

## WHEAT GENETIC DIVERSITY AND DNA MARKERS IN RELATION WITH RESPONSE TO DROUGHT STRESS

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**Summary.** Genetic diversity related to response to drought stress of 26 bread wheat (*Triticum aestivum* L.) cultivars (15 resistant and 11 susceptible) was evaluated at the DNA level using inter simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD) markers. Among 20 primers evaluated using the RAPD assay, 10 produced 103 reproducible DNA fragments. The average number of polymorphic bands per RAPD primers was 5.3. The percentage of polymorphism in RAPD primers ranged from 25% to 90%. Among 21 inter microsatellite primers used in the ISSR assay, 11 primers generated 139 DNA fragments. The average number of polymorphic bands per ISSR primer was 12.6. The percentage of polymorphic bands was higher for ISSRs (63%) than for RAPDs (50%). The clustering dendrogram generated by ISSR based similarity matrix, segregated resistant and susceptible cultivars better than the RAPD based clustering dendrogram. Based on ISSR data, 26 wheat cultivars were clustered into four groups including two resistant and two susceptible groups. In our research, ISSR markers were more efficient than RAPD assays, and we suggest that the use of ISSR markers could be valuable in studies of genetic diversity and in differentiating between resistant and susceptible cultivars.

**Key words:** bread wheat; DNA markers; drought stress; ISSR; RAPD.

**Abbreviations:** ISSR – inter simple sequence repeats; RAPD – random amplified polymorphic DNA.

### INTRODUCTION

Evaluation of the genetic diversity is performed using morphological, molecular and biochemical markers (Chahal and Gosal, 2002). DNA-based markers are considered not only valuable tools for plant breeding programs, but also for studies related to phylogenetics,

evolution and biodiversity conservation. Recently, molecular methods are common procedures for identifying and classifying the genotypes (Moumeni et al., 2001). Molecular markers that provide an excellent tool for obtaining genetic information and their use in the assessment of genetic

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divergence in wheat has increased in the last few years (Manifesto et al., 2001; Corbellini et al., 2002; Almanza- Pizon et al., 2003; Maric et al., 2004; Roy et al., 2004). Also, because molecular markers are not subjected to environmental influence they are considered superior to morphological markers (Maric et al., 2004). At present, microsatellites are one of the most promising molecular marker types able to identify or differentiate between genotypes within a species. Their co-dominant inheritance, high level of polymorphism and easy handling make them extremely useful for many different applications (Devos et al., 1995; Prasad et al., 2000). Inter simple sequence repeat (ISSR) analysis involves the polymerase chain reaction (PCR) amplification of regions between adjacent, inversely oriented microsatellites, using a single simple sequence repeat (SSR) motifs (di-, tri-, tetra-, or penta, nucleotides) containing primers with un-anchored or anchored at the 3' or 5' end by two to four arbitrary nucleotides (Zietkiewicz et al., 1994). Inter simple sequence repeat (ISSR) markers have emerged as an alternative system with the reliability and advantages of microsatellites (SSR) along with the broad taxonomic applicability of RAPDs (Zietkiewicz et al., 1994). ISSRs have been used for cultivar identification in maize (Kantety et al., 1995; Pejic et al., 1998), potatoes (Prevost and Wilkinson, 1999), wheat (Nagaoka and Ogihara, 1997), bean (Metais et al., 2000) and *Diplotaxis* (Martin and Sanchez-Yelamo, 2000). Several studies have indicated that ISSR markers are potentially useful both for cultivar identification and for phylogenetic studies (Wu and Tanksley, 1993; Gupta et al., 1994; Zietkiewicz et al.,

