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Evaluation of Genetic Diversity and Identification of Informative Markers for Morphological Characters in Sardari Derivative Wheat Lines

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Abstract: In this investigation, association between microsatellite markers and morphological traits of 35 'Sardari' derivative wheat lines was evaluated using 7 morphological characters and 60 microsatellite markers. The numbers of observed polymorphic alleles in each locus varied from 2 to 6. Polymorphic information contents of loci were in the range of 0.11 to 0.83. Cluster analysis based on molecular and morphological data separated the lines into 4 and 5 groups respectively. In the most cases, the lines with similar characteristics were grouped into the same clusters. Distribution of lines within obtained groups using molecular data showed more homogeneity as compared to that of morphological data. The result of multiple regression analysis showed a significant correlation for spiklets in spike with 4B chromosome, number of grain in spike with 5B chromosome, thousand grain weight with 3A, 5A and 6A chromosomes, stem height with 3B chromosome, yield with 6A, 3A and 3D chromosomes, percentage of grain protein with 4A, 5B, 3D and 1A chromosomes and primary root length with 1A and 2B chromosomes. Using of informative markers correlated with yield components, may facilitate preliminary selection of high yielding lines, especially for dryland cultivation.

Key words: Sardari wheat, genetic diversity, microsatellite, informative markers

INTRODUCTION

The availability of genetic variability in elite wheat material is pre-requisite for any breeding program. Sardari is one of the most important landrace, has been cultivated in drylands and mountainous area of Iran for more than three decades. Because of high morphological variance observed within this landrace, 35 lines were isolated. These various lines named Sardari morphotypes recently are being crossed with some elite lines in ICARDA in order to widen the gene pool of wheat germplasm (Personal Communication with DARI authorities). However, there are inherent problems with data resulted from morphological traits, because of great environmental influences and genotype-environment interactions. Molecular markers provide a satisfactory alternative because they are almost unlimited in number and are not influenced by the environment. Despite this, studies on variability and diversity in wheat landraces using molecular marker have been relatively few. This can be attributed to the detection of low levels of variability leading to the use of a limited number of polymorphic markers such as (I) restriction fragment length polymorphism/sequence-tagged sites (RFLPs/STSs) for low-copy sequences (Vaccino *et al*., 1993), (II) random amplified polymorphic DNA (RAPDs) for random sequences (He *et al*., 1992; Dweikat *et al*., 1993) and (III) PAGE for such proteins as gliadins (Cox *et al*., 1985). In fact, among DNA markers RFLPs and RAPDs, the two most commonly used markers, detect only low levels of polymorphism (Penner *et al*., 1995; Röder *et al*., 1998; Paull *et al*., 1998). In comparison, microsatellite or simple sequence repeats (SSRs) are more abundant, ubiquitous in presence, hyper variable in nature, more informative and have high polymorphic information content (PIC, Gupta *et al*., 1996). Due to these properties the microsatellite have recently been used to study genetic variability based on DNA polymorphism in a number of crop plants including wheat, soybean, maize, rice, sorghum, barley and etc. It has also been shown that the use of a limited number of microsatellite is adequate to discriminate even the most closely related wheat and barely genotype (Plaschke *et al*., 1995; Russell *et al*., 1997; Struss and Plieske, 1998). In this communication, we

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report the results of a study involving the screening of 35 specific morphotypes derived from Sardari landrace using 60 microsatellite primers. The study was undertaken with the following objectives; (I) to assess the level of microsatellite-based genetic diversity among 35 derived wheat morphotypes that were potentially useful in wheat breeding program, (II) to study the potential of microsatellite markers for detecting the allele-trait association in the most important dryland Iranian landrace as the main objective, (III) to identify probable informative markers related to specific traits

MATERIALS AND METHODS

Plant material and morphological evaluation: In this study, 35 Sardari wheat derived morphotypes were used. Field experiments were carried out at Dryland Agricultural Research Institute of Iran (DARI) in 2004 and the following quantitative morphological variables were measured: spiklet per spike, seed per spike, 1000 grain weight (g), stem height (cm), primary root length (cm), Yield (t/h) and percent of seed protein.

