Bulgarian Journal of Agricultural Science, 20 (No 1) 2014, 87-95
Agricultural Academy

APPLICATION OF RAPD AND ISSR MARKERS TO ANALYSES MOLECULAR RELATIONSHIPS IN AZERBAIJAN WHEAT ACCESSIONS (*TRITICUM AESTIVUM* L.)

S. SADIGOVA[1], H. SADIGOV[1], R. ESHGHI[2], S. SALAYEVA[1] and J. OJAGHI[1*]
[1] *Azerbaijan National Academy of Sciences, Genetic Resources Institute, Az1106, Baku, Azerbaijan*
[2] *Islamic Azad University, Department of Agronomy and Plant Breeding, Science and Research Branch, 31567-56157, Ardabil, Iran*

Abstract

SADIGOVA, S., H. SADIGOV, R. ESHGHI, S. SALAYEVA and J. OJAGHI, 2014. Application of RAPD and ISSR markers to analyses molecular relationships in Azerbaijan wheat accessions (*Triticum aestivum* L.). *Bulg. J. Agric. Sci.,* 20: 87-95

To estimate genetic relationships of 33 wheat accessions originating from different regions of Azerbaijan Republic, RAPD and ISSR analysis was performed with 22 and 20 primers, respectively. The most discriminating primers were OPG-20, OPH-01, OPE-02 and OPG-08 for RAPD and ISSR-8, ISSR-11, ISSR-19 for ISSR which showed the highest values of genetic diversity. A high level of polymorphism was found with both RAPD and ISSR markers, and the mean genetic diversity values were 0.789 and 0.863 for RAPD and ISSR markers, respectively. In RAPD analyses, 319 out of 374 bands (84.89%) were polymorphic. The number of alleles ranged from 8 to 30 per primer, with an average of 17 per primer. In ISSR analyses, a total of 350 alleles were detected, among which 318 alleles (90.85%) were polymorphic. The number of alleles per primer ranged from 10 to 26 with an average of 17.5 alleles per ISSR primer. Cluster analyses indicated that both RAPD and ISSR markers could distinguish all 33 wheat accessions. Although the analysis of RAPD and ISSR markers could successfully be used to investigate the genetic diversity of the wheat accessions, the ISSR markers were superior to RAPD markers in the capacity of revealing more informative bands in a single amplification. In addition, ISSR analyses are more specific than RAPD analyses, due to the longer SSR-based primers with higher primer annealing temperature, which enable higher-stringency and greater band reproducibility amplifications.

Key words: Azerbaijan wheat accessions, genetic diversity, ISSR, RAPD

Introduction

Wheat is a staple food and thus one of the important agricultural crops, which is a foundation of human nutrition and of enormous economic importance both to Azerbaijan and worldwide. It is grown on ~654 thousand hectares of Azerbaijan area with total production of 1.594 million tons and an average yield of 2.437 tons/ha (FAO, 2011).

In Azerbaijan, the wheat crop faces the dual menace of biotic as well as abiotic stresses, which result in lower yields. It has been suggested that information about germplasm diversity and genetic relatedness among elite breeding material is a fundamental element in plant breeding. The value of diverse genetic base for resistance to diseases has recently been advocated in cereals (Garrett and Mundt, 1999; Zhu et al., 2000). Secondly, the future breeding program also depends upon the availability of genetic variability to increase the gain in productivity. Hence, to achieve a level of self-sufficiency and sustainability, there is a need to develop cultivars with diverse genetic base. Therefore, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation in future wheat breeding.

Molecular markers have been proved valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been showed that different markers might reveal different classes of variation (Powell, 1996; Russell, 1997). It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay (D´avila, 1999). The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques

*Corresponding author: javid_804@yahoo.com

such as random amplified of polymorphic DNA (RAPD), simple sequence repeats (SSR or microsatellite), sequence tagged sites (STS), random amplified microsatellite polymorphism (RAMP), and inter simple sequence repeat polymorphic DNA (ISSR) (Wu, 1994). These molecular markers had been used in wheat for detecting genetic diversity, genotype identification, and genetic mapping (Muhammad, 2002; Carvalho, 2009). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost, and the use of small amount of plant material. RAPDs were proved to be useful as genetic markers in the case of self-pollinating species with a relatively low level of intraspecific polymorphism, such as hexaploid wheat (Devos, 1992; Joshi, 1993). Sun et al. (1998), have studied genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. They showed that European spelt wheat and the Tibetan wheat have much higher genetic diversity than Chinese common wheat.

ISSR markers, which involve PCR amplifications of DNA using a primer, composed of a microsatellite sequence anchored at 3□ or 5□ end by 2–4 arbitrary could be used to assess genetic diversity (Qian, 2001). ISSRs have been used for cultivar identification for potatoes (Hu, 2003), wheat (Karaca, 2008), bean (Gonzalez, 2005), and barley (Zvingila, 2012). In this study, we evaluate the level and organization of the genetic diversity and relationship in Azerbaijan wheat cultivated using RAPD and ISSR markers, in order to establish a base line to assist future conservation and breeding programmes of this species. In addition, we aim to report the usefulness of RAPD and ISSR for the assessment of genetic diversity and relationships among wheat accessions.

