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Assessing genetic diversity of promising wheat (*Triticum aestivum* L.) lines using microsatellite markers linked with salinity tolerance

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Abstract

Narrow genetic variability may lead to genetic vulnerability of field crops against biotic and abiotic stresses which can cause yield reduction. In this study a set of 37 wheat microsatellite markers linked with identified QTLs for salinity tolerance were used for the assessment of genetic diversity for salinity in 30 promising lines of hexaploid bread wheat (*Triticum aestivum* L.). A total of 438 alleles were detected with an average allele number of 11.84 per locus using 37 microsatellite markers. The number of alleles per locus ranged from two to twenty, the maximum number of alleles was observed at Xgwm312. Gene diversity statistic for 37 microsatellite loci was varied from 0.66 to 0.94 and also polymorphic information content value was varied from 0.64 to 0.93 for Xgwm445 and Xgwm312 respectively. Result showed Xgwm312 SSR marker with the highest PIC value was distinguished as the best marker for genetic diversity analysis to improve of salinity tolerance. Obtained dendrogram by UPGMA method categorized genotypes in to 3 different groups, which had different reaction to salinity. A wide range of genomic diversity was observed among all the genotypes. Principal Coordinates Analysis (PCoA) also confirmed this pattern of genetic diversity, proving them can be use as the prime candidates in order to improve of salinity tolerance in breeding programs of wheat. The present study also indicates that microsatellite markers permit the fast and high throughput fingerprinting of numbers of genotypes from a germplasm collection in order to assess genetic diversity.

Key words: Genetic diversity, Microsatellite markers, Promising wheat lines, Principal coordinates analysis (PCoA)

Introduction

Estimation of genetic variation level among accessions is prerequisite for germplasm conservation and breeding programs (Fufa *et al*., 2005). Knowledge of genetic diversity in a crop species is fundamental study for its improvement. Genetic studies using molecular markers in elite material of hexaploid bread wheat have been restricted both by the limited number of polymorphic markers and by the low level of variability within this self-pollinated species. A rich and diverse

germplasm collection is the backbone of every successful crop improvement program (Manjarrez-Sandoval *et al*., 1997). The development in molecular genetics in wheat has been relatively slow, especially when compared to other crops such as maize, rice or tomatoes; this is mainly because of wheat's ploidy level, the size and complexity of its genome (Gupta *et al*., 1999). Assessing genetic diversity within a narrow genetic pool of novel breeding germplasm could make crop improvement more efficient by the directed accumulation of desired alleles. This is likely to speed up the breeding process and decrease the amount of plant material that needs to be screened in such experiments (Astarini *et al*., 2004). Efficient and quick screening of such genotypes speedup the process of varietal evaluation, thus molecular marker plays pivotal role in this regard (Asif *et al*., 2005). Identification based on morphological characters is time consuming and requires extensive field trials and evaluation (Astarini *et al*., 2004), while morphological differences may be epigenetic or genetic based characters (Tahir, 2001; Migdadi *et al*., 2004). In barley and wheat, microsatellite markers have been used for analysis of genetic diversity and identification of indigenous landraces and modern cultivars (Khanjari *et al*., 2007; Wang *et al*., 2007). Microsatellites are one of the most promising molecular-marker types able to identify or differentiate genotypes within a species (Prasad *et al*., 2000). Genetic variation in hexaploid wheat has been reported based on restriction fragment length polymorphism (RFLP) (Vaccino *et al*., 1993), Random Amplified Polymorphic DNA (RAPD) (Dweikat *et al*., 1993), specific PCR primers for low copy sequences (Chen *et al*., 1994; Talbert *et al*., 1994), simple sequence repeats (SSRs) (Plaschke *et al*., 1995), and polyacrylamid gel electrophoresis (PAGE) of gliadins (Röder *et al*., 1995). SSR markers have been confirmed as an efficient tool for estimating genetic variation in wheat (Landjeva *et al*., 2006). Several authors reported that microsatellites are more variable than most of other molecular markers that are useful as tools for studying the genetic diversity of germplasm (Haile *et al*., 2012). Increases in salinity tolerance for the world's two staple crops, wheat and rice, are an important goal as the world's population is increasing more quickly than the area of agricultural land to support it (FAO, 2010). In bread wheat germplasm, salinity is considered a major factor in limiting plant growth and crop productivity (Rus *et al*., 2000). Several research have reported information on QTLs attributed to salinity tolerance, since present germplasm has high variation of salinity tolerance therefore the microsatellite markers linked with the identified QTLs for salinity tolerance were used to assess the genetic diversity of bread wheat lines.

