Comparative analyses of the genetic diversity among bread wheat genotypes based on RAPD and SSR markers

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Abstract

Two different DNA-based techniques viz, randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers were used to estimate genetic diversity among bread wheat. A total of 188 clear and repeatable bands were amplified from 17 selected RAPD primers, and 101 fragments were detected from 35 SSR primer pairs. The level of polymorphism was 88% with RAPDs compared to 100% with SSRs. Mean genetic similarity was estimated to be 0.88 based on RAPDs and 0.85 using SSRs. The wide range of genetic similarity was obtained by SSR than RAPD, reflecting the hypervariability of SSR markers and their high resolution power. Matrix correlation analyses suggested that a good representation of the relationships among the bread wheat cultivars/lines can be obtained by using RAPDs alone or in combination with SSRs, but SSRs alone cannot be used for this purpose. Both techniques discriminated the genotypes very effectively. On the hand, RAPDs were able to discriminate the cultivars Alvand and Ghods, whereas the cultivars Sardari and Ghods were discriminated only by SSRs. The use of PCR-based assays having advantage of being quick, easy to use and refractory to many environmental influences can complement traditional methods of germplasm characterization.

Keywords*: Wheat, diversity, RAPD, SSR*

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INTRODUCTION

Knowledge of genetic diversity among adapted cultivars or elite breeding materials has a significant impact on the improvement of crop plants. It can be obtained from pedigree analysis, morphological traits or using molecular markers (Pejic *et al*., 1998).

Molecular markers provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors. In recent years, several molecular assays have been applied to assess genetic diversity among wheat cultivars (Chen *et al*., 1994). These molecular methods are different in principle, application, type, amount of polymorphism detected and in task and time requirements. Assays based on the polymerase chain reaction (PCR) are considered to meet both the technical and genetic requirements for the characterization of plant and animal genetic resources (Powell *et al*., 1995).

Random amplified polymorphic DNA (RAPD) markers can be used to detect DNA polymorphism without the need for predetermined genetic data. Each product is derived from a region of the genome that contains two short segments in inverted orientations, on opposite strands that are complimentary to the primer and sufficiently close together for the amplification to work (Williams *et al*., 1990 and Welsh and McClelland, 1990).

Microsatellite or simple sequence repeats (SSRs) are highly mutable loci which may be present at many sites in a genome (Morgante *et al*., 1998). As the flanking sequence of these sites may be unique, primers can be designed to the flanking sequence (Jones *et al*.,

1997). SSRs provide highly informative markers because they are co-dominant (unlike RAPDs) and generally have high polymorphic information content (Gupta *et al*., 1996).

Comparative studies of RAPD and SSR markers in wide range of crop species, including corn (Smith and Helentejaris, 1998), soybean (Powell *et al*., 1996 and Brown-Guedira *et al*., 2000), barley (Russell *et al*., 1997), sorghum (Agrama and Tuinstra, 2003 and Uptmoor *et al*., 2003), rice (Fugang *et al*., 2003) and wheat (Jones *et al*., 1997) have generally revealed good congruence between the genetics patterns revealed by the two genetic markers.

Our objective was to investigate and compare the genetic relationships among wheat (*Triticum aestivum* L.) cultivars, or lines, using molecular data obtained from RAPD and SSR profiles.

MATERIALS AND METHODS

Plant material and DNA isolation: Nineteen Iranian bread wheat cultivars (Tajan, Golestan, Sardari, Ghods, Ghafghaz, Azar 2, Bolani, Alvand, Tabasi, Roshan, Bezostaya, Falat, Mahdavi, Zarin, Shoaleh, Navid, Omid, Niknezhad and Iniea) and two lines (Shain and Line 518) were selected for this study. All of these samples were obtained from Seed and Plant Improvement Institute, Karaj, Iran. Total genomic DNA was extracted from fresh leaf material by appropriate modifications of the method described by Dallaporta *et al*. (1983). The concentrtion of DNA was determined by spectrophotometry.