Materials and Methods

Plant material. A total of 33 wheat accessions (*Triticum aestivum* L.) originating from different regions of the Azerbaijan Republic, were analyzed. All plant materials were sampled from the wheat collection of the Genetic Resources Institute of the Azerbaijan National Academy of Sciences. The wheat accessions used in this study and their origins are listed in Table 1.

DNA extraction. DNA was extracted from leaves of young plants grown in MS culture medium (Murashige and Skoog, 1962) for about three weeks at -20˚C and photoperiod of 13 hours. Total cellular DNA was extracted from 0.4 g of material using the protocol described by Dellaporta et al. (1983). Final Pellets were solubilized in 250 µl TE solutions and kept at -20˚C. The DNA concentration of all samples was determined in agarose gels comparing the DNA from the samples with l DNA solutions of different concentration.

RAPD and ISSR amplification

The RAPD and ISSR primers were chosen from literature records based on their ability to reveal high levels of

Table 1
Names and origins of wheat accessions investigated in the study

No.	Names	Origin	No.	Names	Origin
1	Barbarossa 143	Absheron	18	Albidum 199	Nakhchivan
2	Cyanotrix 53	Absheron	19	Ps. Hostianum 70	Nakhchivan
3	Qlaucolutescens 21	Absheron	20	Delfi 311	Nakhchivan
4	Ps. Merideonale 74	Absheron	21	Griseum 27	Nakhchivan
5	Murinum 319	Absheron	22	Alborubum 173	Shamakhi
6	Rubnomurinum 54	Absheron	23	Ferrugineum 298	Shamakhi
7	Nigroaristatum 310	Absheron	24	Lutescens 187	Barda
8	Akinçi	Absheron	25	Erithrospermum 86	Barda
9	Qrecum 275	Absheron	26	Erythroleucon 219	Oguz
10	Qlaucolutescens 77	Absheron	27	Leucospermum 317	Oguz
11	Fuliginossium 96	Absheron	28	Grecum 1	Mirbashir
12	Meredionale 111	Nakhchivan	29	Milturum 282	Goranboy
13	Ps. Barbarossa 113	Nakhchivan	30	Velutinum 109	Tovuz
14	Turcicum 127	Nakhchivan	31	Introitum 56	Bilesuvar
15	Pyrotrix 169	Nakhchivan	32	Erithrospermum 246	Qazakh
16	Hostianum 125	Nakhchivan	33	Filfosinerum 313	Qarayazi
17	Renzovatum 28	Nakhchivan			

polymorphism. The sequences of the 22 RAPD and 20 ISSR primers used in the study are shown in Table 2.

The PCR reaction were performed in a 25 µl volume containing the reaction buffer (10 mM Tris–HCl pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$) plus 2 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µ primer, approximately 35 ng of template DNA, and 2 units of Tag DNA polymerase. The thermal cycle used was 94˚C for 3min; then 45 cycles of 94˚C for 1min, 35 C˚ (for RAPD analysis) or 50˚C (for ISSR analysis) for 2min; and finally 72˚C for 5min. A negative control PCR tube containing all components except genomic DNA was included in all thermal cycle runs. Amplification products were fractionated on 1.4% (for RAPD analysis) or 2% (for ISSR analysis) agarose gel. A 100 bp ladder was used as a molecular size standard. Each sample was processed at least twice to confirm genotype reproducibility.

Data analysis. Photographs were performed with a Gel Doc 1000 camera (Molecular Analyst program of Bio-Rad). Data were recorded as presence (1) or absence (0) of each amplification band, to construct the band matrix. After the identification of bands and patterns among genotypes, a genetic diversity index was calculated based on the following formula (Nei, 1973) for each marker: $H = 1 - \Sigma Pi\ 2$, where H – genetic diversity index; Pi – frequency of the ith pattern.

A dendrogram representing genetic similarity was constructed according to Jaccard's similarity index following the

Table 2
Primer sequences, total number of bands, number of polymorphic bands, percent of polymorphic bands and genetic diversity index

