J. Agr. Sci. Tech. (2011) Vol. 13: 905-920

Comparative Assessment of SSAP, AFLP and SSR Markers for Evaluation of Genetic Diversity of Durum Wheat (*Triticum turgidum* L. *var. durum*)

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ABSTRACT

Comparative assessment of genetic diversity of 122 durum wheat genotypes (*Triticum turgidum* L. *var. durum*) was performed using 73 SSAP polymorphic fragments, 123 AFLP polymorphic loci and 104 SSR alleles. SSAP and AFLP data showed a clear demarcation between the cultivars and landraces and SSR data classified cultivars and landraces according to their origin. Furthermore, the estimated genetic diversity of Iranian landraces was higher compared to the foreign entries and a loss of genetic diversity was observed from landraces to cultivars. This study determined that differences in genetic relationships revealed by SSAP, AFLP and SSR distances could not be attributed solely to differences in the level of polymorphism detected by each marker system. The molecular evidence of genetic diversity decrease of the durum wheat gene pool further strengthens the strategic relevance of undertaking appropriate genetic conservation measures for food security.

Keywords: AFLP, Durum, Genetic diversity, SSAP, SSR

INTRODUCTION

Durum wheat (*Triticum turgidum* L. *var. durum*) is an important small-grain cereal, mainly used for human consumption. Recently, this crop has been the subject of renewed interest because of its valuable production and adaptation to low-rainfall, semi-arid environments, and its unique products (Martos *et al.,* 2005). Understanding the extent and nature of genetic variation in crop species has important implications for crop improvement and conservation of plant genetic resources (Soleimani *et al.,* 2002). For many crop plants, continuous cycles of controlled breeding over thousands of years have led to narrowing their genetic basis (Tanksley and McCouch, 1997). Many molecular breeding studies have demonstrated the value of alleles originating from non-cultivar germplasm, which shows that selective breeding has discarded useful alleles in addition to the many useless ones. The challenge for future molecular breeding is to identify these useful alleles and to incorporate them again into cultivated material (Soleimani *et al.,* 2002, Dashti *et al*., 2010, Naghvi *et al*., 2010, Naghvi *et al*., 2009).

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[DOR: 20.1001.1.16807073.2011.13.6.3.6]

Different PCR-based molecular marker techniques, such as amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR), have been used to estimate genetic diversity and relationships among durum wheat accessions (Dograr *et al.,* 2000, Manifesto *et al.,* 2001, Soleimani *et al.,* 2002, Maccaferri *et al.,* 2003, Kudryavtsev *et al.,* 2004, Martos *et al.,* 2005, Perry, 2004, Medini *et al.,* 2005, Figliuolo *et al.,* 2007, Maccaferri *et al.,* 2007). The sequence-specific amplification polymorphism (SSAP) approach (Waugh *et al.,* 1997), also known as transposon display (Casa *et al.,* 2000), is one of the most popular PCR approaches derived from AFLP (Vos *et al.,* 1995). SSAP amplifies the region between a transposon insertion [specificity with an oligonucleotide primer anchored usually on the long terminal repeat (LTR) of the retrotransposon and an adjacent restriction site], and can provide particularly useful markers for the analysis of genetic diversity (Waugh *et al.,* 1997, Ellis *et al.,* 1998, Gribbon *et al.,* 1999, Porceddu *et al.,* 2002, Queen *et al.,* 2004, Tam *et al.,* 2005, Sanz *et al.,* 2007) The transposon insertions are irreversible and they are stable over millions of year (Schulman *et al.,* 2004, Vitte *et al.,* 2004, Jing *et al.,* 2005). The purposes of the present study was the comparative assessment of SSAP, AFLP and SSR markers for the evaluation of genetic diversity and conservation of durum wheat (*Triticum turgidum* L. *var. durum*).

MATERIALS AND METHODS

Plant Material and Genomic DNA Extraction

A total of 122 durum wheat (*Triticum turgidum* L. var. *durum*) genotypes, including 92 Iranian varieties (56 landraces from five local populations (province), 23 traditional cultivars from three local populations and 13 Iranian modern cultivars) and 30 international varieties (11 traditional cultivars and 19 modern cultivars), were used (Table 1). Total genomic DNA was extracted based on the method described by Saghai Maroof et al. (1984).

Molecular Analysis

SSAP analysis was performed using the Ty1-copia group of retrotransposon marker systems based on *BARE*-1, *Thv19* (Waugh *et al.,* 1997, Gribbon *et al.,* 1999), *Tar1* and *Tagermina* (Queen *et al.,* 2004) described previously (modified method of Matsuoka and Tsunewaki, 1997). One microgram of genomic DNA was digested with restriction enzymes *Eco*RI and *Mse*I, and double-stranded adaptors were ligated to the fragment ends. This was followed by a pre-amplification step using non selective primers. Ten selective amplifications were performed with primer pairs that contained two or three selective nucleotides on *Mse*I adaptor primers (Table 2). The AFLP analysis was performed based on the method described by Vos et al. (1995), using the restriction enzyme combination of *Eco*RI and *Mse*I. Eighteen *Eco*RI+NNN/*Mse*I+NNN primer combinations, with three selective nucleotides on the 3′ end of either primer, were used for the selective PCR amplification (Table 3). Nineteen SSR primer pairs were genotyped according to Röder et al. (1998) (Table 4). PCRs were performed on a Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The amplification reaction products were separated on a 5% denaturing polyacrylamide gel, using a Sequi-Gen GT Sequencing Cell 50 cm gel apparatus (Bio-Rad Laboratories Inc.). The resulting images were scored manually. Allele sizes were determined by using molecular mass marker VIII of Roche Molecular Biochemical, USA.