1994; Kantety et al., 1995; Charters et al., 1996; Fang and Roose, 1997; Nagaoka and Ogihara, 1997; Parsans et al., 1997; Blair et al., 1999; Joshi et al., 2000; Prevost and Wilkinson, 1999). Nagaoka and Ogihara (1997) have shown that ISSRs are more informative compared to RFLP and RAPD markers in wheat. The utility of ISSR markers for tagging agronomic important genes was first demonstrated by Akagi et al. (1996) by identifying tight linkage with a nuclear restorer gene in rice. The random amplified polymorphic DNA (RAPD) technique, regardless of its sensitivity to reaction conditions and problems with repeatability and amplifying of non-homologous sequences (Devos and Gale, 1992), has been successfully used for the assessment of genetic diversity in diploid, tetraploid and hexaploid wheat (He et al., 1992; Myburg et al., 1997; Liu et al., 1999; Sivolap et al., 1999). RAPDs have been used for measuring genetic diversity in several plant species, including broccoli and cauliflower (Hu and Quiros, 1991), apple (Landry et al., 1994), wheat (Vierling and Nguyen, 1992; Cao et al., 1999), commercial pea varieties (Bagheri et al., 1995), melon (Garcia et al., 1998), *Gossypium* (Khan et al., 2000), olive (Besnard et al., 2000) and *Oryza granulate* (Qian et al., 2001).

The aims of this research were to study genetic diversity in relation to response to drought stress of wheat cultivars using ISSR and RAPD markers and to compare the results based on ISSR and RAPD markers.

## **MATERIALS AND METHODS**

### **Plant materials**

Twenty six Iranian wheat cultivars

(*Triticum aestivum* L.) (15 resistant and 11 susceptible to drought stress) provided by the Seed and Plant Improvement Institute in Iran were selected based on

their response to drought stress (Table 1). These cultivars were assigned numbers S1 to S11 (susceptible) and R1 to R15 (resistant).

Table 1. Name and code of 26 Iranian bread wheat (*Triticum aestivum* L.) cultivars and their response to drought stress.

Cultivar name	Response to drought stress	Code	Cultivar name	Response to drought stress	Code
Marvdasht	Sensitive	S1	Roshan	Resistance	R1
Mahdavi	Sensitive	S2	Omid	Resistance	R2
Azadi	Sensitive	S3	Pishtaz	Resistance	R3
Ghods	Sensitive	S4	Chamran	Resistance	R4
Shahriyar	Sensitive	S5	Zagros	Resistance	R5
Tous	Sensitive	S6	Kavir	Resistance	R6
Alamout	Sensitive	S7	BakkrasRoshan(bahare)	Resistance	R7
Shiraz	Sensitive	S8	BakkrasRoshan(zemestane)	Resistance	R8
Navid	Sensitive	S9	Alvand	Resistance	R9
Bezostay	Sensitive	S10	Niknejad	Resistance	R10
Gaspard	Sensitive	S11	Sepahan	Resistance	R11
			Sardari	Resistance	R12
			Sabalan	Resistance	R13
			Kras shahi	Resistance	R14
			Verinak	Resistance	R15

### DNA extraction and PCR assays

Fresh leaves were used for DNA extraction according to Pirttila method (Pirttila et al., 2001) with slight modifications. DNA samples were stored at -20°C prior to RAPD and ISSR analysis. The quantity and quality of the extracted total DNA were determined using a spectrophotometer (model 6405 UV-vis) and 0.8% agarose gel.

DNA amplifications were performed in a total reaction volume of 25 µl containing 25 ng template DNA and PCR kit (cinagen). The primers used for RAPD and ISSR reactions were 20 and 21,

respectively. Amplifications were carried out in a TC 512 thermal cycler Techne. PCR programs for RAPD involved an initial denaturation at 94°C for 3 min, followed by 45 cycles of denaturation at 94°C for 1 min, 1 min annealing at 32-35°C (variable for primers) and extension at 72°C for 2 min, plus a final extension at 72°C for 7 min. ISSR reactions were carried out with an initial denaturation for 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 1 min, 1 min annealing at 53-56°C (variable for primers) and extension at 72°C for 3 min plus a final extension

at 72°C for 7 min. An aliquot of 10 µl of each PCR product was separated by electrophoresis on 1.5% agarose gel for 3 h at 80 V, stained with ethidium bromide, visualized and photographed under UV light.