Primer	Sequence (5'-3')	Total number of bands	Number of polymorphic bands	Polymorphism, %	Genetic diversity index
RAPD					
OPH-01	GGTCGGAGAA	28	28	100.0	0.929
OPH-11	CTTCCGCAGT	14	12	85.71	0.733
OPH-15	AATGGCGCAG	21	17	80.95	0.836
OPH-20	GGGAGACATC	18	12	66.66	0.748
OPA-02	TGCCGAGCTG	12	11	91.66	0.725
OPA-06	GGTCCCTGAC	13	10	76.92	0.743
OPG-02	GGCACTGAGG	9	8	88.88	0.693
OPG-05	CTGAGACGGA	10	7	70.00	0.651
OPG-08	TCACGTCCAC	20	18	90.00	0.904
OPG-20	TCTCCCTCAG	30	26	86.66	0.932
OPQ-01	GGGACGATGG	15	14	93.33	0.799
OPO-02	ACGTAGCGTC	11	11	100.0	0.781
OPO-06	CCACGGGAAG	18	17	94.44	0.860
OPO-09	TCCCACGCAA	22	13	59.09	0.811
OPO-18	CTCGCTATCC	16	11	68.75	0.782
OPB-09	TGGGGGACTC	8	8	100.0	0.654
OPB-18	CCACAGCAGT	15	12	80.00	0.821
OPE-02	GGTGCGGGAA	29	29	100.0	0.917
OPN-04	GACCGACCCA	9	9	100.0	0.704
OPN-05	ACTGAACGCC	25	19	76.00	0.862
OPN-08	ACCTCAGCTC	12	9	75.00	0.671
OPI-01	ACCTGGACAC	19	14	73.68	0.823
Mean	-	17	14.5	84.89	0.789
ISSR					
ISSR-1	ATA TAT ATA TAT ATA TT	12	11	91.67	0.763
ISSR-2	AGA GAG AGA GAG AGA GT	15	13	86.67	0.863
ISSR-3	GAG AGA GAG AGA GAG AT	10	8	80.00	0.675
ISSR-4	CTC TCT CTC TCT CTC TT	18	16	88.89	0.837

Table 2 ***Continued***

ISSR-5	GTG TGT GTG TGT GTG TA	14	12	85.71	0.838
ISSR-6	TCT CTC TCT CTC TCT CA	19	16	84.21	0.914
ISSR-7	ACA CAC ACA CAC ACA CT	17	16	94.12	0.919
ISSR-8	TGT GTG TGT GTG TGT GA	26	22	84.61	0.939
ISSR-9	ATA TAT ATA TAT ATA TYA	17	16	94.12	0.861
ISSR-10	AGA GAG AGA GAG AGA GYT	15	15	100.0	0.848
ISSR-11	TAT ATA TAT ATA TAT ART	23	21	91.30	0.932
ISSR-12	GAG AGA GAG AGA GAG AYT	18	17	94.44	0.917
ISSR-13	CTC TCT CTC TCT CTC TRA	17	16	94.12	0.851
ISSR-14	GTG TGT GTG TGT GTG TYC	18	16	88.89	0.909
ISSR-15	ACC ACC ACC ACC ACC ACC	19	17	89.47	0.831
ISSR-17	GGCGGCGGCGGCGGC GGC	16	15	93.75	0.845
ISSR-19	CTA GCT AGC TAG CTA G	24	22	91.67	0.928
ISSR-22	TAG ATC TGA TAT CTG AAT TCC C	17	17	100.0	0.848
ISSR-24	CAT GGT GTT GGT CAT TGT TCC A	15	14	93.33	0.849
ISSR-25	ACT TCC CCA CAG GTT AAC ACA	20	18	90.00	0.892
Mean		17.5	15.9	90.85	0.863

R=Purine, Y=Pyrimidine

UPGMA (unweighted pair group method with arithmetic averages) from the genetic distance matrix, as a graphic representation of the relationships among samples. These analyses were performed using the Power marker software package (Liu, 2005).

Results and Discussions

A set of fifty-four RAPD and forty ISSR primers were used for initial screening of thirty-three wheat accessions (*Triticum aestivum* L.) for genetic analysis. Of the fifty-four RAPD and forty ISSR primers, some primers produced no distinct bands on a smeary background and some of them resulted in very faint bands upon a highly smeared background. The remaining twenty-two RAPD and twenty ISSR primers produced clear amplification patterns. The twenty-two RAPD and twenty ISSR selected primers were used to examine the level of polymorphism detectable in the thirty-three populations of *T. aestivum* sampled in Azerbaijan. As an example, the pattern obtained for some cultivar with OPE-2 and ISSR-8primers is shown in Figure 1.

Marker efficiency and polymorphism degree

By using the twenty-two RAPD primers the size of amplification products selected for statistical analysis ranged from 160 to 2800bp. PCR amplification of the DNA isolated from thirty-three wheat genotypes yielded a total of 374 amplified products, of which 315 were polymorphic (Table 2). For each primer, the number of bands ranged from 8 to 30, with an average of 17. These data were compared with some other reports performed in wheat; Thomas et al. (2006) observed 246 polymorphic fragments with 41 RAPD markers for char-