Materials and Methods

Plant material and DNA extraction

Thirty diverse promising lines of bread wheat (Table 1) which had shown different reaction to salinity were used

for genetic diversity assessment. Experiment was carried out, at Shahid Bahonar university of Kerman (in the Southeast of Iran) in 2011. In order to screen the genotypes in saline conditions, under studied genetic materials had been grown in two field conditions of slain (10 dsm^{-1}) and normal (4 dsm^{-1}) in Yazd and Kerman. Scoring for salinity tolerance during screening with scores of 1 (no injury symptoms) and 9 (plants are dying or dead) showed 30 extreme genotypes among 100 genotypes (Sardouie-Nasab *et al*., 2013). Genomic DNA was extracted from fresh young leaf tissue according to Delaporta (Delaporta *et al*., 1983), and DNA was quantified by spectrophotometer, by absorbance at 260/280nm. And the quality of DNA was further checked on 0.2% agarose gel.

Table 1. Pedigree of lines.

Line No.	Salinity reaction	Pedigree
3	S	Cham4/Tam200//Del 483/3/Mirtos
4	S	Cham4/Tam200//Del 483/3/Mirtos
5	S	Alamoot*2/Kavir
9	S	Alvand*2//Opata*2/Wulp
11	S	Alvand*2/4/Kal/Bb//Cj"s"/3/Hork"s"
20	S	Passarinho//Vee/Nac
22	S	Guadalop/Falat
25	S	Marvdasht/Owl
26	S	TX62A4793/CB809/5/Gds/4/Anza/3/Pi/Nar//Hys/6/Passarinho/7/Alvand
35	S	MV17/Alvd//Chamran/3/Pishtaz
38	S	Mv17/Shiraz
39	S	DH2-390-1563 F3Gds/4/Anza/3/Pi//Hys/5/1-6/6/Tajan/ 7/ Milan/..
40	T	DH2-390-1563 F3Gds/4/Anza/3/Pi//Hys/5/1-6/6/Kauz*2/Opata//….
43	S	Kauz/Sorkhtokhm//Mahooti/ 3 /Bank"s"/Vee "s"
46	S	Bloudan/3/Bb/7C*2//Y50E/Kal*3/4/KRL.14
47	S	DH-line
48	T	Bloudan/3/Bb/7C*2//Y50E/Kal*3/4/Sholeh
49	T	Azd//Tob/Chb/3/Emu"s"/Tjb84/4/Bloudan/3/Bb/7c*2//Y50E/Kal*3
51	T	Alamoot//Opata*2/Wulp
55	T	1-72-92/Col.No.3617//Owl
58	T	Alvd//Aldan/Ias 58/3/1-60-3/5/Kal/Bb//Cj "s"/3/Hork"s"/4/Alvd//Aldan/Ias58
59	T	DH2-390-1563 F3Gds/4/Anza/3/Pi//Hys/5/1-6/6/Tajan/ 7/ Milan/..
67	T	Kauz/Sorkhtokhm//Mahooti/3 /DH-209-1557 F3,Vee "s"/Nac//1-66-22
73	T	DH-line
75	T	DH-line
83	T	Cereal recearch collection , Accession no :2695
87	T	Cereal recearch collection , Accession no :2776
89	T	Cereal recearch collection , Accession no :2812
94	T	Cereal recearch collection , Accession no :2970
97	T	Cereal recearch collection , Accession no :3102