### Data analysis

DNA fingerprints generated by RAPD and ISSR markers were scored as the presence (1) or absence (0) of the bands, each of which was treated as an independent character regardless of its intensity. Data analysis was performed using the NTSYS-pc software (version 2.1; Exeter Biological Software, Setauket, NY, USA). Cluster analysis was performed by the un-weighted pair group method with arithmetic average (UPGMA) using Jaccard's similarity coefficient. To

investigate the correlation between the RAPD and ISSR data sets, the Mantel correspondence coefficient was estimated based on their distance matrices (Mantel, 1967).

## RESULTS

### RAPD band patterns

Among 20 RAPD primers used, 10 produced a total of 103 reproducible DNA fragments, 53 of which were polymorphic. The number of bands generated per primer varied from 8 to 13 for primers 18 and Z16, respectively (Fig. 1). The average number of polymorphic bands per primer was 5.3 (Table 2). The dendrogram constructed using the UPGMA method demonstrated that 26 cultivars were grouped into four main

Table 2. Description of 10 random amplified polymorphic DNA (RAPD) markers used in this study for 26 wheat genotypes.

Primer name	Sequence(5'-3')	Annealing temperature [°C]	N <sup>a</sup>	P <sup>b</sup>	q <sup>c</sup> [%]	PIC <sup>d</sup>
Z-16	TCC CCA TCAC	32	13	7	54	0.33
Z-17	CCT TCC CACT	32	10	7	70	0.33
1	CCT GGG CTT C	35	10	4	40	0.31
9	CCT GCC CTT A	32	12	6	50	0.40
18	GGG CCG TTT A	33	8	2	25	0.39
51	CTA CCC GTG C	34	11	10	91	0.27
28	CCG GCC TTA A	34	11	3	27	0.34
29	CCG GCC TTA C	32	9	6	67	0.31
OPC-06	CCA GAA CGG A	32	8	2	35	0.20
OPAN-13	CTT CCA GGA C	32	11	6	55	0.32
Average			10.3	5.3	50	0.32

<sup>a</sup>Total numbers of reproducible bands scored.

<sup>b</sup>Numbers of polymorphic bands.

<sup>c</sup>Percentage of polymorphic bands.

<sup>d</sup>Polymorphic information content.

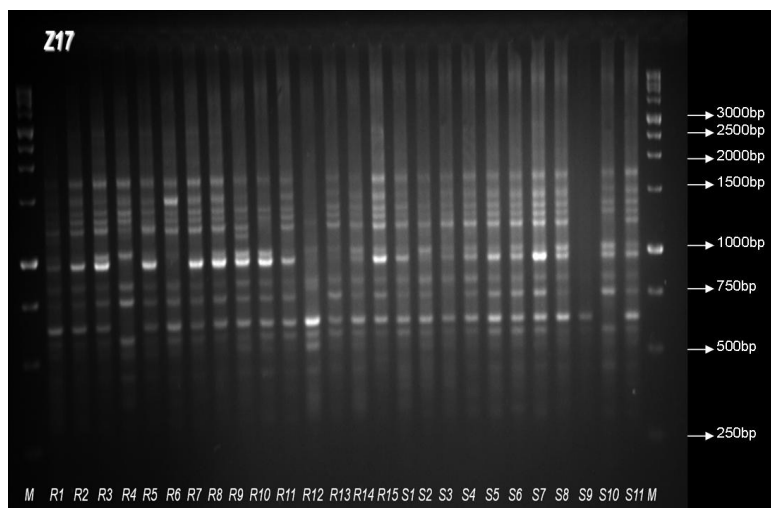


Fig. 1. Genomic DNA amplification pattern in 26 wheat cultivars with 10-mer RAPD primer (Z17), (M molecular-weight marker, R resistant, S susceptible).