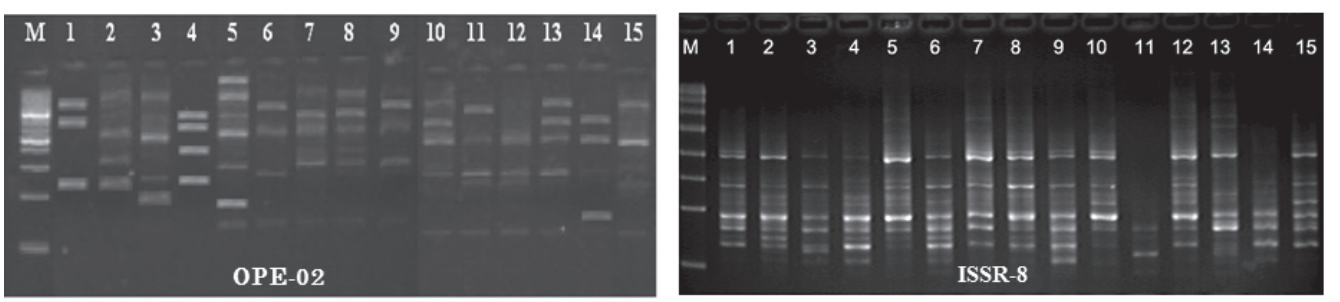

Fig. 1. An example of electrophoregram obtained with primer OPE-02 (RAPD) and ISSR-8 (ISSR); the numbers indicate cultivars as listed in Table 1

acterization of Indian commercial wheat, Ojaghi and Akhundova (2010) determined 273 polymorphic bands among 102 doubled haploid wheat accessions, while Akar and Ozgen (2007) detected 89.96% of polymorphic bands in Turkish durum wheat landraces.

In our study the most and the least replicated DNA fragments were related to primers OPG-20 and OPB-09, respectively. The highest numbers of polymorphic bands were those of primers OPE-02, OPH-01, and OPG-26 and the lowest number of polymorphic band were that of primer OPG-05. The average number of polymorphic bands was 14.3 for each individual primer. The highest percentage of polymorphic bands was detected with the primers OPH-01 (100%), OPE-02 (100%), OPO-02 (100%), OPN-04 (100%), OPB-09 (100%) and OPO-06 (94.44), whereas the lowest percentage of polymorphic bands related to the primers OPO-09 (59.09%), OPH-20 (66.66%) and OPO-18 (68.75%). The average of observed polymorphism per primer was 84.89%. The most discriminating primers were OPG-20, OPH-01, OPE-02 and OPG-08, which showed the highest values of genetic diversity (0.932, 0.929, 0.917 and 0.904, respectively). The lowest values of diversity pertained to the markers OPG-05 (0.651), OPB-09 (0.654) and OPN-08 (0.671). In total, the primers OPG-20, OPH-01 and OPE-02 with the highest number of polymorphic bands and the highest value of genetic diversity were recognized to be the most appropriate primers for studies related to genetic diversity of wheat accessions.

The twenty ISSR primers amplified a total of 350 bands in the set of thirty-three wheat accessions, of which 318 bands were polymorphic. The number of bands varied from twelve (ISSR-1) to twenty-six (ISSR-8).

The percentage of polymorphic bands ranged between 80 and 100 with an average of 90.85% (Table 2). The mean numbers of bands and polymorphic bands per primer were 17.5 and 15.9, respectively. Variable efficiencies of different marker systems for detecting DNA polymorphism in wheat have been reported. Joshi and Nguyen (1993) observed 1.8 polymorphic bands per RAPD primer among 15 wheat cultivars, while SSRs with 6.2 alleles/bands were more polymorphic (Plaschke, 1995). The number of RFLP polymorphic bands per probe/enzyme combination in 124 bread wheat cultivars was 3.3 (Paul, 1998). Altintas et al. (2008) observed 47% polymorphism among 22 bread wheat cultivars using five AFLP and three SAMPL primer pairs with an average of 20.4 polymorphic loci per primer pair. Nagaoka and Ogihara (1997) detected 3.7 polymorphisms per ISSR primer, while Carvalho et al. (2009) reported 12.9 polymorphic bands per primer using 18 ISSR primers in 48 wheat accessions. We detected a high level of polymorphism among the wheat genotypes using ISSRs, indicating high efficiency of the marker technique to reveal genetic diversity in the case of wheat. The lowest polymorphism value (80%) was obtained with the ISSR-3 primer $(GA)_8T$ (Table 2).

The highest genetic diversity index among the primers used in this study and among all the genotypes was that of primer ISSR-8, ISSR-11, ISSR-19 and the lowest genetic diversity was that of primer ISSR-3. The mean of total genetic diversity among all the primers and for all the samples was calculated as 0.863, which indicates that the population under study has had a considerable diversity at DNA level. All the 20 used primers could identify all Azerbaijan wheat genotypes. The suitability of the ISSR technique for genetic diversity studies and germplasm evaluations has been shown in many studies (Rizkalla et al., 20012; Rekha et al., 2012; Karaca et al., 2008; Sofalian et al., 2008).

Genetic relationship and distance between the genotypes

Jaccard similarity matrix based on RAPD binary data was used to group the wheat accessions using the complete linkage (CLINK) method (Figure 2). The dendrogram obtained from the method, in comparison with the UPGMA method had higher cophenetic correlation and no chaining. According to the results obtained from the dendrogram, all the genotypes were divided into five separate groups at a similarity index 0.5. Cluster 1included genotypes Pyrothrix169 and Renovatum28 from Nakhchivan region, İntroitum56 from Bilesuvar region and the rest genotypes belonged to the *Absheron peninsula*. As observed, 57.14% of the genotypes in this cluster are originating from the *Absheron peninsula*.

