Bulgarian Journal of Agricultural Science, 20 (No 6) 2014, 1413-1420
Agricultural Academy

EVALUATION AND COMPARISON OF ISSR AND RAPD MARKERS FOR ASSESSMENT OF GENETIC DIVERSITY IN TRITICALE GENOTYPES

F. A. TONK*, M. TOSUN, E. ILKER, D. ISTIPLILER and O. TATAR
Department of Field Crops, Faculty of Agriculture, Ege University, Bornova, Izmir, Turkey

Abstract

TONK, F. A., M. TOSUN, E. ILKER, D. ISTIPLILER and O. TATAR, 2014. Evaluation and comparison of ISSR and RAPD markers for assessment of genetic diversity in triticale genotypes. *Bulg. J. Agric. Sci*., 20: 1413-1420

Assessments of genetic diversity in a gene pool facilitate effective selection and shorten the breeding time. Two PCR-based marker approaches, Inter-simple sequence repeat (ISSR) and Random amplified polymorphic DNA (RAPD) were used to evaluate genetic diversity of 16 triticale genotypes grown in Turkey and relative efficiencies of the marker systems were compared. From 28 and 21 primers were utilized in ISSR and RAPD markers, respectively. Effective multiplex ratio (EMR), marker index (MI), resolving power (Rp) and polymorphic information content (PIC) of the primers were calculated for the two marker systems and all the parameters examined found to be higher in ISSR system. The Mantel test revealed a highly significant correlation (r = 0.74) between the Jaccard's similarity matrices of RAPD and ISSR. The most remarkable result of the current study is that cluster analysis on ISSR and RAPD data clearly discriminated the genotypes in terms of their growth habit and origin, respectively and triticale genotypes were clustered from two different points of view and both of clustering provides consistent results of their qualifications. On the basis of results of this study, it can be concluded that in future study of genetic diversity like here especially in breeding programs of triticale genotypes, more than one marker systems should be used for higher genetic resolution of the genome.

Key words: genetic diversity, triticale, ISSR, RAPD

Introduction

Triticale (x*Triticosecal*e Wittmack) is a self-pollinated crop derived from a synthetic hybrid made by crossing of wheat and rye (*Secale cereale*). Tetraploid wheat (*Triticum durum* L.) has been used as a parent to obtained hexaploid triticale that is commonly used. When hexaploid wheat (*T. aestivum* L.) contained the genes not found or rarely found in *T. durum*, octoploid triticale has been made using hexaploid wheat as female parent. Compared to the other cereals, triticale is able to resist same abiotic stresses related to climatic (drought, extreme temperatures, etc.) and soil conditions (extreme pH levels, salinity, trace elements deficiency or toxicity, etc.) and thus has advantages to produce high yield under marginal land conditions (Mergoum et al., 2004). According to the Food and Agriculture Organization (FAO), triticale production was made in 3.7 million ha area in 36 countries across the world in 2012. The primary producers of triticale are Belarus, Chine, France, Germany and Poland (FAOSTAT, 2012). It is used as grain, silage and forage crop in animal feeding (Myer and Lozano del Rio, 2004) and in making of variety of breads, cookies, biscuits and cakes etc. in food uses (Pena, 2004).

Even though wheat and rye are still sometimes utilised as genetic resources of triticale, an immediate and a major gene pool for triticale improvement is available triticale varieties and germplasm (Kuleung et al., 2006). Thus, having information about genetic diversity of this gene pool would facilitate effective parent selection and shorten the breeding time. DNA-based molecular markers play an essential role to identify genetic diversity in plants and have some advantages in contrast with morphological markers and pedigree method. Because of detecting of variation at the DNA level, they are not influenced environmental factors. Knowledge of pedigree information of studies genotypes is not necessary and number of markers does not have limitation. RAPD (Random amplified polymorphic DNA) markers, which depends on random amplification of DNA with short primers (Wil-

*Corresponding author: fatma.aykut@ege.edu.tr

liams et al., 1990), and ISSR (Inter-simple sequence repeat) markers that amplify the inter-SSR sequences of different sizes (Zietkiewicz et al., 1994) are useful PCR (Polymerase chain reaction) techniques for estimating the genetic diversity in plants. Both markers are widely used for genetic diversity studies in many species such as wheat (Liu et al., 1999; Sofalian et al., 2008), barley (Tanyolaç, 2003), cotton (Rana et al., 2006), triticale (Sözen, 2010), Turkish oregano (Tonk et al., 2010) and St. John's work (Tonk et al., 2011). Though both spring and winter varieties of triticale are grown in Turkey, there is limited number of studies investigated the genetic diversity of the varieties. Therefore, our objectives in this study were to evaluate genetic diversity of some triticale genotypes grown in Turkey and to compare relative efficiencies of ISSR and RAPD markers in respect to applicability in genetic diversity researches of triticale genotypes.

