0.11900	0.0430	0.0000	0.0822
14	PPER	0.0455 (2)	0.0000	0.0000	0.0152
	APER	0.0476	0.0000	0.0000	0.0273
15	PPER	0.0455 (2)	0.0000	0.0000	0.0152
	APER	0.0476	0.0000	0.0000	0.0273
24	PPER	0.0000	0.0000	0.0000	0.0000
	APER	0.0000	0.0000	0.0000	0.0000

[a] Posterior probability error rate estimates; [b] Apparent error rate estimates, [c] Number in parenthesis is the number of misclassified clones in group.

stability after water stress and could be used for increasing the frequency of progenies with better performance under drought in a wheat breeding program. Nachit *et al*. (2000) showed that grain yield, yield components, physiological traits were associated with some RFLP markers in durum wheat (*Triticum turgidum* L. var.

durum), and markers *CDO395* and *BCD1661* were associated with higher grain yield. Molecular markers are particularly useful for traits that are highly affected by environmental variations.

However, few studies using DA procedure has been applied for this purpose (Capdevielle 2001). A series of agricultural applications of DA to combine molecular marker and agronomic data from cultivar field trials has suggested a connection between QTL analysis and marker selection (Capdevielle, 2001; Mcharo *et al*., 2005; Zhang, 2005; Miano *et al*., 2008). Results from this work indicate the possibility of using DA for selecting markers that may be useful to breeders. This is a new tool for germplasm improvement providing a discriminative model to integrate the information from markers selected to classify *STIs*. The model can be used to facilitate the allocation of new genotypes into groups with distinct performance for drought tolerance, as well as to identify additional markers associated with the trait. Results obtained to date suggest that the complementation of DA and QTL analysis in STIs could be a good strategy to identify informative markers. Young (1999) argues the need for caution when approaching crop improvement through marker assistance and more so through QTL analysis. *STI* as a quantitative trait is likely to be influenced by several loci. Our results suggest that there are dominant AFLP markers associated with low, medium, and high *STI*. Although this was not a gene mapping study, the markers identified were expected to be closely associated with QTLs responsible for expression of this trait. This is supported by Capdevielle (2001) who investigated the linkage between marker classification and differential response of rice to sheath blight disease and Mc Haro *et al*. (2005) that showed log regression and discriminative analysis for AFLP markers that had a strong and significant association with the southern root-knot nematode resistance in sweet potato. Bonamico *et al*. (2009) also identified an array of SSR markers associated with traits related to common symptoms of Mal De Rio Cuarto virus by means of DA analysis. In this study, maize Recombinant Inbreed Lines (RILs) with distinct reactions to disease were analyzed and results suggest that the complementation of DA and QTL analysis would provide a good strategy to identify informative markers. However, accuracy was slightly compromised when classification models were based on two or three markers. For a crop whose genome has not been mapped, novel techniques like discriminative functions combined with molecular marker data could accelerate progress in breeding. Applications of such protocols include screening of large germplasm collections for desired quantitative traits, phenotypic class identification, and verification of clones assigned to particular classes.

AFLP, simple sequence repeat (SSR), sequence tagged site (STS), sequence characterized amplified region (SCAR) or single nucleotide polymorphism (SNP) markers that are linked to a gene or quantitative trait locus (QTL) are extremely useful for marker assisted selection (MAS) (Shan *et al*., 1999; Sanchez *et al*., 2000; Sharp *et al*., 2001). AFLP markers were used extensively for MAS in cereals (van Berloo *et al*., 2001; Chen *et al*., 2001; Zhou *et al*., 2005). Zhou *et al*. (2001) applied AFLP marker, associated with powdery mildew resistance, as a selection marker and concluded that the AFLP method was more efficient. Quarrie *et al*. (2003) studied marker-assisted selection of improved drought responses in wheat with a number of simple-sequence repeat (SSR) microsatellites. They found justified associations between specific alleles and variation in the expression of traits important for drought resistance.

In conclusion, remarkable genetic variation in the so called Sardari cultivar has been shown. Although DARI′s breeder released Saradari as a pure cultivar three decades ago, this cultivar is clearly based on a complex mixture of genotypes. This variation might have resulted from selection

made by farmers in response to stressful growing conditions in the various cultivation areas of Iran.

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Abbreviations

DA: Discriminant analysis; AFLP: Amplified fragment length polymorphism; SSR: Simple sequence repeat; STI: Stress tolerance index; UPGMA: Un-weighted pair-group method with arithmetic averages; MVSP: Multivariate statistical package; PCA: Principle component analysis; Popgene: Population genetic analysis; PIC: Polymorphism index content; MI: Marker index; QTL: Quantitative trait loci, DARI: Dry land agricultural research institute.

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شناسایی نشانگر AFLP مرتبط با شاخص مقاومت به خشکی در اکوتیپ های گندم سرداری

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چکیده

گندم سرداری یکی از مهمترین نژادهای گندم معمولی (*Triticum aestivum* L.) است که که در زمینهای خشک و نواحی کوهستانی ایران کشت می شود. علاوه بر این سطح بالایی از تنوع را نشان داده است. در این پژوهش مقاومت به تنش حشکی مجموعه‌ای از ۷۳ اکوتیپ گندم سرداری ارزیابی شده است. تنوع ژنتیکی توسط نشانگر AFLP با به کار بردن سه جفت ترکیب پرایمری اندازه گیری شد. از حدود ۲۴۳۱باند AFLP پیدا شده، ۱۵۸۲ مورد چند شکل بود، با میانگین درصد چند شکلی ۷۳/۹۲٪. تجزیه خوشه ای بر اساس نتایج AFLP، همه اکوتیپ‌ها را به ۸ گروه اصلی تقسیم کرد. همچنین اکوتیپ‌ها در ارتباط با مقاومت به خشکی تنوع زنتیکی نشان دادند. اولین گروه ۴۲ مورد از ۷۳ نژاد را تشکیل داد که شاخص مقاومت به خشکی (STI) پایینی با دامنه‌ای از ۰/۱۶۵ تا ۰/۴۰۱را دارا بودند، در حالیکه دومین (۲۳ نژاد) و سومین گروه (۷ نژاد) به ترتیب شاخص مقاومت به خشکی متوسط و بالا با دامنه ۰/۴۲۵ تا ۰/۶۴۰ و ۰/۶۶۲ تا۰/۸۱۷ داشتند. تجزیه تابع تشخیص ۲۴ نشانگر مولکولی از ۲۱۸ نشانگر AFLP بر مبنای اختلاف حاصل از ۳ گروه فنوتیپی را تشخیص داد. با به کار بردن مارکرهای انتخاب شده، تجزیه تابع تشخیص گروه‌های فنوتیپی را با میزان اشتباه صفر تایید کرد. نتایج مشاهده شده از درجه بالا تنوع ژنتیکی میان اکوتیپ‌های گندم سرداری، نشان می دهد که سرداری حاوی اکوتیپ‌های مختلف است و بنابراین میتواند به عنوان یک منبع اصلاحی در جهت آزادسازی کولتیوارهای مطلوبتر به کار برده شود.