S: Sensitive; T: Tolerant

SSR Assays: Thirty- seven polymorphic SSR markers were used for genetic diversity analysis (Table 2). SSR primers were obtained based on wheat physical maps (hattp://wheat.pw.usda.gov). PCR reactions were carried out in 10 l reaction mixture containing 50ng/ l of genomic DNA, 0.25 l of 50mM MgCl2, 0.25 l of 10mM dNTPs, 1U of Taq polymerase (0.25 from 5U/ l) and 0.5 l of 5 mM primer in 1x reaction buffer. The amplification reactions were performed in the Eppendorf Master cycler with an initial denaturation for 3 minute at 94^{oC}, then 35 cycles: 1 minute denaturation at 94^{oC}; 1 minute annealing at 55^{oC}; 2 minute extension at 72^{oC}. Final extension was carried out at 72^{oC} for 5 minutes. The PCR products were electrophoresed on 8% polyacrylamid gels containing 1x TBE (Tris Borate EDTA) for 2 hours.

Data analysis

For each locus, SSR allelic composition was determined in the genotypes. SSRs data were scored by AlphaEaseFC4 software. A total of 37 informative SSR markers were scored with high quality. The program Power Marker version 3.25 (Anderson *et al*., 1993) was used to calculate allele frequencies, alleles per locus and observed heterozygosity for each locus. Polymorphic information content (PIC) values which indicating the ability to distinguish between genotypes for each primer combination, was calculated as expected heterozygosity for polymorphic bands with the following formula (Nei *et al*., 1983) using Power Marker version 3.25 (Anderson *et al*., 1993).

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} p_{ij}^2$$

Where n is the total number of alleles detected for a locus of a marker and P_{ij} is the frequency of the jth allele for marker i, and summation extends over n alleles. Cluster analysis was performed according to the unweighted pair-group method with arithmetic average (UPGMA) with the Nei (Roussel *et al*., 2004) similarity index using Power Marker version 3.25 (Anderson *et al*., 1993). Principal coordinates analysis (PCoA) was performed on the matrix of Dice similarity coefficients (Dice 1945) using the modules DCENTER and EIGEN of the NTSYS-PC ver. 2.1 (Rohlf 2000) and the 2D plot was done using the MOD2D module of the program.

Results

Thirty- seven SSR markers that are linked with QTL identified for salinity tolerance were used to assess the genetic diversity of thirty wheat genotypes. Result showed a total of 438 alleles with an average of 11. 84 alleles per locus over 37 SSR markers (Table 2). Roussel *et al* (2004) reported a total of 609 alleles with 41 microsatellite markers and Huang *et al* (2002) reported a total of 470 alleles with 24 SSRs. The average number of alleles per locus and mean gene diversity were 11.84 and 0.84, respectively. The average number of alleles per locus (7; range, 3-14) obtained in this study was less than the 18.1 alleles reported by Huang (Huang *et al*., 2002). Zhan *et al*

(2002) obtained a total of 501 alleles with 90 SSRs in 43 wheat varieties. The number of alleles per locus ranged from two to twenty, the maximum number of alleles was observed at Xgwm312 (Table 2).

Table 2. Polymorphic information content (PIC), genome location, amplified alleles, and primer designation of 37 microsatellites.