Among the samples in this group, the Renovatum28 genotype and İntroitum56 sample appeared very close genetically, with a similarity index 0.78.

The second group contained 5 genotypes, i.e. 15.33% of all genotypes; 3 of them were originating from Nakhchivan region (Ps.Barbarossa-113, Hostianum-125 and Albidum-199), but the Alborubum173 and Erithrospermum86 samples were originating from Shamakhi and Barda regions, respectively. The highest level of genetic similarity among the samples in this group related to Erithrospermum86 and Albidum199 with a similarity index 0.78.

In the third group, the lowest genetic distance was observed between Meredionale111 sample originating from Nakhchivan and Lutescens187 sample originating from Barda region. The lowest genetic similarity index was studied between Griseum27 from Nakhchivan and Cyanotrix53 sample from Absheron region, with a similarity index 0.52. In addition, one genotype from Georgia (Qarayazi) has resided in this group.

The fourth group included only three genotypes from Nakhcivan, Shamakhi and Qazakh regions. Therefore, this

cultivar belongs to a separate group, which indicates the genetic distance of these samples from the other investigated genotypes.

Finally, the fifth group consisted of 12 genotypes, which made up 36.36% of all examined genotypes. In this group genotypes representative from different regions like Nakhchivan, Oguz, Mirbashir, Goranboy and Tovuz were grouped. The highest similarity index was studied between Graecum1 from Mirbashir region and Turcicum127 from Nakhchivan region. In contrast, the lowest level of genetic similarity belonged to the Qlaucolutescens77 from Absheron and Erythroleucon219 from Oguz region.

A dendrogram representing Jaccard genetic similarity by ISSR markers among all selected samples is demonstrated in Figure 3. If the cutting is done from a distance of 0.5, the genotypes will be classified into 5 groups. An average genetic similarity among all studied samples was 0.567. The first cluster contained 7 accessions which made up 21.21% of all examined wheat genotypes, all originating from the Nakhchivan region with the exception of one genotype (Erythroleucon219) from the Oguz region. The other 4 genotypes of Nakhchivan region were grouped into the second (Renovatum28, Albidum199) and fourth (Ps. Hostianum70, Delfi311) cluster. In this cluster, the highest genetic similarity was ob-