Molecular markers assays

RAPD assays: A total of seventeen 10-mer oligonucleotides from UBC (University of British Colombia) series were selected according to the number and consistency of amplified fragments (Table 1). PCR reactions were carried out in a 25-µl reaction volume containing 1 unit of *Taq* polymerase, 5 ng of template DNA, 0.2 µM of primer, 200 µl of each of dATP, dCTP, dGTP and dTTP, 1X PCR reaction buffer. Amplifications were performed in a DNA thermocycler (Perkin-Elmer) programmed for 45 consecutive cycles each consisting of 1 min at 92°C, 1 min at 37°C and 72°C for 2 min. Following amplification, the amplification products were subjected to electrophoresis in 6% acrylamide gels in TAE buffer running at 200v for 2.5h. The gels were stained using ethidum bromide and viewed under ultra- violet light.

SSR assay: Thirty five primer.s pairs (from MWG Biotech, Germany) give the information from where the primers were obtained or designed, were used to characterize loci containing microsatellite sequences among the 21 bread wheat (Table 1). PCR amplification was performed in a 25-µl reaction volume containing the following components: 20 ng of template DNA, 200 µM of each of the four dNTPs, 1X *Taq* polymerase buffer, 1 unit *Taq* polymerase, 2 mM $MgCl_2$ and 0.25 µM of each of the two primers. Amplifications were performed in a Perkin-Elmer thermocycler. The amplification cycles consisted of 26 cycles with denaturation at 94°C for 30 s, annealing at 52°C for 45 s and extension at 72°C for 1 min. The final extension was done at 72°C for 7 min. Amplification products were separated on denaturing 6% polyacrylamide gel electrophoresis. For fragment detection, silver staining of the gels was done.

Data scoring and statistical analysis: For subsequent statistical analysis, polymorphic bands detected by both molecular marker systems were coded in a binary by giving character state (1) or (0) for presence or absence of bands, respectively, in each cultivar. The generated data matrixes were subjected to statistical analyses using NTSYS-pc version 2.0 software (Rohlf, 1998). Genetic similarities were calculated for RAPD, SSR and the combined data using Dice similarity index as in Nei and Li (1979). Dendrograms showing genetic relationships of the 21 cultivars and lines were constructed using the unweighted pair-group method on arithmetic averages (UPGMA). For each dendrogram the cophenetic coefficients between the matrix of genetic similarities and the matrix of cophenetic values were computed and the significance of cophenetic correlation was tested using Mantel matrix correspondence test (Mantel, 1967).

RESULTS

Variation for molecular markers: A summary of effectiveness of RAPD and SSR markers in detecting polymorphism of wheat genotypes is given in table 2. In the RAPD analysis out of 46 decamer primers screened for amplification of all the genotypes, 17 primers gave reproducible and scorable amplification products. Hence they were used for the further analysis. A total of 213 bands were obtained (average of 11 bands per primer) among which 188 were polymorphic (88%) across the 21 wheat genotypes. The highest and the lowest number of polymorphic bands per

Table 1. List of RAPD and SSR primers used.

RAPD ten-mer primer name and sequences (5' -3')			
UBC1	CCTGGGCTTC	UBC110	TAGCCCGCTT
UBC3	CCTGGGCTTA	UBC112	GCTTGTGAAC
UBC9	CCTGCGCTTA	UBC114	TGACCGAGAC
UBC13	CCTGGGTGGA	UBC116	TACGATGACG
UBC104	GGGCAATGAT	UBC117	TTAGCGGTCT
UBC105	CTCGGGTGGG	UBC126	CTTTCGTGCT
UBC106	CGTCTGCCCG	UBC128	GCATATTCCG
UBC108	GTATTGCCCT	UBC129	GCGGTATAGT
UBC109	TGTACGTGAC		