Molecular analyses: DNA was isolated from leaves according to Dellaporta method (Dellaporta *et al.*, 1983). For each chromosome, more than 2 microsatellite markers (WMS) were selected to guarantee an even coverage of total wheat genome (Röder *et al.* 1998). PCRs were performed on a BioRad thermocycler (BioRad Laboratories Inc., Hercules, CA, USA) according to Röder *et al.* (1998). Amplification reaction products were resolved on a 6% denaturing polyacrylamide gel using a Sequi-Gen GT Sequencing Cell 30×38 cm gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA). Only clearly different bands were accepted and resulting images were scored manually and independently by two persons.

Statistical analyses: The morphological data were analyzed for simple statistics, i.e., standard deviation, minimum and maximum. The average Polymorphic Information Content (PIC) using molecular data was calculated for each wheat microsatellite (WMS) locus (Botstein *et al.*, 1980). Cluster analyses according to guide lines of SPSS and JMP software were performed using morphological and microsatellite data by UPGMA algorithm. To test the effectiveness of association between marker alleles and traits means, stepwise multiple regression analysis was assessed using each morphological and molecular data as dependent and independent variable, respectively.

RESULTS

Morphological analysis: Table 1 Results evident that there is a large amount of diversity between Sardari wheat morphotypes. Cluster analysis based on 7 quantitative traits, separated lines into 5 distinct clusters (Fig 1).

Table 1: Summary of the morphological characters scored for 35 Sardari wheat lines

Character	Mean	Std. Dev.	Minimum	Maximum
Spiklet per spike	13.87	1.73	11.50	19.50
Seed per spike	30.95	6.19	23.50	45.50
1000 grain weight (g)	43.63	5.12	34.0	52.00
Stem height (cm)	8.15	8.45	65.50	100.00
Root length (cm)	10.70	2.28	5.70	16.40
Yield (t h^{-1})	2.53	0.40	1.69	3.15
Seed protein (%)	12.95	1.26	10.50	16.00

Table 2: Primer pairs used in SSR analysis

Marker	Chromosome	Motifs	No. of alleles	PIC
GWM44	7D	(GA)28	2	0.358
GWM55	6D.2B-L	(TC)3 (T)3 (CT)17	5	0.772
GWM111	7D-L	(CT)32 (GT)17	2	0.472
GWM114	3D	(GA)53	4	0.475
GWM120	2B	(CT)11 (CA)18	2	0.499
GWM132	6B	(GA)24 (GAA)6	2	0.395
GWM133	6B	(CT)39	2	0.290
GWM148	2B	(CA)22	2	0.382
GWM149	4B	(GA)23	3	0.608
GWM153	1B	(GA)18	2	0.32
GWM155	3A	(CT)19	2	0.156
GWM156	5A	(GT)14	3	0.827
GWM160	4A	(GA)21	2	0.408
GWM164	1A	(CT)16	2	0.284
GWM190	5D	(CT)22	2	0.358
GWM194	4D	(CT)32	2	0.495
GWM247	3B	(GA)24	2	0.395
GWM249	2D	(GA)11 (GGA)8	2	0.352
GWM259	1B	(GA)17	2	0.265
GWM314	3D	(CT)25	2	0.224
GWM334	6A	(GA)19	3	0.644
GWM335	5B	(GA)14 (GCGT)3	2	0.107
GWM337	1D	(CT)5 (CACT)6 (CA)4	3	0.592
GWM340	3B	(GA)26	3	0.522
GWM357	1A	(GA)18	2	0.466
GWM359	2A	(CT)20 (CTT)13	2	0.472
GWM369	3A	(CT)11(T)2 (CT)21	2	0.244
GWM372	2A	(GA)51	2	0.480
GWM389	3B	(CT)14 (GT)16	2	0.432
GWM397	4A	(CT)21	2	0.498
GWM437	7D	(CT)24	2	0.320
GWM448	2A	(GA)29	2	0.489
GWM459	6A	(GA)28	2	0.197
GWM469	6D	(CT)19 (CA)10	2	0.472
GWM471	7A	(CA)34	3	0.663
GWM493	3B	(CA)43	5	0.766
GWM497	1A,2A,3D	(GT)29	2	0.491
GWM540	5B	(CT)3 (CC)(CT)16	4	0.743
GWM565	5D	(CA)10	2	0.244
GWM577	7B	(CA)14 (TA)6	2	0.426
GWM611	7B	(GA)32	2	0.502
GWM613	6B	(CT)23	2	0.475
GWM626	6B	(CT)5 (GT)13	2	0.485
GWM635	7A	(CA)10 (GA)14	2	0.611
GWM642	1D	(GT)14	2	0.415