Data Analysis

Amplification reactions from all individuals were scored, and the following statistics of genetic variation within different

[DOR: 20.1001.1.16807073.2011.13.6.3.6]

Table 1. Grouping of durum wheat genotypes (landraces, traditional and modern cultivars) used in the present study.

Landrace (L)			Traditional cultivar (TC)			Modern cultivar (MC)		
Pop. Code	Location	Accessions No./Name	Pop. Code	Location	Accessions No./Name	Pop. Code	Location	Accessions No./Name
L-Az1	Azarbayejan	969	TC-Az1	Azarbayjan	142	MC-F-Algeria	Algeria	PG-RCID-961-Algeria – 76
L-Az2	Azarbayejan	960	TC-Az2	Azarbayjan	141	MC-F-Egypt1	Egypt	Dagahliga
L-Az3	Azarbayejan	440209	TC-Az3	Azarbayjan	140	MC-F-Egypt2	Egypt	Dagahliga
L-Az4	Azarbayejan	461181	TC-Az4	Azarbayjan	172	MC-F-Hungary1	Hungary	PKGST-25
L-Az5	Azarbayejan	460997	TC-Az5	Azarbayjan	133	MC-F-Hungary2	Hungary	TBVD-9
L-Az6	Azarbayejan	3649	TC-Az6	Azarbayjan	233	MC-F-Hungary3	Hungary	1KMVD-51-1266
L-Il1	Ilam	1252450	TC-Az7	Azarbayjan	220	MC-F-Hungary4	Hungary	MBVD-06
L-Il2	Ilam	1489186	TC-Ham1	Hamedan	458	MC-F-Hungary5	Hungary	DKGST-16
L-Il3	Ilam	370	TC-Ham2	Hamedan	464	MC-F-Hungary6	Hungary	MBVD-21
L-Il4	Ilam	1417782	TC-Ham3	Hamedan	478	MC-F-Hungary7	Hungary	ND-58-1934
L-Il5	Ilam	4-2237 (2103)	TC-Ham4	Hamedan	473	MC-F-India	India	Indian - 35
L-Il6	Ilam	3-2237 (2102)	TC-Ham5	Hamedan	465	MC-F-Lybia	Libya	DGR-(ICD-96)-Libia-301
L-Il7	Ilam	3-376 (2091)	TC-Ham6	Hamedan	471	MC-F-Mexico	Mexico	Don Pedro 87
L-Ker1	Kermanshah	1783	TC-Ham7	Hamedan	454	MC-F-Morocco	Morocco	DGR-(ICD-96)-Moroco-32
L-Ker2	Kermanshah	1780	TC-Ham8	Hamedan	457	MC-F-Russia1	Russia	Co-Russia-138
L-Ker3	Kermanshah	1759	TC-Ker1	Kermanshah	434	MC-F-Russia2	Russia	Co-Russia – 13B
L-Ker4	Kermanshah	1793	TC-Ker2	Kermanshah	413	MC-F-Syria1	Syria	Omrabis
L-Ker5	Kermanshah	1794	TC-Ker3	Kermanshah	337	MC-F-Syria2	Syria	Blikh 2
L-Ker6	Kermanshah	1791	TC-Ker4	Kermanshah	409	MC-F-Turkey	Turkey	ND-S7-1425
L-Ker7	Kermanshah	1547625	TC-Ker5	Kermanshah	115	MC-IGB1	Iran	45305
L-Ker8	Kermanshah	1547566	TC-Ker6	Kermanshah	108	MC-IGB2	Iran	45307
L-Ker9	Kermanshah	1597997	TC-Ker7	Kermanshah	193	MC-IGB3	Iran	45127
L-Ker10	Kermanshah	1643318	TC-Ker8	Kermanshah	1-351	MC-IGB4	Iran	D-76-7
L-Ker11	Kermanshah	6-370	TC-F-Mexico1	Mexico	A47	MC-IGB5	Iran	Co- t 9 998
L-Ker12	Kermanshah	8-6138 (1842)	TC-F-Mexico2	Mexico	A 52	MC-IGB6	Iran	A48
L-Ker13	Kermanshah	1175	TC-Foreign1	Foreign	D-76-2	MC-IGB7	Iran	428
L-Khuz1	Khuzestan	2458	TC-Foreign2	Foreign	D-76-2D	MC-IGB8	Iran	73
L-Khuz2	Khuzestan	2449	TC-Foreign3	Foreign	D-76-17	MC-IGB9	Iran	495
L-Khuz3	Khuzestan	2466	TC-Foreign4	Foreign	D-76-18	MC-IGB10	Iran	236
L-Khuz4	Khuzestan	2461	TC-Foreign5	Foreign	D-76-g	MC-IGB11	Iran	219

Table 1 continued

Table 1. Continued.