Materials and Methods

Plant materials and DNA extraction

Triticale genotypes used in this study are listed in Table 1 with their origins and pedigrees. First ten genotypes of triticale were obtained from CIMMYT (International Maize and Wheat Improvement Center) in the past years and selected as superior genotypes in respect to yield in field trials. Seeds of Focus, Mikham-2002, Tatlıcak-97 and Melez-2001 were provided from Bahri Dağdaş International Agricultural Research Institute, Konya and the seeds of Tacettinbey and Ege Yıldızı were obtained from Çukurova University, Faculty of Agriculture, Adana and Aegean Agricultural Research Institute, Izmir, respectively. The varieties of Focus, Mikham-2002, Tatlıcak-97 and Melez-2001 have winter growth habit while other genotypes have spring growth habit. The seeds of each genotype were sown in small pots and grown under controlled conditions for DNA extraction. Genomic DNA from young leaves of each genotype was isolated by DNA mini-extraction from fresh leaf tissue (Doyle and Doyle 1987). Isolated DNA was treated with RNAse and Proteinase K in order to remove RNA and protein. Quantification of the DNA concentration was done using Biophotometer (Eppendorf) at absorbencies of 260 and 280 nm. Dilutions that gave 4 ng/µl for ISSR and 5 ng/µl for RAPD analyses were calculated from the absorbance measured at 260 nm.

ISSR and RAPD analyses

ISSR analysis was carried out in a total reaction volume of 15 µl containing 20 ng genomik DNA, 80 µM dNTP, 0.2 mM primer (University of British Columbia), 1x *Taq* polymerase reaction buffer (100 mM Tris-HCL, pH: 8.3, 500 mM KCL, 15 mM $MgCl_2$ and 0.01% gelatin), 1 unit of Taq DNA polymerase (Sigma). PCR amplification was performed in initial denaturation for 2 min at 96°C, 35 cycles each consisting of 1 min at 94°C, 1 min at 50-56°C, 2 min at 72°C, and final extension for 5 min at 72°C.

In RAPD analysis, polymerase chain reactions (PCR) were performed in 15 µl reaction mixture that containing 25

Table 1
Triticale lines and varieties used in this study, with origin and pedigree

Genotypes	Origin	Pedigree
9	CIMMYT	KER-6/FARAS-1//BULL-2/3/ERİZO-11/YOGUI-3
18	CIMMYT	POLLMER-4/5/TAPIR-YOGUI-1//*2MUSK/3/
33	CIMMYT	YOGUI- 3/ERİZO-11//ONA-2//POOS-1-2
805	CIMMYT	POLLMER-2.1.1
815	CIMMYT	DAHBI/COATI-1//ASAD/FAHAD
829	CIMMYT	STIR-22-1/NIMIR-3/6/IA-T/M2A//PI3/BG1/5/…
835	CIMMYT	POLLMER-2.2.1*2//FARAS/CMH84.4414
Eronga-83	CIMMYT	DRIRA//KISS/ARM
Juanillo	CIMMYT	DRIRA//KISS/ARM
Beaguelita	CIMMYT	BGL/3/BGL//ITA/LEO-T
Focus	Turkey	Unavailable
Mikham-2002	Turkey	BDMT-19/JGS1 BDKT910036-3F4BD-0BD
Tacettinbey	Turkey	Unavailable
Tatlıcak-97	Turkey	Unavailable
Ege Yıldızı	Turkey	Unavailable
Melez-2001	Turkey	GT-AD-1/91//CWT1988/79/10 BDKT910018-3F4BD-0BD