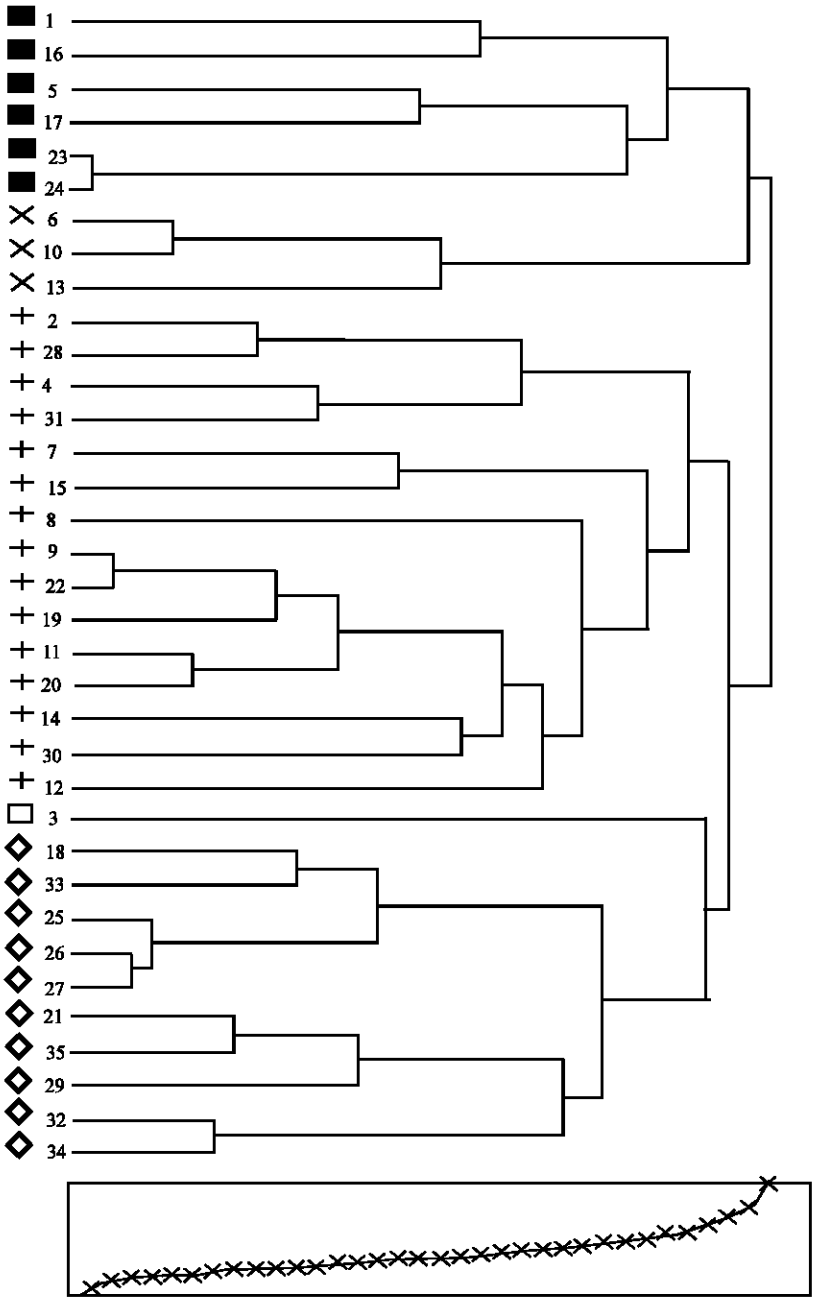

Fig. 1: Grouping of Sardari wheat lines using UPGMA algorithm based on morphological data

SSR analysis: In the SSR analysis, out of 60 primer pairs, 45 produced polymorphic banding patterns and a total of 104 polymorphic alleles were detected. The number of alleles varied from 2 to 6, resulting in an average number of 2.31 alleles per locus (Table 2). Genetic similarity assessment based on microsatellite data ranged from 0.34 to 0.88 (the table is not shown). The highest number of polymorphic alleles occurred in B genome (41%) and the lowest was in D genome (29%). Using the present set of primers, the most polymorphic loci with 8 alleles were found on the short arm of chromosome 3B. PIC values were ranged from 0.107 to 0.829 (Table 2) with an average of 0.447. The average PIC values for A, B and D genomes were 0.463, 0.466 and 0.432, respectively. PIC values for long and short arms of chromosomes averaged 0.436 and 0.524, respectively. Although a great effort was devoted to select equal number of markers on long and short chromosomes, the numbers of polymorphic markers on