Landrace (L)			Traditional cultivar (TC)			Modern cultivar (MC)		
Pop. Code	Location	Accessions No./Name	Pop. Code	Location	Accessions No./Name	Pop. Code	Location	Accessions No./Name
L-Khuz5	Khuzestan	2457	TC-Foreign6	Foreign	D-76-3	MC-IGB12	Iran	438
L-Khuz6	Khuzestan	2453	TC-Foreign7	Foreign	D-76-9	MC-IGB13	Iran	502
L-Khuz7	Khuzestan	2468	TC-Foreign8	Foreign	D-76-3			
L-Khuz8	Khuzestan	2479	TC-Foreign9	Foreign	D-76-1			
L-Khuz9	Khuzestan	2478						
L-Khuz10	Khuzestan	2504						
L-Khuz11	Khuzestan	2500						
L-Khuz12	Khuzestan	2505						
L-Khuz13	Khuzestan	2509						
L-Khuz14	Khuzestan	2536						
L-Khuz15	Khuzestan	2530						
L-Khuz16	Khuzestan	2533						
L-Khuz17	Khuzestan	2524						
L-Khuz18	Khuzestan	2525						
L-Khuz19	Khuzestan	2526						
L-Khuz20	Khuzestan	2534						
L-Lor1	Lorestan	2249						
L-Lor2	Lorestan	2242						
L-Lor3	Lorestan	2228						
L-Lor4	Lorestan	2232						
L-Lor5	Lorestan	2241						
L-Lor6	Lorestan	2254						
L-Lor7	Lorestan	2200						
L-Lor8	Lorestan	2285						
L-Lor9	Lorestan	2271						
L-Lor10	Lorestan	2280						
L-Khuz5	Khuzestan	2457	TC-Foreign6	Foreign	D-76-3	MC-IGB12	Iran	438

groups of durum wheat genotypes (landraces, traditional and modern cultivars) were computed as binary data, using the GENAlEX 6 software (Peakal and Smouse, 2006): number of observed bands, mean number of bands per unit assay, polymorphism percentage and mean average expected heterozygosity (*He*) were computed according to Nei (1978). Genetic distances were estimated according to Nei (1978) and the resulting similarity matrix was subjected to principal coordinate analysis (PCoA). Mantel's test (1967) was used to assess the correlation between the calculated distance matrices and the test statistic tested for significance against 999 random permutations. Pairwise genetic similarity was estimated using SIMJACARD of the software package NTSYS-pc: 2.11 (Rohlf, 2004). The similarity matrix was used to construct the dendrogram for all 122 accessions using SHAN of NTSYS-pc based on unweighted pair group method with arithmetic mean (UPGMA).

RESULTS

Levels of Polymorphism

10 SSAP primer combinations yielded 73 polymorphic bands. The number of polymorphic fragments ranged from three to 11, with an average of 7.3 per primer combination (Table 2). Eighteen AFLP primer combinations revealed 123 polymorphic amplified DNA fragments. The number of polymorphic fragments ranged from two to 12, with an average of 6.8 per primer combination (Table 3). A total of 104 fragments were obtained from the 19 SSR primer pairs. The number of alleles per locus ranged from two (*Xgwm508*) to 10 (*Xgwm540 and Xgwm132*), with an average of 5.5 alleles per locus (Table 4). For considering Iranian durum wheat landraces, traditional and modern cultivars, the average numbers of AFLP polymorphic bands were 122, 103 and 99, respectively (Table 5). The average number of bands per assay for the Iranian traditional cultivars was higher than those for the international datasets, whereas Iranian modern cultivars, in comparison

Table 2. SSAP markers number, selective primer sequence and number of polymorphic fragments.

Marker No.	Selective primer sequence [a]	No. of Polymorphic fragments
1	Thv19/M-ACA	10
2	Tagermina/M-ACA	11
3	Thv19/M-CAT	12
4	Tar1/M-CG	8
5	Tar1/M-ACA	6
6	BARE-1/M-CAT	6
7	BARE-1/M-ACA	8
8	BARE-1/M-CG	5
9	Tagermina/M-CAT	9
10	Tar1/M-CAT	6
Mean	-	7.3

[a] M, *Mse*I adaptor, *Thv19*, *Tar1*, *Tagermina* and *BARE-1/ Wis-2-1A* adaptors.

Table 3. AFLP markers number, selective primer sequence and number of polymorphic fragments.

Marker No.	Selective primer sequence [a]	No. of Polymorphic fragments
1	M-CCC/E-AGG	10
2	M-CAA/E-ACT	11
3	M-CAA/E-AGG	12
4	M-GAG/E-ACT	8
5	M-CAA/E-CTG	6
6	M-CCC/E-CTG	6
7	M-GAG/E-AAC	8
8	M-GAG/E-GTC	5
9	M-GAG/E-AGG	9
10	M-CAT/E-AAC	6
11	M-CAT/E-GTC	4
12	M-CCC/E-ACT	5
13	M-CAA/E-GTC	5
14	M-CAT/E-AGG	12
15	M-CCC/E-AAC	2
16	M-CAT/E-ACT	3
17	M-CCC/E-GTC	7
18	M-CAA/E-AAC	4
Mean	-	6.8

[a] M, *Mse*I adaptor, E, *Eco*RI adaptor.