Marker	Genome location	Major Allele Frequency	Allele NO.	Gene diversity	PIC	Size band range(bp)
xgwm10	2A	0.30	11	0.85	0.83	124-198
xcfa2043a	2A	0.20	13	0.89	0.88	176-243
xgwm445	2A	0.57	11	0.66	0.64	189-224
xbarc353.2	2A	0.33	13	0.84	0.83	183-233
xwmc261	2A	0.30	7	0.81	0.78	90-116
xcfa2058	2A	0.33	11	0.82	0.80	173-219
xgpw2206	2A	0.17	16	0.91	0.90	211-309
xwmc109d	2A	0.27	13	0.86	0.85	177-208
xgwm47.2	2A	0.27	19	0.90	0.89	101-167
xgwm294b	2A	0.23	11	0.85	0.84	127-202
xcfa2121b	2A	0.37	15	0.83	0.82	163-231
xgwm372	2A	0.33	7	0.75	0.71	102-124
xgwm339	2A	0.17	13	0.90	0.89	156-180
xgwm515	2A	0.30	7	0.80	0.77	127-148
xwmc296	2A	0.20	14	0.88	0.87	144-171
xgwm95	2A	0.27	14	0.88	0.87	105-142
xgwm249	2A	0.33	11	0.80	0.77	158-190
xgwm328	2A	0.40	7	0.77	0.75	167-193
xwmc17o	2A	0.23	14	0.89	0.88	215-236
xgwm312	2A	0.13	20	0.94	0.93	178-243
xwmc11	3A	0.30	15	0.86	0.84	143-185
xgwm674	3A	0.13	14	0.91	0.90	138-173
xgwm108	3B	0.23	14	0.89	0.88	130-174
xwmc326	3B	0.37	9	0.79	0.77	168-200
xwmc291	3B	0.33	11	0.81	0.79	216-246
xcfa2170	3B	0.20	13	0.90	0.89	98-150
xbarc84	3B	0.33	6	0.77	0.74	98-107
xbarc206	3B	0.23	13	0.88	0.87	201-269
xwmc687	3B	0.47	11	0.74	0.72	191-229
xwmc206	3B	0.37	13	0.81	0.80	190-254
xbarc48.4	4D	0.20	11	0.88	0.87	139-204
xgwm194	4D	0.23	10	0.86	0.84	124-157
xgwm609	4D	0.50	9	0.71	0.69	220-237
xgpw345	4D	0.20	8	0.85	0.84	185-215
xgwm624	4D	0.30	9	0.83	0.81	133-156
xbarc196	6D	0.33	11	0.82	0.80	162-195
xwmc416	6D	0.27	14	0.88	0.87	193-237
Mean		0.29	11.84	0.84	0.83	

The polymorphic information content value was varied from 0.64 to 0.93 for Xgwm445 and Xgwm312 respectively. The smallest size of amplified fragments by all the primers was varied between 90-309 bp. The lowest size was belonged to Xwmc261 (90bp) and the biggest was 309 bp for Xgpw2206. (Table 2). Gene diversity statistic for 37 microsatellite loci was varied from 0.66 to 0.94 for Xgwm445 and Xgwm312 respectively. PCoA showed that the first ten eigenvalues explained 44.25% of the cumulative variation (Table 3), which were then plotted to identify the diversity of the genotypes (Figure. 1). The dendrogram constructed on the basis of the similarity matrix showed that the wheat varieties were divided into three groups (Figure. 2). The 1st cluster contained line No. 25 while Lines No. 3, 4, 9, 11, 22, 26, 35, 47, 51 and 73 were grouped in the 2nd cluster. Other lines were grouped in the 3nd cluster. These three major cluster further partitioned in different sub-clusters. Genotypes that classified in same group were genetically close to each other. Three groups had different salinity tolerance.

Table 3. Eigenvalue, proportion and cumulative variance of extract factors.

Cumulative	Proportion	Eigenvalue
10.55	10.55	3.16
14.86	4.31	1.29
19	4.13	1.24
22.93	3.93	1.18
26.7	3.76	1.13
30.31	3.61	1.08
33.92	3.6	1.08
37.41	3.49	1.04
40.88	3.47	1.04
44.25	3.36	1.01

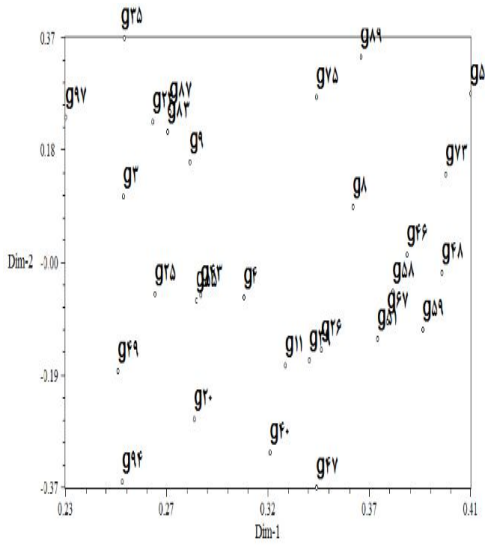

Figure 1. Scattergram of principal coordinates (PCo) based on 37 wheat SSRs.