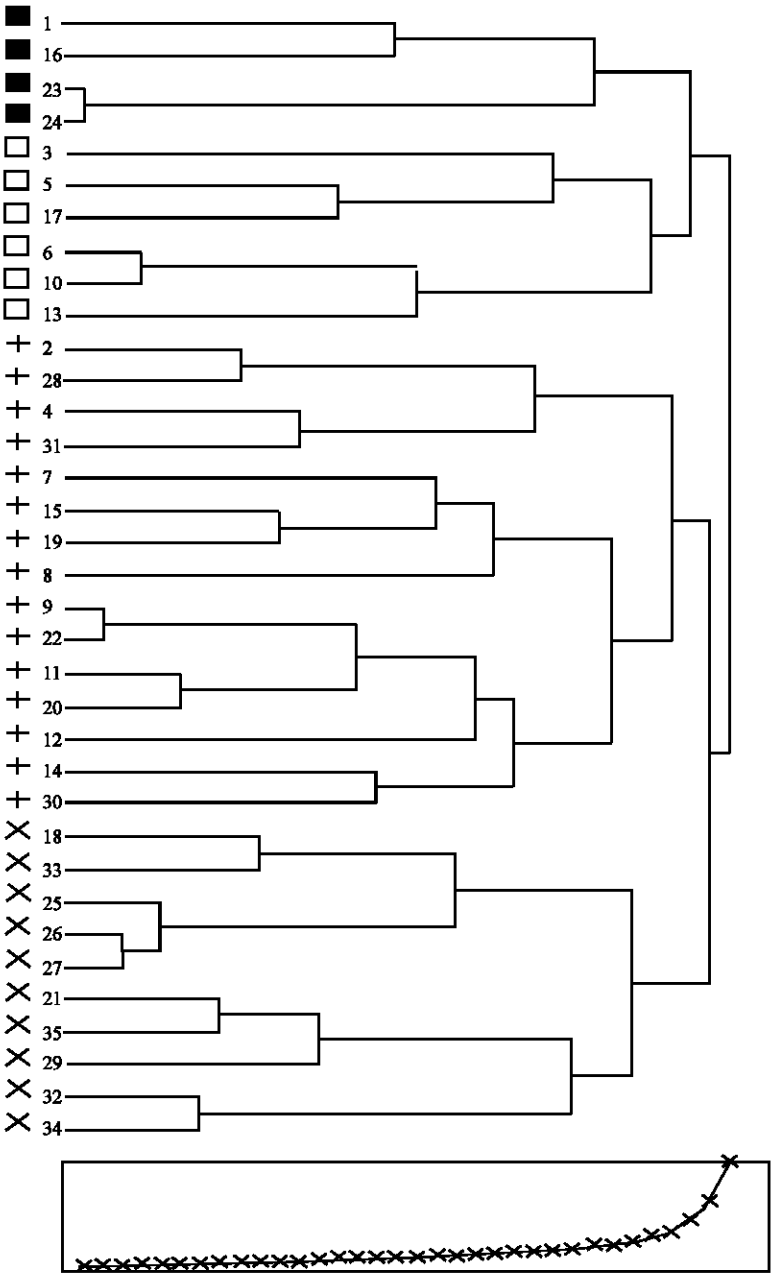

Fig. 2: Grouping of Sardari wheat lines using UPGMA algorithm based on molecular data

long arms (70%) were more than those of short arms (22.2%). Cluster analysis using molecular data assigned the 35 lines into 4 groups (Fig. 2).