ng DNA template, 1x *Taq* polymerase reaction buffer (100 mM Tris-HCl, pH: 8.3, 500 mM KCl, 15 mM MgCl2 and 0.01% gelatin), 100 mM each dNTP, 15 ng primer (Operon Technologies, USA), and 1 unit of Taq DNA polymerase (Sigma). PCR program consisted of initial denaturation for 30 s at 94°C, followed by 35 cycles of 25 s at 94°C, 45 s at 35°C, 1 min at 72°C, and final extension for 5 min at 72°C. Both ISSR and RAPD amplifications were performed in Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany). ISSR and RAPD products were separated by with 1x TAE buffered gel electrophoresis in 1.5% and 1.8% (w/v) agarose, respectively. Then, the gels were stained with ethidium bromide and visualized under UV transillumination (Bio-Vision 1000). λDNA double digest (*Hind*III/*Eco*RI) was used as molecular weight marker.

Data scoring and analysis

ISSR and RAPD bands were scored for their presence (1) or absence (0) across 16 triticale genotypes. For each primer used in RAPD and ISSR analyses, the total number of bands scored, the number of polymorphic bands and the percentage of polymorphism were determined. By making a pair wise comparison between all genotypes using the Simqual module of NTSYS-pc software version 2.01e (Rohlf, 1998), genetic distances based on the Jaccard coefficient (Jaccard, 1908) were calculated. The distance coefficients obtained were used to construct dendrograms using UPGMA (the unweighted pair group method with arithmetic averages) employing the SAHN (sequential, agglomerative, hierarchical, and nested clustering) algorithm in same software package. The goodness of fit of the clustering to the basic data matrix, the cophenetic correlation coefficient was calculated using the normalized Mantel statistics Z test (Mantel, 1967) via the COPH and MXCOMP procedures of NTSYS-pc version 2.01e (Rohlf, 1998).

Effective multiplex ratio (EMR) and marker index (MI) for both marker systems were calculated in order to measure the usefulness of the marker system according to Powell et al. (1996). The multiplex ratio (MR) was estimated by dividing the total number of bands amplified by the total number of assays. The effective multiplex ratio (EMR) is the number of polymorphic fragments detected per assay. Using the formula of Roldán-Ruiz et al., (2000), polymorphic information content (PIC) or heterozygosity (H) was calculated: PIC = 2fi (1-fi), where fi is the frequency of the amplified allele. Average heterozygosity (Hav) is estimated by taking the average of PIC values obtained for all the markers. Marker index (MI) was calculated by multiplying the average heterozygosity (Hav) with EMR (Powell et al., 1996).

Resolving power (Rp) which shows the ability of the most informative primers to differentiate between the genotypes was assessed according to Prevost and Wilkinson, (1999) using:

$$Rp = \sum I_b$$

where I_b is the band informativeness with $I_b = 1 - [2 \times (0.5-p)]$ and where p is the proportion of clones containing the band. The resolving power is based on the distribution of detected bands within the sampled genotypes. All the statistical analyses were performed for the results of both marker systems.

Results

Polymorphism detected by ISSR and RAPD markers

In ISSR analysis of triticale genotypes, used ISSR primers, total number (n) and number of polymorphic bands (np), percentage of polymorphic bands (%P) of ISSR primers are shown in Table 2. Twenty eight ISSR primers amplified a total 244 scorable fragments with average 8.71 bands per primer (MR). In these fragments, 134 (55%) bands were observed as polymorphic. The number of the polymorphic bands (np) yielded by ISSR primers changed from 1 (ISSR 817, 827, 850 and 851) to 12 (ISSR 824). The percentage of polymorphism (P %) across the triticale varieties and lines ranged from 11.11% (ISSR 851) to 85.71% (ISSR 824) with the average polymorphism of 53.80% per primer.

Table 3 gives RAPD primers used in the study, their sequences, total number (n) and number of polymorphic bands (np), percentage of polymorphic bands (% P). The 21 arbitrary 10-mer primers yielded a total of 145 scorable fragments (an average of 6.90 bands per primer, MR), of which 70 (50%) were found to be polymorphic. The number of the polymorphic bands (np) generated by each used RAPD primers ranged from 1 (OPB-13 and OPC-04) to 11 (OPF-12). The percent of polymorphism (P%) changed from 16.67% (OPB-13) to 81.82% (OPC-07) with average 41.28% polymorphism. Any polymorphic band was not observed in the five of the RAPD primers (OPB-11, OPC-01, OPC-05, OPC-06 and OPC-08).