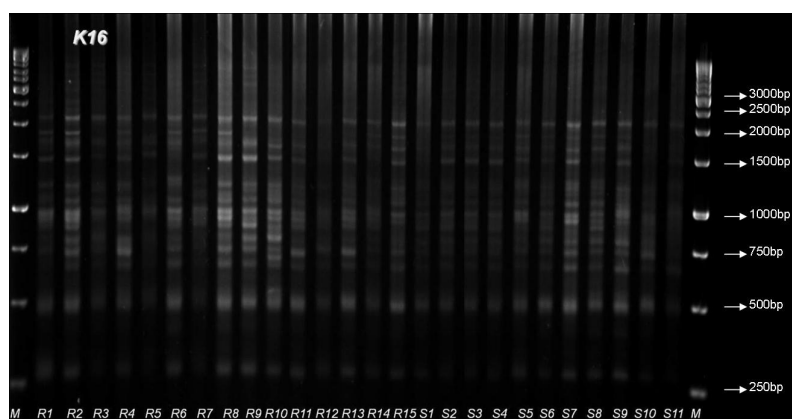


Fig. 2 Genomic DNA amplification pattern in 26 wheat cultivars with ISSR primer (K16), (M molecular-weight marker, R resistant, S susceptible).

subclusters (Fig. 3a). The cophenetic correlation coefficient of this dendrogram was  $r=0.78$ . Group I included Roshan, Bakkras Roshan (winter), Bakkras Roshan (spring) and Omid, which were resistant to drought. Group II included Pishtaz, Alvand, Chamran, Niknejad, Verinak, Tous, Bezostay, Sepahan, Alamout and Shiraz. Most of them were resistant to drought. Group III included Kavir, Sabalan, Mahdavi, Ghods, and

Shahriyar, three of them being susceptible to drought. Finally, Marvdasht (sensitive to drought) formed group IV. The results of the cluster analysis were confirmed by PCA analysis. The first three most informative PC components explained 82% of the total variation. Based on three-dimensional plot (Fig. 4a), five cultivars S11, S3, S9, R12 and R14 (similar to RAPD cluster dendrogram) appeared to be distinct from the other cultivars.

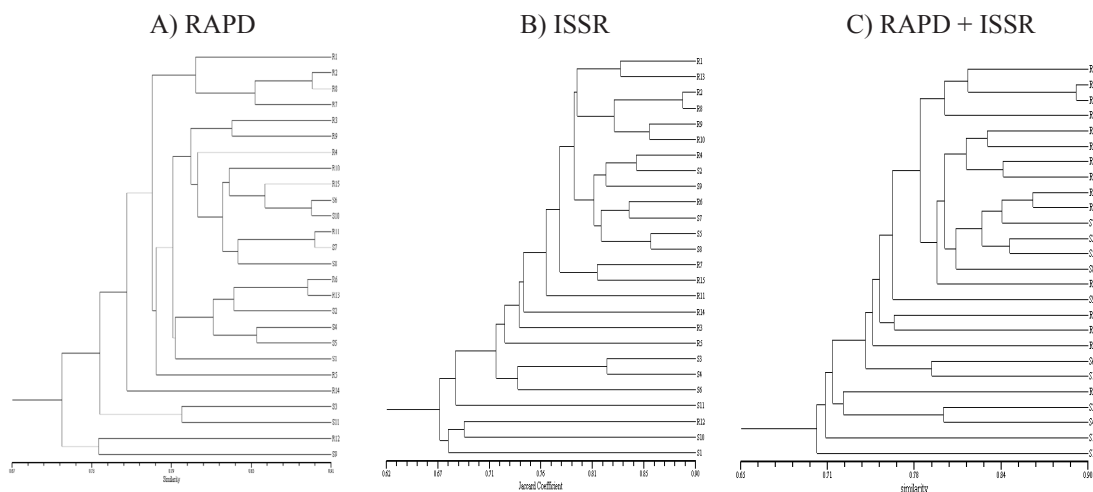


Fig. 3. Dendrograms established from Jaccard's similarity coefficients between cultivars, using UPGMA method based on RAPD, ISSR, and RAPD + ISSR data.