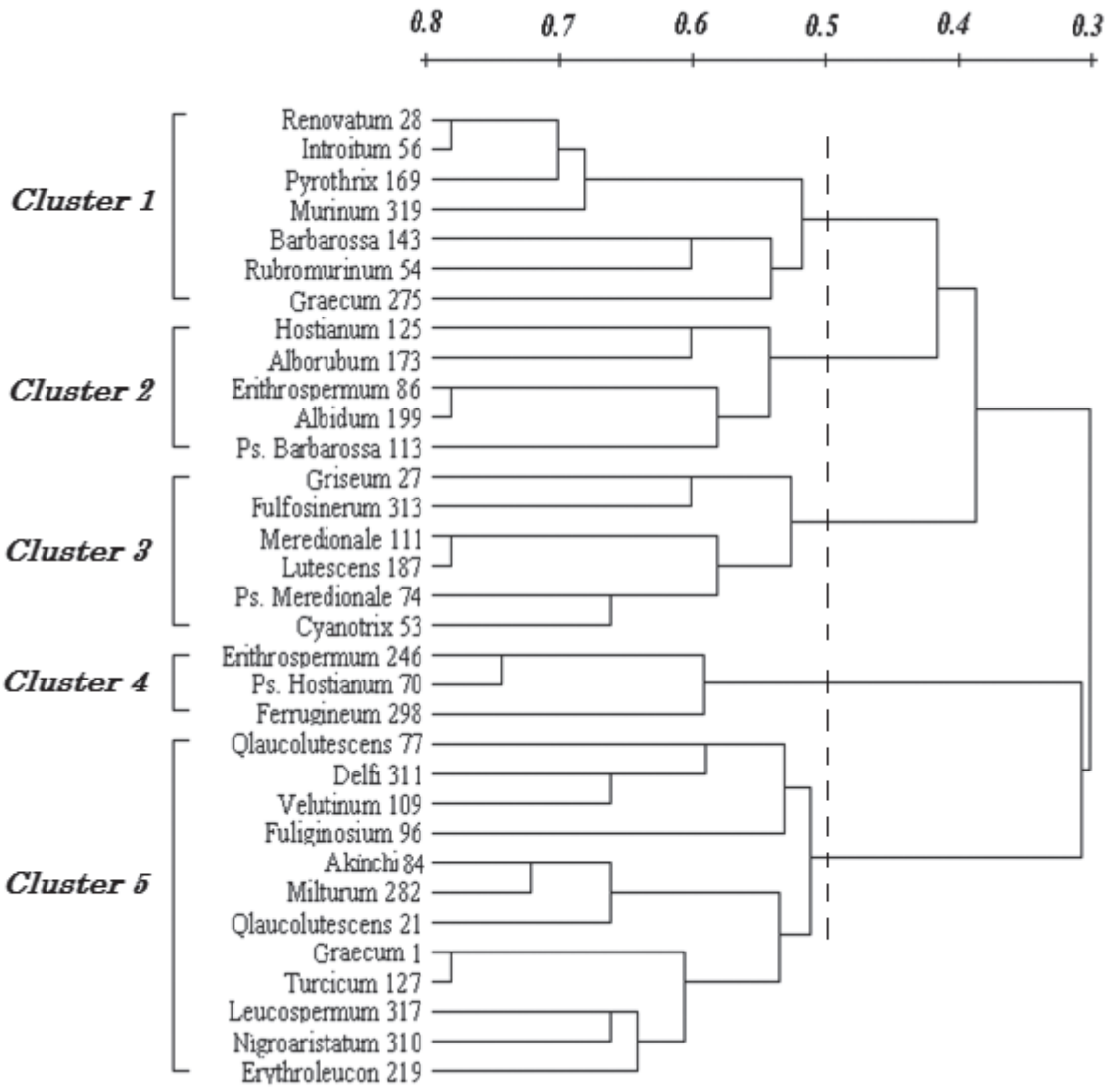

Fig. 2. Dendrogram showing the genetic relationship among investigated Azerbaijan wheat accessions by RAPD markers

served between the Turcicum127 and the Ps.Barbarossa133 with a similarity index of 0.78. In contrast, the lowest level of similarity was studied between Meredionale111 and Erithroleucon219, with a similarity index of 0.53.

In the second group, as well as two genotypes from Nakhchivan region, two genotypes from Absheron Peninsula (Fuliginosium96 and Qlaucolutescens77), one genotype from each region of Barda and Tovuz (Erithrospermum86 and Velutinum109, respectively) were included.

The fourth cluster was assigned the highest number of genotypes, i.e. 39.4% of all genotypes, among the five separated groups. As observed, 81.18% of the genotypes from Absheron Peninsula (Barbarossa143, Cyanotrix53, Qlaucolutescens21, Ps. Meredionale74, Murinum319, Rubromurinum54, Nigroaristatum310, Akinchi84 and Graecum275) were grouped in this cluster. In addition to the two samples from Nakhchivan region (Delfi311 and Ps. Hostianum70), one genotype from each region of Oguz and Gazakh (Leucospermum317 and Fulfosinerum313, respectively) were located in this group. The highest level of genetic similarity among the samples in this group related to Ps.Meredionale74 and Akinchi84. The lowest level of genetic similarity belonged to the Delfi311 and Qlaucolutescens21.

Finally, the fifth group included only two genotypes originating from Shamakhi region (Ferrugineum298 and Albo-