Genetic relationships and cluster analysis

The Jaccard's similarity matrix was calculated for both ISSR and RAPD data. Genetic similarity (GS) obtained from ISSR data across the 16 triticale genotypes varied from 0.65 (835 vs. Mikham-2002) to 0.93 (Juanillo vs. Beaguelita). In the case of RAPD, GS ranged from 0.73 (829 vs. Focus) to 0.97 (18 vs. 805). The average GS values of triticale genotypes were found 0.77 and 0.83 for ISSR and RAPD markers, respectively. UPGMA cluster analyses by using genetic

similarities obtained from ISSR and RAPD data are shown in Figure 1 and Figure 2, respectively. The genotypes clustered similarly with little differences in the both dendrograms. In ISSR dendrogram, the genotypes grouped in two main clusters, the first of which consisted of two subclusters (Figure 1). The first subcluster included the lines and varieties originated from CIMMYT while the other involved two varieties (Tacettinbey and Ege Yıldızı) originated from Turkey. In addition, the lines and varieties grouped in the first cluster have spring growth habit. However, the varieties grouped in the second cluster have winter growth habit and originated from Turkey. In RAPD dendrogram (Figure 2), the major difference is that all varieties originated from Turkey grouped in same cluster except the variety Focus. This variety was separately placed in the dendrogram. Similarly, the lines and varieties from CIMMYT were grouped in a same cluster in RAPD dendrogram. The Mantel test revealed that the Jaccard similarity matrices of ISSR and RAPD markers were always significantly correlated with the respective dendrograms (goodness of fit: Z = 0.88, P = 0.001 and Z = 0.83, P =

Table 2
Total number (n) and number of polymorphic bands (np), percentage of polymorphism (%P), main values of proportion of accessions containing band (mp), main values of band informativeness (mI_b), resolving power (Rp) and polymorphic information content (PIC) of ISSR primers among the triticale genotypes

ISSR Primer	Sequence	n	np	%P	mp	mI_b	Rp	PIC
ISSR 807	$(AG)_8$T	12	6	50.00	0.29	0.42	2.50	0.30
ISSR 808	$(AG)_8$C	7	2	28.57	0.50	0.38	0.75	0.31
ISSR 811	$(GA)_8$C	7	4	57.14	0.66	0.31	1.25	0.23
ISSR 814	$(CT)_8$A	5	3	60.00	0.56	0.38	1.50	0.30
ISSR 815	$(CT)_8$G	10	6	60.00	0.33	0.54	3.25	0.39
ISSR 817	$(CA)_8$A	3	1	33.33	0.44	0.88	0.88	0.49
ISSR 818	$(CA)_8$G	7	3	42.86	0.29	0.58	1.75	0.38
ISSR 822	$(TC)_8$A	11	5	45.45	0.46	0.48	2.38	0.32
ISSR 824	$(TC)_8$G	14	12	85.71	0.55	0.50	6.00	0.35
ISSR 827	$(AC)_8$G	2	1	50.00	0.41	0.38	0.38	0.31
ISSR 830	$(TG)_8$G	4	3	75.00	0.41	0.42	1.25	0.32
ISSR 834	$(AG)_8$YT	7	2	28.57	0.44	0.25	0.50	0.21
ISSR 835	$(AG)_8$YC	16	8	50.00	0.41	0.53	4.25	0.36
ISSR 836	$(AG)_8$YA	3	2	66.67	0.41	0.81	1.63	0.47
ISSR 840	$(GA)_8$YT	13	5	38.46	0.16	0.48	2.38	0.35
ISSR 841	$(GA)_8$YC	12	6	50.00	0.22	0.42	2.50	0.31
ISSR 842	$(GA)_8$YG	3	2	66.67	0.33	0.88	1.75	0.49
ISSR 843	$(CT)_8$RA	9	7	77.78	0.57	0.38	2.50	0.28
ISSR 844	$(CT)_8$RC	9	4	44.44	0.56	0.31	1.25	0.25
ISSR 845	$(CT)_8$RG	12	9	75.00	0.30	0.53	4.75	0.36
ISSR 846	$(CA)_8$RT	11	6	54.55	0.48	0.46	2.75	0.35
ISSR 847	$(CA)_8$RC	10	5	50.00	0.67	0.38	2.25	0.27
ISSR 848	$(CA)_8$RG	15	11	73.33	0.49	0.47	5.13	0.31
ISSR 849	$(GT)_8$YA	10	7	70.00	0.41	0.59	4.13	0.37
ISSR 850	$(GT)_8$YC	5	1	20.00	0.81	0.38	0.38	0.31
ISSR 851	$(GT)_8$YG	9	1	11.11	0.94	0.13	0.13	0.12
ISSR 854	$(TC)_8$RG	6	5	83.33	0.60	0.60	3.00	0.40
ISSR 859	$(TG)_8$RC	12	7	58.33	0.41	0.47	4.25	0.36
Total		244	134					
Average		8.71^{MR}	4.79^{EMR}	53.80		0.48	2.34	0.33