SSR locus name and primer pairs sequences			
Xgwm153-1B	GATCTCGTCACCCGGAATTC TGGTAGAGAAGGACGGAGAG	Xgwm369-3A	CTGCAGGCCATGATGATG ACCGTGGGTGTTGTGAGC
Xgwm609-2D	ACATTCTGTGTGGGGCC GATCCCTCTCCGCTAGAAGC	Xgwm120-2B	GATCCACCTTCCTCTCTCTC GATTATACTGGTGCCGAAAC
Xgwm340-3B	GCAATCTTTTTTCTGACCACG ACGAGGCAAGAACACACATG	Xgwm264-3B	GAGAAACATGCCGAACAACA GCATGCATGAGAATAGGAACTG
Xgwm334-6A	AATTTCAAAAGGAGAGAGA AACATGTGTTTTTAGCTATC	Xgwm495-4B	GAGAGCCTCGCGAAATATAGG TGCTTCTGGTGTTCCTTCG
Xgwm111-7D	TCTGTAGGCTCTCTCCGACTG ACCTGATCAGATCCCACTCG	Xgwm44-7D	GTTGAGCTTTTCAGTTCGGC ACTGGCATCCACTGAGCTG
Xgwm642-1D	ACGGCGAGAAGGTGCTC CATGAAAGGCAAGTTCGTCA	Xgwm469-6D	CAACTCAGTGCTCACACAACG CGATAACCACTCATCCACACC
Xgwm148-2B	GTGAGGCAGCAAGAGAGAAA CAAAGCTTGACTCAGACCAAA	Xgwm55-2B	GCATCTGGTACACTAGCTGCC TCATGGATGCATCACATCCT
Xgwm149-4B	CATTGTTTTCTGCCTCTAGCC CTAGCATCGAACCTGAACAAG	Xgwm130-7A	AGCTCTGCTTCACGAGGAAG CTCCTCTTTATATCGCGTCCC
Xgwm372-2A	AATAGAGCCCTGGGACTGGG GAAGGACGACATTCCACCTG	Xgwm55-6D	GCATCTGGTACACTAGCTGCC TCATGGATGCATCACATCCT
Xgwm539-2D	CTGCTCTAAGATTCATGCAACC GAGGCTTGTGCCCTCTGTAG	Xgwm132-6B	TACCAAATCGAAACACATCAGG CATATCAAGGTCTCCTTCCCC
Xgwm540-5B	TCTCGCTGTGAAATCCTATTTC AGGCATGGATAGAGGGGC	Xgwm389-3B	ATCATGTCGATCTCCTTGACG TGCCATGCACATTAGCAGAT
Xgwm156-5A	CCAACCGTGCTATTAGTCATTC CAATGCAGGCCCTCCTAAC	Xgwm613-6B	CGACCCGACCTACTTCTCT TTGCCGTCGTAGACTGG
Xgwm190-5D	GTGCTTGCTGAGCTATGAGTC GTGCCACGTGGTACCTTTG	Xgwm271-5D	CAAGATCGTGGAGCCAGC AGCTGCTAGCTTTTGGGACA
Xgwm383-3D	ACGCCAGTTGATCCGTAAAC GACATCAATAACCGTGGATGG	Xgwm371-5B	GACCAAGATATTCAAACTGGCC AGCTCAGCTTGCTTGGTACC
Xgwm133-6B	ATCTAAACAAGACGGCGGTG ATCTGTGACAACCGGTGTGA	Xgwm493-3B	TTCCCATAACTAAAACCGCG GGAACATCATTTCTGGACTTTG
Xgwm325-6D	TTTCTTCTGTCGTTCTCTTCCC TTTTTACGCGTCAACGACG	Xgwm359-2A	CTAATTGCAACAGGTCATGGG TACTTGTGTTCTGGGACAATGG
Xgwm357-1A	TATGGTCAAAGTTGGACCTCG AGGCTGCAGCTCTTCTTCAG	Xgwm443-5B	GGGTCTTCATCCGGAACTCT CCATGATTTATAAATTCCACC
Xgwm608-2D	ACATTGTGTGTGCGGCC GATCCCTCTCCGCTAGAAGC		

assay unit were 6 and 18, respectively. In the SSR assay, a total of 199 fragments were obtained from the 35 SSR assay units. The number of polymorphic bands per assay unit ranged from 2 to 10 with an average of 5.3. An example of RAPDs and SSRs banding pattern has been shown in figure 1 and figure 2.

Estimates of genetic similarity: A summary of the genetic similarity estimates between pairs of genotypes, calculated for each marker system, is shown in Table 3. Microsatellite data gave lower average similarity than RAPDs. Estimates of genetic similarity of RAPD based on the 111 polymorphic markers between 21 wheat cultivars ranged from 0.40 for Navid/Zarin to 0.83 for Ghods/Sardari with an average of 0.45. The estimates revealed by the 175 polymorphic SSR bands showed the lowest average value (0.25) and also the highest range of genetic similarity (from 0.12 up to 0.76, for Bolani/Nikneghad and Ghods/Alvand respectively) reflecting their hypervariability and their high resolution power.