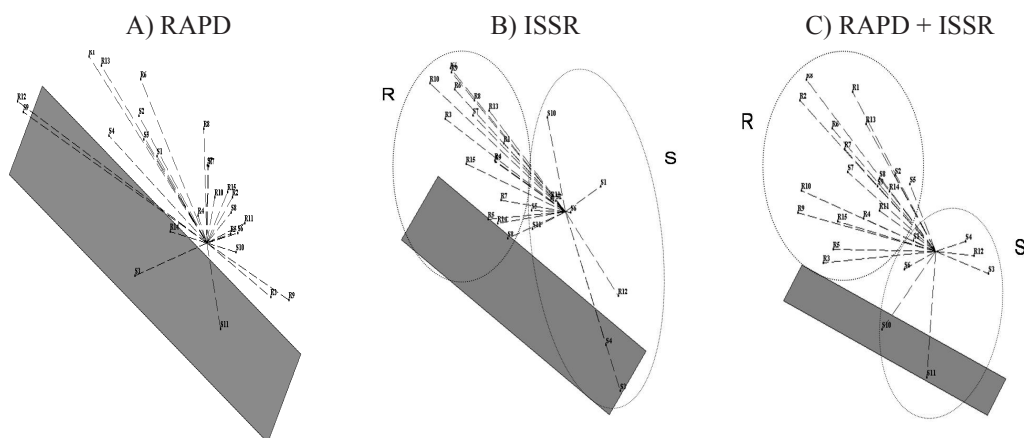


Fig. 4. Three-dimensional plot of principal component analysis of 26 wheat cultivars examined (R resistant and S susceptible) based on RAPD, ISSR and RAPD +ISSR data.

### ISSR band patterns.

Among 21 ISSR primers used, 11 generated 139 DNA fragments and 92 were polymorphic. In the group of dinucleotide motifs, GA repeats primers indicated lower level of polymorphism in contrast to other primers, as previously mentioned by Akkaya et al. (1992) with respect to microsatellite primer in soybean. The number of bands varied from 6 to 19 for primers ISSR Hb10 and K13,

respectively (Fig. 2). The average number of polymorphic bands per primer was 12.6 with an average polymorphism of 63% across all the cultivars (Table 3). The dendrogram constructed by the UPGMA method with ISSR data is shown in Fig. 3b. Coefficients of Jaccard's similarity between cultivars ranged from 0.62 to 0.90, and 26 wheat cultivars were clustered into four groups, including two resistant

groups and two susceptible groups (Fig. 3b). Group I included Roshan, Sabalan, Omid, BakkrasRoshan (winter), Alvand and Niknejad. All of the cultivars clustered in this group were resistant. Group II included Chamran, Mahdavi, Navid, Kavir, Alamout, Shahriyar and Shiraz, five of them being susceptible. Group III included BakkrasRoshan (spring), Verinak, Sepahan, Kras shahi, Pishtaz and Zagros. All of them were resistant. Azadi, Ghods and Tous that were susceptible formed the fourth groups. The results of PCA analysis confirmed the cluster results. Based on PCA analysis, the first three most informative PC components explained 80% of the total variation, and similar to the cluster dendrogram, three cultivars S1, S10, and R12 appeared distinct from the other cultivars (Fig. 4b).