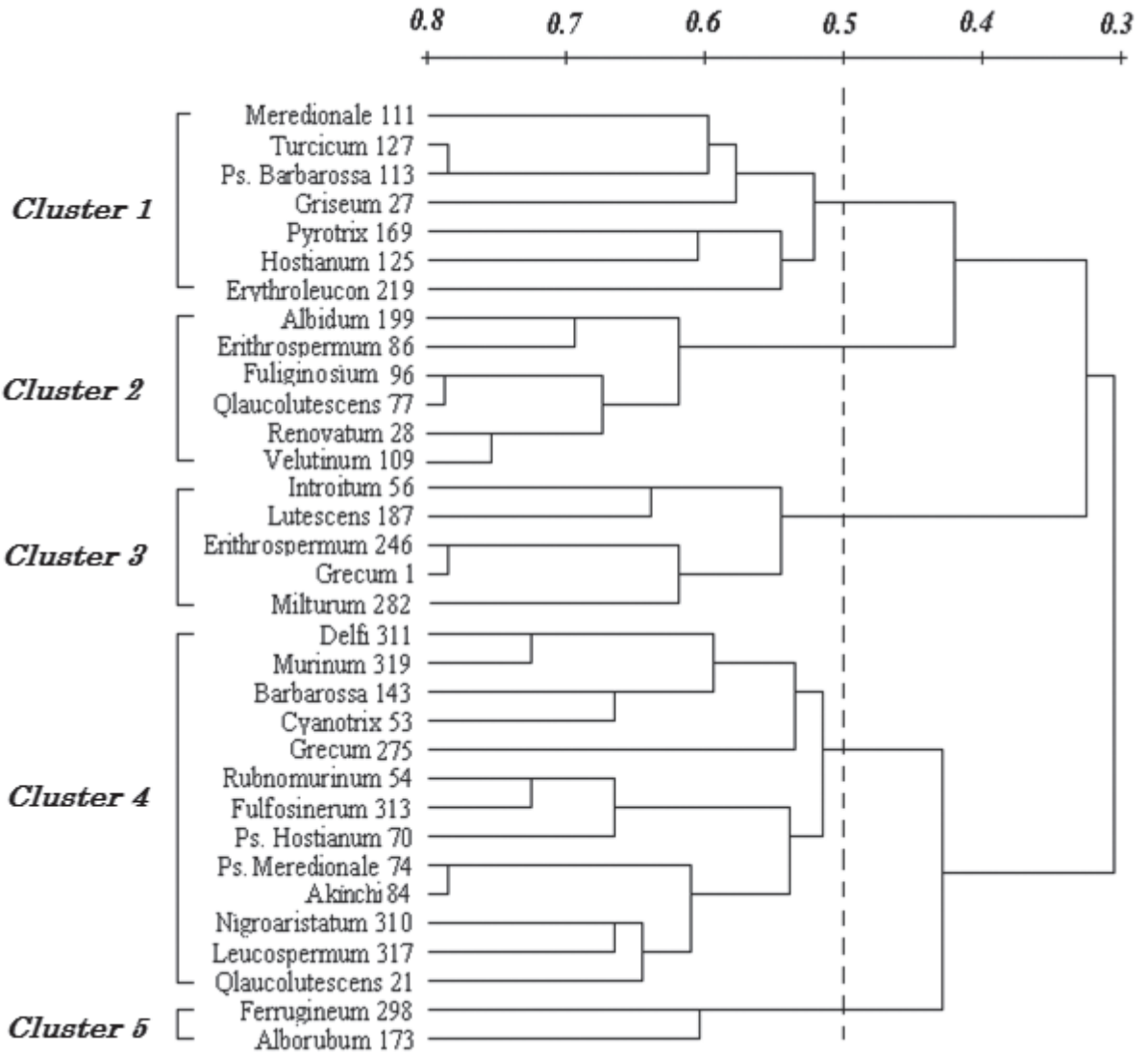

Fig. 3. Dendrogram showing the genetic relationship among investigated Azerbaijan wheat accessions by ISSR markers

rubum173), which shows the existence of genetic distance between these genotypes and other investigated samples.

In the present study, the dendrogram generated using Jaccard's similarity index showed that the observed genetic diversity of wheat material sampled in Azerbaijan is not completely geographically structured.

Conclusion

Comparing the results obtained from ISSR and RAPD showed that there was no significant correlation between the RAPDs and ISSRs matrices. Therefore, genetic diversity pattern apparently differed based on different methods and classifying the germplasm yielded different results when different methods were applied.

It seems that since each of these methods demonstrates different aspects of diversity in different populations, simultaneous application of these methods can present researchers a brighter view of diversity. However, the assessment of genetic variation in large samples of plant genetic resources requires a high costing time and amount of consumables. Thus, reliable, affordable and economical techniques should be preferred, at least in the first screening of genetic variability. Obviously, assessment of agro-morphological traits takes several months and requires considerable costs; even in some cases, die to influential role of environment and mutual effects of genotype × environment in the emergence of these traits, it is necessary to repeat the experiments throughout several years and in different places. The results indicated that, although the total number of amplification RAPD bands (374) was more than that of ISSR (350), the mean number of polymorphic bands (15.9) detected by ISSR primers was higher than that of the RAPDs (14.5). In addition, the percentage of polymorphic bands (90.85%) and the mean genetic diversity index (0.863) in ISSR was higher than the RAPD. Therefore, the results in this study suggested that, although the analysis of RAPD and ISSR markers could successfully be used to investigate the genetic diversity of the wheat accessions, the ISSR markers were superior to RAPD markers in the capacity of revealing more informative bands in a single amplification. In addition, ISSR analyses are more specific than RAPD analyses, due to the longer SSR-based primers with higher primer annealing temperature, which enable higher-stringency and greater band reproducibility amplifications. Therefore, if resources are the main limiting factor, we find that ISSR markers must be the technique of choice for the first estimation of genetic diversity in wheat resources collections.

References

Akar, T. and M. Ozgen, 2007. Genetic Diversity in Turkish Durum Wheat Landraces. *Wheat Production in Stressed Environments Developments in Plant Breeding*, **12:** 753-760.

Altintas, S., F. Toklu, S. Kafkas, B. Kilian, A. Brandolini and H. Ozkan, 2008. Estimating genetic diversity in durum and bread wheat cultivars from Turkey using AFLP and SAMPL markers. *Plant Breed*., **127:** 9-14.

Carvalho, A., J. Lima-Brito, B. Macas and H. Guedes-Pinto, 2009. Genetic diversity and variation among botanical varieties of old Portuguese wheat cultivars revealed by ISSR assays. *Biochem. Genet*., **47:** 276-294.

D´avila, J. A., Y. Loarce, L. Ramsay, R. Waugh and E. Ferrer, 1999. Comparison of RAMP and SSR markers for the study of wild barley genetic diversity. *Hereditas*, **131:** 5-13.

Dellaporta, S. L., J. Wood and J. B. Hicks, 1983. A plant DNA mini-preparation: version II. *Plant Mol. Biol. Rep.*, **1:** 19.

Devos, K. M. and M. D. Gale, 1992. The use of random amplified polymorphic DNA markers in wheat. *Theoretical and Applied Genetics*, **84:** 567-572.

FAO (Food and Agriculture Organization of the United Nations), 2011. FAOSTAT. http://faostat.fao.org/.