Table 2. Effectiveness of RAPD and SSR markers in detecting polymorphism of wheat cultivars/lines.

	RAPD	SSR
Number of assay units	17	35 (pairs)
Total bands scored	213	199
Polymorphic fragments scored	188	199
Percentage of polymorphism	88	100
Number of loci	188[a]	50
Minimum polymorphism scored per primer/primer pair	6	2
Maximum polymorphism scored per primer/primer pair	18	10
Average polymorphism scored per primer/primer pair	11	5.3

[a] Theoretical number of loci

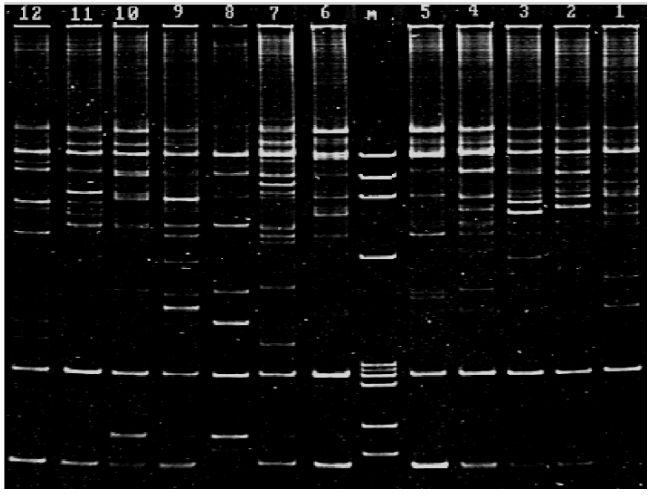

Figure 1. An example of RAPD banding pattern obtained from primer UBC129 on twelve genotypes of wheat. M is DNA size markers.

Table 3. Average, minimal and maximal values of Dice similarity coefficients using 3 combinations of marker system (RAPDs, SSRs and RAPDs+ SSRs) among 21 wheat cultivars/lines (210 pairwise comparisons).

Parameter	Marker system		
	RAPDs	SSRs	RAPDs+ SSRs
Average	0.65	0.33	0.68
Minimum	0.40	0.12	0.60
Maximum	0.84	0.76	0.80

Correlation between measures: The correlation coefficients used to compare similarity matrices and cophenetic matrices were statistically significant for all three combinations. But these correlation coefficients were lower for RAPD and SSR comparing (0.19 and 0.40 for comparing of cophenetic and similarity matrices, respectively) than those obtained with the other comparings. The cophenetic correlation coefficient between the dendrogram and the original distance matrix for RAPDs was significant and relatively high (0.92) but these were significant and relatively low for SSRs and RAPDs+SSRs (0.72 and 0.79 respectively). These results indicated that only cophenetic correlation coefficient between the dendrogram and the original distance matrix for RAPDs was a good representation of the relationships among the genotypes, as reported by Rolf (1998), earlier where a correlation of 0.82 was considered to be significant.

Cluster analyses: Three dendrograms were constructed to express the results of cluster analyses based on data obtained by RAPD, SSR, and RAPD+SSR (Fig.3) ampilifcation products. The dendrograms obtained with SSR and SSR+RAPD markers were more similar to each other than to the dendrogram based on RAPD.

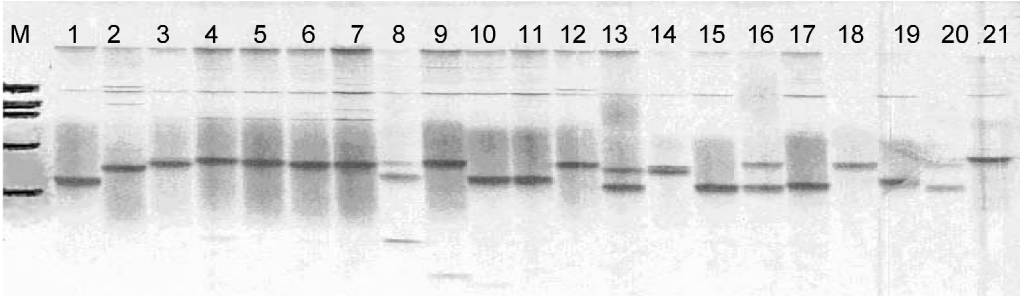

Figure 2. An example of SSRs banding pattern obtained using primer pair Xgwm190-5D in 21 genotypes of wheat. M is DNA size markers.