### RAPD and ISSR data

The RAPD and ISSR data were combined for UPGMA cluster analysis. The dendrogram constructed by the combined RAPD and ISSR data gave a relatively different clustering pattern. The pattern in clustering of the cultivars remained more or less the same in ISSR and ISSR + RAPD data, whereas the dendrogram based on RAPD showed some variations in the accurate grouping of resistant and susceptible cultivars. The cophenetic correlation value for the dendrogram based on RAPD + ISSR data was high ( $r=0.81$ ). In addition, the results of the PCA analysis were comparable with the cluster analysis and four cultivars S11, S3, S4 and R12 appeared to be distinct from the other cultivars in the 3D plot (Fig. 4c).

Table 3. Description of 11 polymorphic Inter-simple sequence repeats (ISSR) markers used in this study for 26 wheat genotypes.

Primer name	Sequence(5'-3')	Annealing temperature [°C]	N <sup>a</sup>	P <sup>b</sup>	q <sup>c</sup> [%]	PIC <sup>d</sup>
K 13	(AG) <sub>8</sub> YT	54	19	14	74	0.41
K 16	(CA) <sub>8</sub> RC	54	14	13	93	0.34
ISSR 17899 A	(CA) <sub>6</sub> AG	44	15	8	53	0.36
ISSR 17899 B	(CA) <sub>6</sub> AT	44	11	9	82	0.37
UBC 818	G(GGGGT) <sub>3</sub>	55	8	6	75	0.39
UBC 834	TT(AG) <sub>8</sub>	55	11	6	55	0.33
UBC 840	TT(GA) <sub>8</sub>	53	18	13	72	0.40
ISSR HB 10	(GA) <sub>6</sub> CC	44	6	0	0	0.00
UBC 856	AA(TC) <sub>8</sub>	56	14	8	57	0.25
UBC 872	(GATA) <sub>2</sub> (GACA) <sub>2</sub>	47	11	7	64	0.32
K 10	(AC) <sub>8</sub> YG	54	12	8	67	0.25
Average			12.6	8.36	63	0.34

<sup>a</sup>Total numbers of reproducible bands scored.

<sup>b</sup>Numbers of polymorphic bands.

<sup>c</sup>Percentage of polymorphic bands.

<sup>d</sup>Polymorphic information content.

## DISCUSSION

The percentage of polymorphic bands was higher for ISSRs (63%) than for RAPDs (50%). The mean number of amplification products obtained with RAPDs per primer (10.3) was fewer than with ISSRs (12.6). Also the mean of polymorphic information content for ISSRs (0.34) was higher than RAPDs (0.32). The dendrogram generated by the ISSR data matrix isolated resistant and susceptible cultivars better than RAPD data. In fact, ISSRs have a high capacity to reveal polymorphism and offer great potential to determine resistant and susceptible cultivars as compared to other arbitrary primers like RAPDs (Zietkiewicz et al., 1994). The ISSR technique has been used in genetic relationships in several other crops (Reddy et al., 2002). Based on the Mantel correspondence coefficient, the correlation between RAPD and ISSR Jaccard's similarity matrices was low in magnitude. The ability to resolve genetic variation among different genotypes may be more directly related to the number of polymorphisms detected with each marker technique rather than a function of which technique is employed. Clustering of cultivars within the groups was not similar when RAPD- and ISSR-derived dendrograms were compared. These differences may be attributed to a marker sampling error and/or the level of polymorphism detected, reinforcing again the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al. 1996). ISSR markers were more efficient than the RAPD assay as they detected 63% polymorphic DNA

markers as compared with RAPD markers which were 50%. Similar results were obtained for several other plant species like wheat (Nagoaka and Ogihara 1997). In accordance with Sofalian et al. (2008) ISSR markers were efficient tools for estimating genetic diversity in wheat. The highest polymorphism was observed in the case of K16 and ISSR 17899B primers. With regard to our goal to differentiate between resistant and susceptible cultivars to drought stress, ISSR three-dimensional plot was almost separated to two areas consisting of resistant and susceptible cultivars. In conclusion, the ISSR markers could be efficient for determination of genetic diversity and differentiating between resistant and susceptible wheat cultivars.

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