[DOR: 20.1001.1.16807073.2011.13.6.3.6]

Table 4. SSR locus, chromosomal location and number of polymorphic fragments**.**

Marker No.	SSR locus	Location	No. of Polymorphic fragments
1	GWM164	1A	5
2	GWM249	2A	4
3	GWM160	4A	4
4	GWM304	5A	4
5	GWM639	5A, 5B	6
6	GWM169	6A	5
7	GWM334	6A	6
8	GWM427	6A	5
9	GWM459	6A	7
10	GWM573	7B, 7A	6
11	GWM274	1B, 7B	5
12	GWM148	2B	6
13	GWM340	1B	5
14	GWM389	3B	4
15	GWM493	3B	6
16	GWM251	4B	4
17	GWM540	5B	10
18	GWM132	6B	10
19	GWM508	6B	2
Mean	-	-	5.5

with the international ones, showed a lower average number of bands per assay. Overall, Iranian germplasm showed a higher number of polymorphic bands compared to the international entries (Table 5).

Genetic Diversity

Across all analyzed datasets of durum wheat (landraces, and traditional and modern cultivars), the mean values of the genetic variation (*He*) indicated a considerable amount of genetic variation within each category. Furthermore, *He* values were different among the three marker systems (Table 5). The SSRs showed twofold higher *He* values than SSAP and AFLP. The highest percentages of polymorphic bands per primer set were observed for SSAP, being higher than those for AFLP and SSR.

For the SSR assays, the *He* values were different when Iranian populations were considered separately, ranging from 0.36 to 0.62, whereas the SSAP and AFLP assays generated close *He* values for the same entries, which indicated suitability of SSRs for population diversity studies (Table 5). A comparison of the improved cultivars (traditional and modern) versus the landraces based on SSAP, revealed a slightly lower diversity in the former set. The Iranian landraces had the mean *He* of 0.28 and an average of 7.2 alleles per locus, while the Iranian improved cultivars (traditional and modern) had the mean *He* of 0.27 and 0.26 and an average of 7.1 and 6.7 alleles per locus, respectively. The difference between the Iranian landraces and the improved cultivars (traditional and modern) was more pronounced using AFLP and SSR. Iranian landraces had the mean *He* of 0.33 whereas the improved cultivars (traditional and modern) had the mean *He* of 0.29 and 0.27, respectively, when using AFLPs. The same trend was also observed with the SSRs, with the mean *He* value for Iranian landraces (0.62) being higher than that for traditional (0.59) and modern cultivars (0.57). Interestingly, *He* value originated from SSRs was slightly higher in the Iranian traditional cultivars compared to foreign cultivars, whereas, based on SSAP and AFLP, this *He* value was slightly lower for Iranian modern cultivars.

Genetic Differentiation

PCoA using SSAP data showed that, on the basis of the first principal coordinate, which accounted for 28.8% of the total variation, the traditional cultivars were clearly separated from modern cultivars and the landraces. The latter were separated from the modern cultivars across the second principal coordinate, which explained 23.9% of the total variation, with the exception of the Ilam population. Based on AFLP data, the first principal coordinate, which accounted for 30% of the total variation, separated all cultivars from landraces, with the same exception of the Ilam population. The former was subsequently separated clearly based on the second principal

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Table 5. Genetic variation values for SSAP, AFLP and SSR durum wheat datasets.

Marker	Population	Landraces						Traditional cultivars					Modern cultivars	
		Azarbayejan	Ilam	Kermanshah	Khuzestan	Lorestan	Iran	Azarbayejan	Hamedan	Kermanshah	Iran	Foreign	Iran	Foreign
SSAP	Total No. of observed bands	53	60	67	67	64	72	54	63	65	71	63	67	70
	Mean No. of bands per unit assay	5.3	6	6.7	6.7	6.4	7.2	5.4	6.3	6.5	7.1	6.3	6.7	7
	Percentage of polymorphic bands	59.5	66.2	86.5	86.5	83.8	96	63.5	74.3	82.4	93.2	75.7	83.8	93.2
	Mean average of expected heterozygosity (*He*)	0.22	0.22	0.25	0.26	0.26	0.28	0.22	0.23	0.25	0.27	0.25	0.26	0.27
	SE of Mean *He*	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02
AFLP	Total No. of observed bands	100	95	115	117	108	122	96	80	90	103	91	99	108
	Mean No. of bands per unit assay	5.6	5.3	6.4	6.5	6	6.8	5.1	5.1	4.4	5.7	5.1	5.5	6
	Percentage of polymorphic bands	71.5	58.5	90.2	93.5	82.9	98.4	65	48.8	63.4	82.1	61.8	74.8	85.4
	Mean average of expected heterozygosity (*He*)	0.27	0.24	0.3	0.3	0.29	0.33	0.23	0.18	0.25	0.29	0.23	0.27	0.31
	SE of Mean *He*	±0.02	±0.02	±0.02	±0.02	±0.02	±0.01	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.02
SSR	Total No. of observed alleles	45	47	73	74	74	96	58	59	64	81	63	75	71
	Mean No. of alleles per unit assay	2.4	2.5	3.8	3.8	3.8	5.1	3.1	3.2	3.7	4.3	3.4	4	3.8
	Percentage of polymorphic alleles	37.1	40	67.6	69.5	67.6	100	54.3	53.3	55.2	94.7	54.3	68.5	66.7
	Mean average of expected heterozygosity (*He*)	0.4	0.36	0.56	0.54	0.57	0.62	0.52	0.47	0.53	0.59	0.52	0.57	0.55
	SE of Mean *He*	±0.06	±0.07	±0.05	±0.05	±0.06	±0.05	±0.06	±0.07	±0.06	±0.05	±0.04	±0.05	±0.04

coordinate, which explained 27.1% of the total variation. In contrast to SSAP and AFLP systems, PCoA using SSR data classified cultivars and landraces according to their origin. Modern and traditional cultivars having originated from foreign countries were clustered together. Iranian modern cultivars were positioned closer to the Iranian traditional cultivars and landraces. A combined PCoA based on all SSAP, AFLP and SSR markers presented a similar picture to PCoA derived from AFLP data. The first and second principal coordinates accounted for 27.2% and 6.2% total variation, respectively (Figure 1).