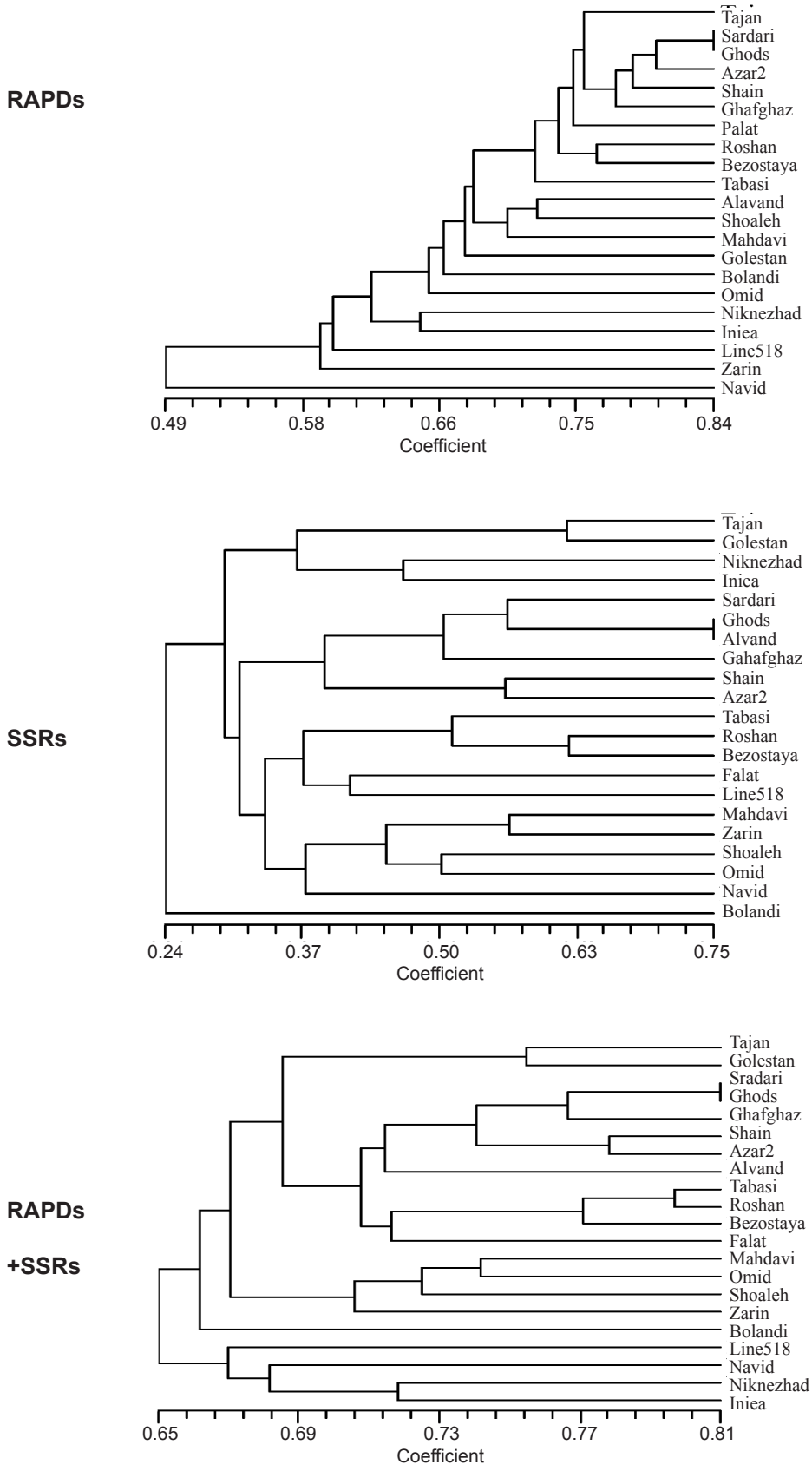

Figure 3. Dendrograms of 21 wheat cultivars/lines obtained using RAPDs, SSRs and RAPDs+SSRs.

In other words, there are some common associations of cultivars between the dendrograms of RAPD and SSR+RAPD. For instance, cultivars Tajan and Golestan cluster together in the same subgroup using both markers, and also cultivars Tabasi, Roshan and Bezostaya gave similar results. But some differences were found between the dendrograms of RAPD and SSR+RAPD, as well as in the SSR dendrogram cultivar Flat and Line518 grouped together, whereas these are in different subgroup in the SSR+RAPD dendrogram.