Correspondence between morphological and molecular data: Stepwise multiple regression analysis for determining relationship between genomic regions and some traits, showed positive or negative significant relevance and revealed informative markers related to some morphologically important traits as following:

GWM 149 marker located on 4B chromosome with spiklet per spike, GWM 540 marker located on 5B chromosome with number of kernel per spike, GWM 369, GWM 459 and GWM 156 markers located respectively on 3B, 5A and 6A chromosomes with thousand grain weight, GWM 493 marker located on 3B chromosome with stem height, GWM 357 and GWM 55 markers located respectively on 1A and 2B chromosomes, with primary root length, GWM 369, GWM 164, GWM 540 and GWM 314 markers located respectively on 4A, 2A, 5B and 3D

Table 3: Informative marker for morphological traits in 'Sardari' wheat

Adjusted R^2	p-value	Regression coefficient	Chromosome	Marker	Trait
0.12	0.023	-1.56	4B	GWM 149	Spiklet in spike
0.19	0.005	-7.02	5B	GWM 540	Seed in spike
0.52	0.000	-9.84	3A	GWM 369	Weight of 1000 seed
	0.002	4.61	6A	GWM 459	
	0.023	3.24	5A	GWM 156	
0.17	0.009	9.67	3B	GWM 493	Stem height
0.30	0.002	2.21	1A	GWM 357	Primary root length
	0.015	-1.75	2B	GWM 55	
0.64	0.000	-0.63	3A	GWM 369	Yield
	0.001	0.31	6A	GWM 334	
	0.008	0.47	3D	GWM 314	
0.48	0.001	-1.40	4A	GWM 160	Seed protein (%)
	0.006	1.13	1A	GWM 164	
	0.024	1.09	5B	GWM 540	
	0.039	0.99	3D	GWM 314	

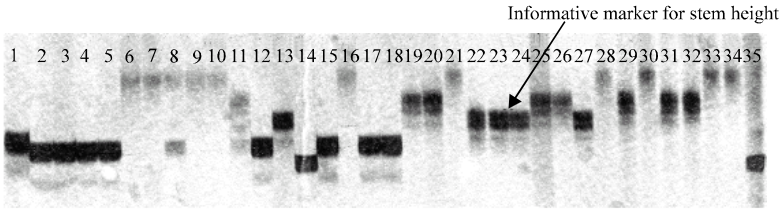

Fig. 3: SSR analysis (GWM 493) in 35 Sardari wheat lines

with yield (Table 3). An example of SSR analysis (GWM493) in 35 Sardari wheat morphotypes along with informative marker related to stem height represented in Fig. 3.

DISCUSSION

Microsatellite markers have been used to investigate genetic diversity of a large number of cultivars in rice (Yang *et al*., 1994), soybean (Rongwen *et al*., 1995), wheat (Plaschke *et al*., 1995) and maize (Senior *et al*., 1998). According to latest study, the number of alleles amplified per locus ranged from 2 to 23 for rice, 11 to 26 for soybean, 2 to 16 for wheat and 2 to 23 for maize. In the present study the level of microsatellite polymorphism and the number of allele per locus in Sardari landrace is much lower than the other crops. One possible reason is that the materials used in present investigation were all from one specific landrace restricted in drylands of the country, thus have a relatively narrow genetic bases. In a similar study conducted by Rongwen *et al.* (1995) on genetic diversity of soybean, 11 to 26 allele per microsatellite primer pair were amplified from 96 soybean genotypes, while this number was reduced to 5 to 10 alleles per locus in 26 cultivars collected from a restricted region of North America. Röder *et al.* (1995) studying 15 WMS in 12 breeding lines found an average of 3.2 alleles, while Bryan *et al.* (1997) in ten wheat varieties with 49 WMS found an average of 3.5 alleles per locus. The higher variation in Plaschke *et al.* (1995) material can be explained partly by the higher resolution power of their detection system using automated laser fluorescence.

We also found unremarkable null alleles in our investigation, while Stachel *et al.* (2000) reported a great number of null alleles at 16 loci especially in *wms*169 locus that occurred with maximum frequency. Null alleles for MS in wheat were previously described by Devos *et al.* (1995), Plaschke *et al.* (1995) and Donini *et al.* (1998), while alleles of MS are the result of change in the number of repeats, null alleles are the consequence of polymorphism in the primer binding site (Tautz *et al*., 1986; Stachel *et al*., 2000). Thus our results suggest that the lowest amount of change occurred in flanking regions of simple sequence repeat in our material. The complete lack of variation in 15 loci over the 35 lines shows that they may not be selectively neutral and possibly have functional role (Gupta *et al*., 1994).