In order to illustrate the relatedness between the 122 durum wheat genotypes, we developed a combined UPGMA cluster, based on the genetic similarity matrix calculated by the combination of 302 polymorphic bands obtained with SSAP, AFLP and SSR data (Figure 2). Similar to the PCoA scatter plots, the dendrogram resulting from cluster analysis clearly separated the foreign accessions from Iranian ones, as well as accessions assigned to different durum wheat populations of landraces or cultivars. Correlation coefficients among pair wise genetic distance matrices generated by the different marker systems were calculated using Mantel's test (Figure 3). SSAP and AFLP showed the highest correlation (R^2 = 0.498, P<0.01), whereas lower correlations were detected between SSAP and SSR (R^2 = 0.264) and AFLP and SSR (R^2 = 0.116).

DISCUSSION

In the present study, we compared the genetic diversity of durum wheat landraces and improved cultivars (modern and traditional) originating from Iran or other countries around the world. The three DNA marker techniques used (SSAP, AFLP and SSR) were able to discriminate different durum wheat groups clearly. According to our results, the mean values of the *He*, for each marker type, indicated a considerable amount of genetic variation for autogamous species. SSR was the most informative system for revealing genetic diversity among populations. Much higher *He* estimated from SSR markers reflects the higher mutation rates found in SSR markers. The average number of alleles per assay unit and the estimated genetic diversity of Iranian genotypes were higher compared to the foreign entries. This indicates that the Iranian durum wheat landraces may be a good source of genetic variability, to be explored in crosses with elite durum wheat germplasm.

Estimates of genetic relationships, based on the different SSAP, AFLP and SSR datasets, were significantly correlated, which demonstrated the reliability of the marker techniques in durum wheat. Correlations among marker techniques have also been shown in earlier investigations. Good correlations between datasets have been reported (dos Santos *et al.,* 1994, Thormann *et al.,* 1994, Lu *et al.,* 1996, Nagaoka and Ogihara, 1997, Tom *et al.,* 2005), while others have reported lower correlations (Beer *et al.,* 1993, Powwell *et al.,* 1996, Pejic *et al.,* 1998, Giancola *et al.,* 2002). Powell et al. (1996) reported that SSRs are correlated well with AFLPs and RFLPs data only at the interspecies level. However, SSRs are very useful for germplasm assessment and evolutionary studies because of their greater resolving power (Yang *et al.,* 1994, Olufowote *et al.,* 1997, Pejic *et al.,* 1998, Giancola *et al.,* 2002). High correlation has been reported by Tom et al. (2005) between SSAP and AFLP indicating their suitability for inferring genetic relationships, which is important in germplasm management. However, they observed much lower correlation between the former two marker systems and SSRs. This suggests that co-dominant SSRs are able to infer genetic relationships based on specific genome sites, most probably caused by their sensitivity to neutrality and/or linkage disequilibrium, and would be more useful for studies of breeding material with special characteristics. Indeed, the three

[DOR: 20.1001.1.16807073.2011.13.6.3.6]