DISCUSSION

In this study, two of the polymerase chain reaction (PCR)-based systems (RAPD and SSR) have been used and compared for studding the genetic diversity between 19 cultivars and 2 lines of wheat. Each system is different in principle, type and amount of polymorphism detected. The level of polymorphism was 88% with RAPDs compared to 100% with SSRs (Table 2). In previous study in Iranian wheat cultivars 58% polymorphism was reported using 8 RAPD primers (Abdolahie *et al*., 2003). The highest levels of polymorphism for SSRs system compare to other systems also reported in previous studies (Belaj *et al*., 2003; Russel *et al*., 1997; Bohn *et al*., 1999; Gomes *et al*., 1998; Maguire *et al*., 2002; Palombi and Damiano, 2002; Rajora and Rahman, 2003; Ferreria, *et al*., 2004). This high level of polymorphism, associated with SSR markers, is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage. Replication slippage is thought to occur more frequently than single nucleotide mutations and insertion\deletion events, which generated the polymorphisms detected by RAPD analysis (Powell *et al*., 1996). The codominant nature of SSR markers also permits the detection of a high number of alleles per locus and contributes to higher levels of expected heterozygosity being reached than would be possible with RAPD markers. However, this result also depends on species under study (Belaj *et al*., 2003).

No association was detected between each marker data and pedigree information (data have not shown) that may be due to inaccuracies in pedigree information. Several previous comparisons of pedigree and molecular markers data indicated that correlation coefficients between data sets generated from these two diversity estimation methods are highly variable.

The finding of lower, but yet significant, correlation

Table 4. Correlation[a] between matrices.

Marker system	Marker system		
	RAPDs	**SSRs**	**SSRs+RAPDs**
RAPDs	**0.92*****	0.19*	0.69***
SSRs	0.40**	**0.72*****	0.65***
SSRs+RAPDs	0.74***	0.81***	**0.79*****

*** Significant at P < 0.001, ** significant at P < 0.01, * significant at P < 0.05.

[a] Below diagonal, original similarity matrix comparison; diagonal (in bold), goodness of fit of a cluster analysis to the similarity matrix on which it was based; above diagonal, cophenetic value matrix comparison (after UPGMA analysis).

similarities observed in the present study for SSRs (Table 4) might be due to their codominant nature. The type of genetic polymorphism detected by the two markers and the number of primers used may also affect the correlations among different markers (Belaj *et al*., 2003).

In this study RAPD markers were able to discriminate cultivars Alvand and Ghods, and also SSR were able to discriminate cultivars Sardari and Ghods (Fig. 3). Although two marker systems discriminated most genotype very effectively, whereas, SSR markers were more discriminating than RAPD markers. This high level of discrimination is also reported by Belaj *et al*. (2003) and Rajora and Rahman. (2003).

The two molecular markers used in this study have shown an aptitude in the differentiation of the cultivars, but for RAPD analysis, the problems of reliability and transferability among laboratories should be considered (Jones *et al*., 1997). Reliable RAPD data can be generated following a standard protocol, replication of amplification reactions and a conservative criterion of band selection (Belaj *et al*., 2003). The RAPD technique is quick (Colombo *et al*., 1998) cost effective (Fugang *et al*., 2003) and the ability to perform analysis without the need for prior sequencing of the genome (Huff *et al*., 1993). However, problems with reproducibility in amplification of RAPD markers and with data scoring have been reported (Jones *et al*., 1997). Although major bands from RAPD reactions are highly reproducible, minor bands can pose difficulty to repeat due to random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers (Tessier *et al*., 1999). But SSR markers are attractive for DNA fingerprinting studies for several reasons.

SSR markers are the markers of choice for variability studies in many of crops as they are transferable, highly polymorphic, simple to interpret and they are multiallelic and co-dominant (Rafaleski *et al*., 1996; Agrama and Tuinstra, 2003).

The congruence between SSR and RAPD data sets suggest that either methods, or a combination of both, applicable to expended the diversity studies in wheat cultivars/lines. There are different strengths and limitations for two marker systems, and knowledge of these may be used to guide the choice of techniques.

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