Garret, K. A. and C. C. Mundt, 1999. Epidemiology in mixed host populations. *Phytopathology*, **89:** 984-990.

Gonzalez, A., A. Wong, A. Delgado-Salinas, R. Papa and P. Gepts, 2005. Assessment of Inter Simple Sequence Repeat Markers to Differentiate Sympatric Wild and Domesticated Populations of Common Bean. *Crop Sci*., **45:** 606-615.

Hu, J., M. Nakatani, A.G. Lalusin, T. Kuranouchi and T. Fujimura, 2003. Genetic analysis of sweet potato and wild relatives using inter-simple sequence repeats (ISSRs). *Breed Sci,* **53:** 297-304.

Joshi, C. P. and H.T. Nguyen, 1993. RAPD (random amplified polymorphic DNA) analysis based on intervarietal genetic relationship among haploid wheat. *Plant Sci*., **93:** 95-103.

Karaca, M. and A. Izbirak, 2008. Comparative analysis of genetic diversity in Turkish durum wheat cultivars using RAPD and ISSR markers. *Journal of Food, Agriculture & Environment*, **6:** 219-225.

Liu, K. and S.V. Muse, 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics,* **21:**2128-2129.

Muhammad Sahid, M., R. Mehboob-ur and Y. Zafar, 2002. Assessment of genetic diversity among wheat (*Triticum aestivum* L.) cultivars from a range of localities across Pakistan using random amplified polymorphic DNA (RAPD) analysis. *Euphytica,* **128:** 417-425.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultivars. *Physiol. Plant*, **15:** 437-497.

Nagaoka, T. and Y. Ogihara, 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet*., **94:** 597-602.

Nei, M, 1973. Analysis of gene diversity in subdivided population. *Proceedings of the National Academy of Sciences*, **70**: 3321-3323.

Ojaghi, J. and E. Akhundova, 2010. Genetic diversity in doubled haploids wheat based on morphological traits, gliadin protein

patterns and RAPD markers. *African Journal of Agricultural Research*, **5:** 1701-1712.

Paul, J. G., K. J. Chalmers, A. Karakousis, J. M. Kretschmer, S. Manning and P. Langridge, 1998. Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. *Theor. Appl. Genet*., **96**: 435-446.

Plaschke, J., M. W. Ganal and M. S. Roder, 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor. Appl. Genet*., **91:** 1001-1007.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski, 1996. "The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, **2:** 225-238.

Qian, W., S. Ge and D. Y. Hong, 2001. Genetic variation within and among populations of a wild rice Oryza granulata from China detected by RAPD and ISSR markers. *Theoretical and Applied Genetics*, **102:** 440-449.

Rekha, M., S. Sindhu, K. Sushila and S. Jag, 2012. The use of SSR and ISSR markers for assessing DNA polymorphism and genetic diversity among Indian bread wheat cultivars. *Indian Journals*, **12:** 82-89.

Rizkalla, S. A., A. Attia, A. Abd E-Hady, N. S. Hanna and J. E. Nasseef, 2012. Genetic Diversity Based on ISSR and Protein Markers Associated with Earliness Trait in Wheat. *World Applied Sciences Journal*, **20:** 23-33.

Russell J. R., J. D. Fuller, M. Macaulay, B. G. Hatz, A. Jahoor, W. Powell and R. Waugh, 1997. "Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. *Theoretical and Applied Genetics*, **95:** 714-722.

Sofalian, O., N. Chaparzadeh, A. Javanmard and M. S. Hejazi, 2008. Study the Genetic Diversity of Wheat Landraces from Northwest of Iran Based on ISSR Molecular Markers. *International Journal of Agriculture & Biology*, **10:** 466–468.

Sun, Q., Z. Ni, Z. Liu, J. Gao and T. Huang, Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. *Euphytica*, **99:** 205-211.

Thomas, G., T. Mohapatra, A. R. Rao and R. P. Sharma, 2006. Distinguishing Indian commercial wheat varieties using RAPD based DNA fingerprints. *Indian Journal of Biotechnology*, **5:** 200-206.

Wu, K.S., R. Jones, L. Danneberger and P.A. Scolnik, 1994. Detection of microsatellite polymorphisms without cloning. *Nucleic Acids Research*, **22:** 3257-3258.

Zhu, Y., H. Chen, J. Fan, Y. Wang, Y. Li, J. Chen, J. Fan, S. Yang, L. Hu, H. Leung, T. W. Mew, P. S. Teng, Z. Wang and C. C. Mundt, 2000. Genetic diversity and disease control in rice. *Nature,* **406:** 718-722.

Zvingila, D., V. Vaitkuniene, J. Patamsyte, A. Leistrumaite, M. Staniute, L. Balciuniene, T. Cesniene, V. Kleizaite, R. Siuksta and R. Rancelis, 2012. DNA polymorphism and agronomic traits of revertants from barley (*Hordeum vulgare* L.) mutant *tw. Agriculture*, **99:** 139-148.

Received April, 10, 2013; accepted for printing December, 2, 2013.