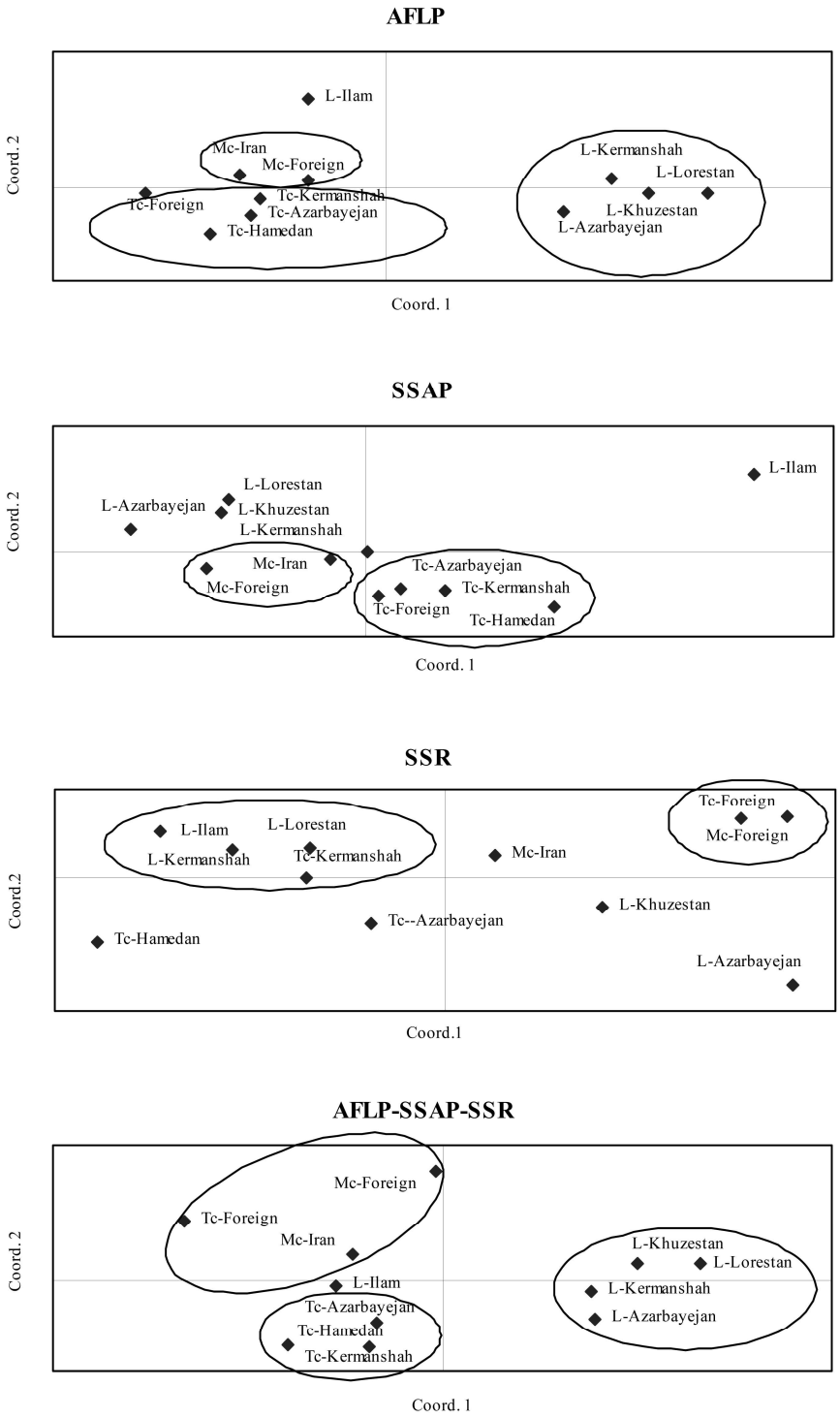

Figure1. Two dimensional graph based on the ordination scores of the principal coordinate analyses. See the codes in Table 1.

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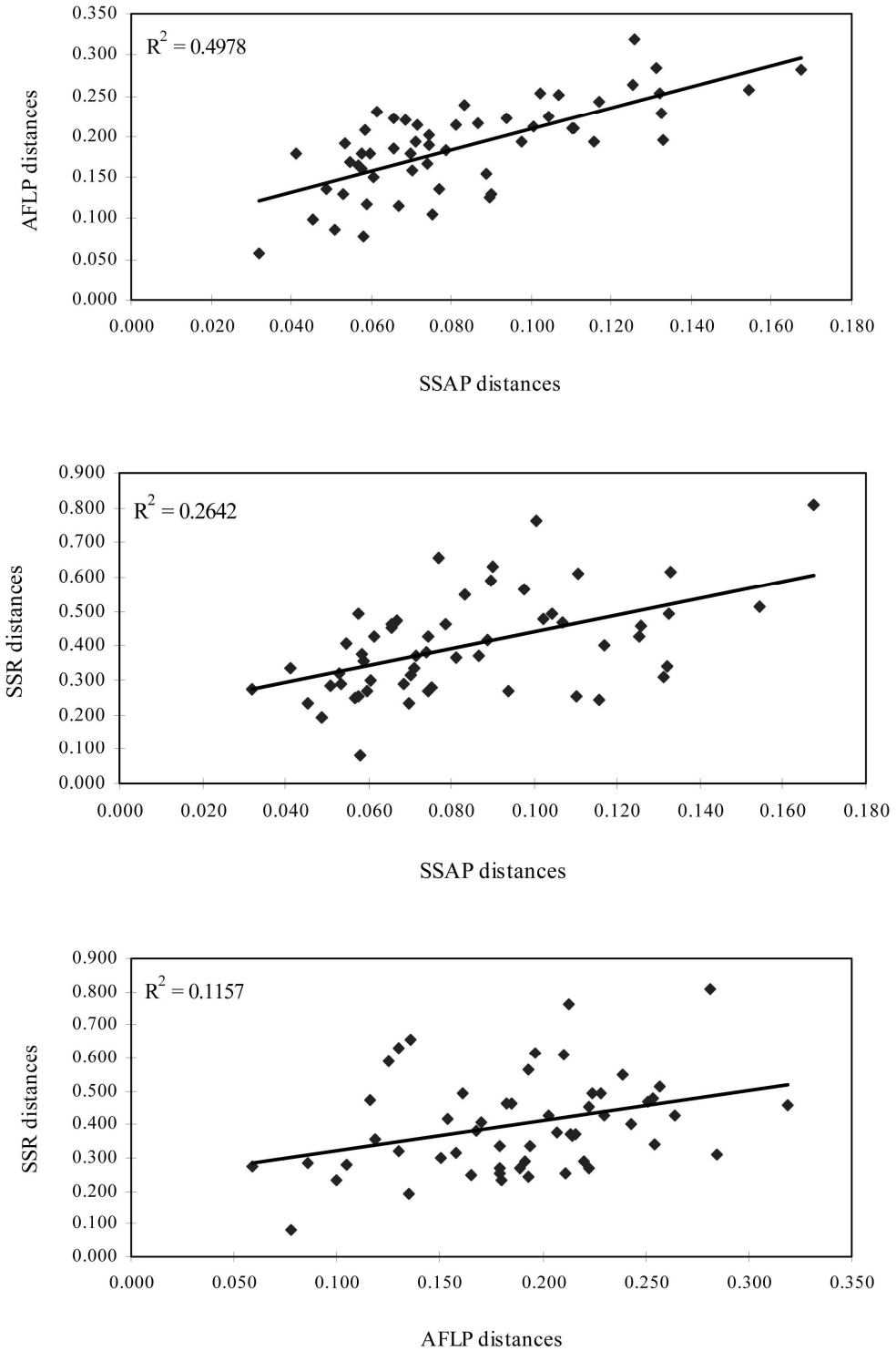

Figure2. Scatter plot of pair wise SSAP, AFLP and SSR based distances.