Also in despite of the fact that, great efforts were devoted to select equal number of markers on A, B and D genome, the lowest number of allele and lowest amount of polymorphism observed in D genome. It is not surprising because Stachel *et al.* (2000) studied genetic diversity amongst 60 agro-ecologically different wheat cultivars using microsatellite markers and reported the lowest percentage of polymorphism in D genome. Although there was no complete concordance between grouping using morphological and molecular data (Fig. 1 and 2), in the most cases, the lines with similar characteristics were

grouped into the same clusters. Dillmann *et al.* (1997), Moser and Lee (1994) studied the coincidence between the results derived from molecular and morphological data. In most cases it has been proved that, when the molecular analysis shows a high genetic similarity value between two individuals, they necessarily have similar morphological values (Burstin and Charcosset, 1997). For instant lines number 23 and 24 with highest genetic similarity value, showed a very close morphological identity. In the other hand, great different of morphological characters was observed between lines number 1 and 34 with the lowest genetic similarity value. Consequently in this study, cluster analysis based on microsatellite data corresponded appropriately morphological characters in most cases. Several studies have compared the use of morphological and molecular markers to examine genetic relatedness and most of these showed that relationships between two methods were low (Semagn *et al.*, 2002; Kjar *et al.*, 2004; Martínez *et al.*, 2003; Vollmann *et al.*, 2005). Two reasons have been mentioned by Semagn *et al.* (2002) for these relationships (i) molecular markers cover a larger proportion of the genome, including coding and non-coding regions, than the morphological markers and (ii) molecular markers are not subjected to artificial selection compared to morohological markers.

In our investigation, significant associations between some traits and chromosomal regions revealed. The stepwise multiple regression analyses of the relationships between the seven morphological traits and the markers data (Table 3) showed that, A genome is important for yield components. Significant association between yield and the first allele was present for GWM334 (~114 bp) located on the long arm of chromosome 6A. Field trials showed that, all of the lines carry this allele have mean yield around 3 t/ha in dry lands. Blanco *et al.* (2002) reported a QTL (PsP 3071) for yield ($p< 0.01$) around the centromere of chromosome 6A in durum wheat and also Quarrie *et al.* (2003) reported a significant allele-trait association with yield, located on long arm of chromosome 6A. Further studies on allele associated with yield component revealed a significant effect ($p = 0.002$) of the first allele of Xgwm459 (~126 bp) situated on chromosome 6A, with thousand grain weigh (TGW). Field trials showed that, all of the wheat lines carry this allele, have an average TGW more than 50 g. In this respect Quarrie *et al.* (2003) reported significant association between PsP3071 allele and TGW ($p<0.001$). Negatively significant effect ($p = 0.000$) of Xgwm369 locus (~184 bp), located on chromosome 3A, on TGW and yield shows possible rule of A genome in productivity. Also Campbell *et al.* (2003) reported the positive significant association between Xtam55, located on chromosome 3A, with TGW in wheat recombinant inbred lines population, but in this study no significant association observed between TGW and 3A chromosome. The differences observed between our result and Campbell *et al.* (2003) may be cause of two essentially different investigated population. Negatively significant association ($p< 0.001$) observed between third allele of Xgwm493 (~179 bp) locus, located on chromosome 3B and stem height. The lines carried this allele showed the lowest stem height (68.5 cm) while lack of this allele caused the most stem height (about 100 cm). Significant association observed between some alleles on chromosomes 1A, 4A, 5B, 3D and grain protein ($p = 0.001$), while the most significant effect were detected with the first allele of Xgwm160 locus (~184 bp), located on chromosome 4A. Although there is no report regarding QTL related to grain protein percent on this chromosome, high negative effect of this allele on this trait were evidently clear in field trials.

The obtained results suggest that microsatellite markers are useful (I) for estimation of genetic diversity in closely related genotypes, specially for Sardari as an important tolerant wheat landrace and in future (II) for sampling strategies and identification of suitable parental lines in order to widen the gene pool of wheat germplasm. In conclusion, wide range of genetic similarity (0.34 to 0.88) offers a remarkable genetic variation among so called Sardari cultivar. Although DRI's breeder released Sardari as a pure cultivar three decade ago, but the recent high level of variation within this cultivar shows a remarkable variation occurred for the genome of this cultivar. These variations might be caused by stressful condition that Sardari is cultivated in. The huge effect of stress on genome structure and regulation in various plants has been widely discussed in a review by Madlung and Coma (2004). Other possible reasons for this differentiation might be: wrong selection, mechanical mixture and misdoing or unperformance of single spike selection.

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