Discussion

Diversity analysis is important for deciphering genetic relationships including parentage and for the efficient management of germplasm and thereby, use in breeding of improved varieties (Al-Doss *et al*., 2011). Pyramiding crosses are suggested to increase the genetic diversity in the population (Siedler *et al*., 1994) and will be helpful in developing improved wheat cultivars. In the present study, we compared the genetic diversity of bread wheat lines using markers linked with QTL for salinity tolerance. Study of genetic diversity showed a high percentage of polymorphic loci and considerable genetic diversity among lines in the present study. Previous studies have shown that SSRs are highly polymorphic in wheat (Manifesto *et al*., 2001; Zhang *et al*., 2002). According to Bohn *et al* (1999) RFLP analysis revealed 4.7 polymorphisms per probe/enzyme combination among 81

European cultivars, whereas RAPDs primers generated only 1.8 polymorphisms/primer among 15 wheat cultivars. Our results indicate that microsatellite markers are much more informative than RFLPs and RAPDs in wheat. The PIC value refers to the relative value of each marker with respect to the amount of polymorphism exhibited (Iqbal *et al*., 1997). In this research Xgwm312 SSR marker with the highest PIC value at this research was distinguished as the best marker for genetic diversity analysis (Table 2).

To better understand the relationships among these genotypes, in the present study, PCoA was carried out using the genetic similarities data set. Principal coordinate analysis showed that the first ten eigenvalues explained 44.25% of the cumulative variation, which were then plotted to identify the diversity of the genotypes, the low amount of cumulative variance can be due to dispersion of SSR markers in Genome A, B and D (Figure. 1). It demonstrated the genetic relationship between the lines' that had also been observed through dendrogram analysis (Figure. 2). A wide range of genetic diversity among all genotypes was observed. These findings clearly demonstrate the reliability, usefulness, and efficiency of SSRs in analyzing genomic diversity. Thus, it should be possible to establish a collection of highly polymorphic SSRs for genetic diversity studies, genomic diversity estimates a potentially valuable predicting source for selecting diverse parent genotypes for favorable heterotic combinations in a wheat improvement program that aims to broaden the genetic basis and progeny performance for complex traits such as yield or partial disease resistance (Bohn *et al*., 1999). Based on this study, markers linked with major QTLs of salinity tolerance will also allow estimating genetic similarity between different genotypes.

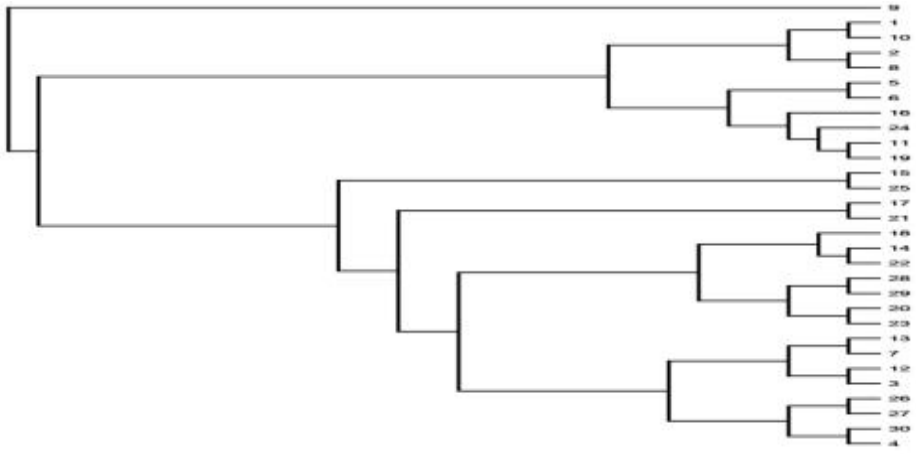

Figure 2. Dendrogram of 30 wheat genotypes showing the genetic similarity based on 438 alleles detected by 37 SSRs using Jaccard's coefficient and UPGMA cluster analysis.