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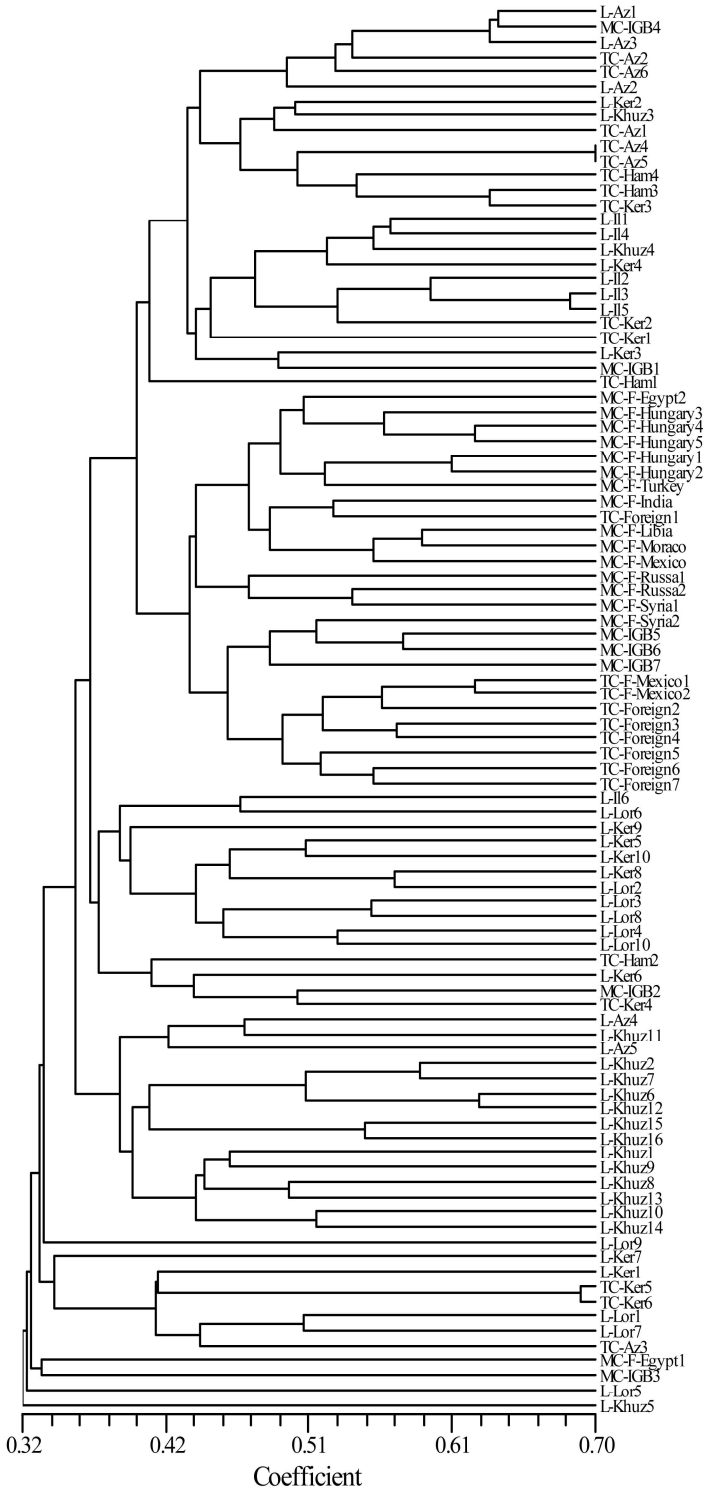

Figure 3. Dendrogram of 122 Iranian and foreign landraces and cultivars by UPGMA cluster analysis. See the codes in Table 1.

[DOR: 20.1001.1.16807073.2011.13.6.3.6]

marker systems used in this study were derived from different regions of the genome with different mutation rates occurring at different targeted sequences, which may have contributed to the low correlation between SSAP and SSR, and between AFLP and SSR. The SSAPs were developed from retrotransposons and the methods used to assay these markers were similar to AFLP. Thus, the polymorphism can be detected if there are mutations at the restriction sites, and/or indels are present in the elements, which may partly explain the similarity found in *He* values estimated for landrace and cultivars.

It is important to note that, by using SSAP and AFLP as well as combined data, landraces, and traditional and modern cultivars were separated from each other and were placed in different clusters. The dendrogram based on the combination of SSAP, AFLP and SSR data also showed the same separation. A clear demarcation between cultivars and landraces, based on different marker systems, has been reported in previous studies (Prashanth *et al.,* 2002, Medini *et al.,* 2005, Eivazi *et al.,* 2008). Despite the fact that most of the cultivars are related by pedigree, they share several agro-morphological characters that are different from those observed in landraces (plant height, yield, earliness and insensitivity to day-length). These characters can be used to distinguish cultivars and landraces from each other.

The genetic relationship among the entries used revealed by SSR markers corresponded well with the geographical origin of Iranian and foreign durum wheat gene pools, and classified these gene pools as independent. However, Iranian landraces and cultivars were also distantly related and classified in relatively different groups. Similar results have been reported for soybeans (Ude *et al.,* 2003) and for durum wheat (Martos *et al.,* 2005), which reveals that geographically different accessions are quite different genetically as well. The eco-geographical separation of the different durum wheat sample sets using SSRs may be explained by the polygenic inheritance of the adaptive traits to certain ecological conditions. A strong relationship between marker and adaptive distance is expected only if there is linkage disequilibrium. More importantly, we wish to highlight that different marker systems can reflect different aspects of genetic relationships. Molecular markers have the ability to discriminate between close similarity as a consequence of different breeding sources and that caused by high relatedness (Dillman *et al.,* 1997).

We found a high similarity in the genetic relationship of the groups depicted by SSAP and AFLP, which indicates that the SSAP and AFLP marker systems can extract information from related areas of the genome. In using SSRs, we detected an overly high level of polymorphism, as shown by high values of *H*e, but a moderate number of alleles per locus, not related to the geographic origin of the accessions. Therefore, we do not believe that differences in genetic relationships revealed by SSAP/AFLP and SSR distances, respectively, can be attributed solely to differences in the level of polymorphism detected by each marker system; rather they reflect the complexity in the inheritance of the adaptability characters. Discordance between different marker systems can be very informative for understanding genetic relationships within the study group.

It is generally believed that modern breeding practices have led to a significant decrease of genetic diversity in modern cultivars (Vellvé, 1993). In the present study, the mean *H*e values of genetic diversity of different groups of Iranian durum wheat genotypes (from landraces to traditional and modern cultivars) decreased from 0.28 to 0.27 and 0.26 (using SSAP), 0.33 to 0.29 and 0.27 (using AFLP) and 0.62 to 0.59 and 0.57 (using SSR), which revealed lower diversity in the set of improved cultivars. Considering that genetic differentiation among different groups of Iranian durum wheat germplasm (landraces versus cultivars) is statistically significant, it is possible to conclude that, on average,

[DOR: 20.1001.1.16807073.2011.13.6.3.6]

breeding processes can cause a considerable genetic diversity reduction from landraces to cultivars. These results are in line with previous reports (Boggini *et al.,* 1987, Figliuolo and Zeuli, 2000, Medini *et al.,* 2005, Figliuolo *et al.,* 2007).

In contrast, Martos et al. (2005) and Prashanth et al. (2002) have indicated that the genetic variability of durum wheat accessions seems to have been maintained. In the present study, when we considered only the data derived from SSAP, the absence of a significant reduction in the mean *H*e values of durum wheat cultivars indicated that the overall genetic diversity remained unchanged throughout the genetic improvement. Discordance between different marker systems can be very informative for understanding genetic relationships within the study groups. Overall, genetic diversity estimates from different marker systems provide different levels of information that should cater for the different needs of plant breeding programs and the management of germplasm resources.

In conclusion, the present analysis revealed that Iranian durum germplasm is highly variable and genetically distinct from the foreign germplasm. The genetic diversity of Iranian durum landraces was higher compared to that of the foreign entries and they may be considered for the genetic improvement in durum wheat program. Apparently, a loss of genetic diversity was observed from Iranian durum landraces to cultivars. This can be attributed to particular breeding pressures and the limited interchange of genetic material and supports the case for the implementation of more intense characterization and conservation strategies.

ACKNOWLEDGEMENTS

This study was supported by the Agricultural Biotechnology Research Institute of Iran (ABRII).

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[DOR: 20.1001.1.16807073.2011.13.6.3.6]

بررسی مقایسه‌ای نشانگرهای SSAP ، AFLP وSSR جهت ازریابی تنوع ژنتیکی گندم دوروم

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چکیده

بررسی مقایسه‌ای تنوع ژنتیکی ۱۲۲ ژنوتیپ گندم دوروم با استفاده از ۷۳ نشانگر چند شکل SSAP، ۱۲۳ نشانگر چند شکل AFLP و ۱۰۴ آلل SSRانجام شد. داده‌های دو نشانگر SSAP و AFLP به طور مشخص ارقام زراعی و ژنوتیپ های بومی را از همدیگر تفکیک کردند و داده‌های نشانگر SSR ارقام زراعی و ژنوتیپ‌های بومی را بر اساس منشا آنها تقسیم بندی کردند. همچنین تنوع ژنتیکی برآورد شده ژنوتیپ‌های بومی در مقایسه با نمونه‌های خارجی بیشتر بود و کاهش تنوع ژنتیکی از ژنوتیپ های بومی تا ارقام زراعی مشاهده شد. این مطالعه نشان داد که اختلاف های مشاهده شده در فواصل ژنتیکی به وسیلهٔ نشانگرهای SSAP ، AFLP و SSR تنها به دلیل اختلاف در چند شکلی مشاهده شده برای هر نشانگر نمی باشد. شواهد مولکولی کاهش تنوع خزانه ژنتیکی گندم دوروم بیش از پیش بر ارائه راهکارهایی برای حفاظت ژنتیکی مناسب این گیاه جهت ایمنی غذایی تاکید می‌کند.

[DOR: 20.1001.1.16807073.2011.13.6.3.6]