The information about genetic similarity will be helpful to avoid any possibility of elite germplasm becoming genetically uniform. Efficiency and speed of plant breeding programs can be accelerated by (MAS) and permit persistent progress in the advancement of selected material. The information gathered here would be helpful in genomic mapping studies and for the development of wheat cultivars with wider and diverse genetic background to obtain improved crop productivity.

Conclusion

In summary, our data showed significant variation in microsatellite DNA polymorphisms among wheat varieties. This study by using wheat microsatellite markers revealed considerable amount of genetic diversity among thirty wheat varieties that can be used in selecting diverse parents in breeding program and in maintaining genetic variation in the germplasm. Obtained dendrogram by UPGMA method categorized lines in to 3 different groups, that had different reaction to salinity, since our goal was, select for salt tolerance, therefore these lines may have different genetic mechanism of salinity tolerance and it can be one promising approach for understanding of plant salt tolerance mechanisms during the course of breeding for salinity tolerance and it showed there are known some information markers in the study for salinity tolerance.

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ارزیابی تنوع ژنتیکی لاین‌های امید بخش گندم (.Triticum aestivum L) با استفاده از نشانگرهای ریزماهواره مرتبط با تحمل به شوری

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چکیده

کاهش تنوع ژنتیکی منجر به آسیب‌پذیری ژنتیکی گیاهان زراعی در برابر تنش‌های زیستی و غیر زیستی و در نتیجه منجر به کاهش عملکرد می‌شود. در این مطالعه جهت ارزیابی تنوع ژنتیکی ۳۰ لاین امید بخش گندم هگزاپلوئید (Triticum aestivum L) از ۳۷ نشانگر ریزماهواره مرتبط با QTLهای شناسایی شده تحمل به شوری استفاده گردید. ۴۳۸ آلل با میانگین تعداد ۱۱/۸۴ آلل در هر لوکوس با استفاده از ۳۷ نشانگر ریزماهواره آشکار شدند. تعداد آلل برای هر مکان ژنی در محدوده ۲ تا ۲۰ بود که بیشترین تعداد آلل متعلق به جایگاه Xgwm312 بود. آماره تنوع ژنتیکی برای ۳۷ نشانگر ریزماهواره از ۰/۶۶ تا ۰/۹۴ متفاوت بود، همچنین محتوی اطلاعات چندشکلی از ۰/۶۴ تا ۰/۹۳ به ترتیب برای نشانگرهای Xgwm445 و Xgwm312 متفاوت بود. نتایج نشان داد نشانگر Xgwm312 با داشتن بیشترین محتوی اطلاعات چند شکلی به عنوان بهترین نشانگر برای آنالیز تنوع ژنتیکی جهت بهبود تحمل به شوری شناسایی گردید. دندروگرام بدست آمده با استفاده از روش UPGMA ژنوتیپ‌ها را به سه گروه متفاوت گروه‌بندی کرد که واکنش متفاوتی به شوری داشتند. تجزیه به مولفه‌های اصلی نیز این الگوی تنوع ژنتیکی را تایید کرد و نشان داد که ژنوتیپ‌های مورد بررسی می‌توانند به عنوان کاندیدهای خوبی برای بهبود تحمل به شوری در برنامه های اصلاحی گندم مورد استفاده قرار گیرند. مطالعه حاضر همچنین نشان داد که نشانگرهای ریزماهواره پتانسیل بالایی در انگشت‌نگاری تعداد زیادی ژنوتیپ به منظور ارزیابی تنوع ژنتیکی دارند.

کلمات کلیدی: تجزیه به مولفه‌های اصلی (PCoA)، تنوع ژنتیکی، لاین‌های امید بخش، نشانگرهای ریزماهواره.