*R = A, G; Y = C, T

0.001 for ISSR and RAPD, respectively). Also, the correlations between the ISSR and RAPD matrices were high level (Z = 0.74, P = 0.001).

Resolving power, polymorphic information content and marker index

The main values of proportion of the genotypes containing band (mp), main values of band informativeness (mI_b), resolving power (Rp) and polymorphic information content (PIC) of each ISSR and RAPD primer are presented in Table 2 and Table 3, respectively. The mp and mI_b values in RAPD varied from 0.06 to 0.88 and from 0.13 to 0.75 while same parameters in ISSR changed from 0.16 to 0.94 and from 0.13 to 0.88, respectively. In ISSR primers, the resolving power values varied from 0.13 for ISSR 851 to 6.00 for ISSR 824 with average 2.34. PIC values of the primers changed from 0.12 for ISSR 851 to 0.49 for ISSR 817 and ISSR 842. The average PIC value was identified 0.33. The effective multiplex ratios (EMR) and marker index (MI) of ISSR analysis was detected 4.79 and 1.58, respectively.

The resolving power (Rp) of the RAPD primers ranged from 0.13 for OPC-04 to 4.63 for OPF-12 (Table 3). The value of 1.94 was detected for average resolving power of all primers. The polymorphic information content (PIC) value of RAPD primers varied from 0.12 for OPB-14 and OPC-04 to 0.47 for OPC-02 with an average of 0.28. For RAPD analysis in triticale genotypes, effective multiplex ratios (EMR) were estimated at 3.33, while marker index (MI) was identified 1.23.

Discussion

In this study, we investigated genetic diversity of some triticale genotypes grown in Turkey using two different marker systems and compared relative efficiencies of them in assessing the levels of genetic diversity among the genotypes.

Table 3
Total number (n) and number of polymorphic bands (np), percentage of polymorphism (%P), main values of proportion of accessions containing band (mp), main values of band informativeness (mI_b), resolving power (Rp) and polymorphic information content (PIC) of RAPD primers among the triticale genotypes

RAPD Primer	Sequence	n	np	%P	mp	mI_b	Rp	PIC
OPA-04	5’-AATCGGGCTG-3’	5	3	60.00	0.42	0.25	0.75	0.21
OPA-20	5’-GTTGCGATCC-3’	11	4	36.36	0.31	0.38	1.50	0.27
OPB-02	5’-TGATCCCTGG-3’	11	7	63.64	0.63	0.46	3.25	0.30
OPB-08	5’-GTCCACACGG-3’	13	3	23.08	0.35	0.63	1.88	0.41
OPB-11	5’-GTAGACCCGT-3’	2	0	-	-	-	-	-
OPB-12	5’-CCTTGACGCA-3’	10	8	80.00	0.42	0.50	4.00	0.36
OPB-13	5’-TTCCCCCGCT-3’	6	1	16.67	0.88	0.25	0.25	0.22
OPB-14	5’-TCCGCTCTGG-3’	4	3	75.00	0.65	0.13	0.38	0.12
OPB-16	5’-TTTGCCCGGA-3’	5	3	60.00	0.75	0.42	1.25	0.28
OPB-20	5’-GGACCCTTAC-3’	4	2	50.00	0.84	0.31	0.63	0.26
OPC-01	5’-TTCGAGCCAG-3’	3	0	-	-	-	-	-
OPC-02	5’-GTGAGGCGTC-3’	3	2	66.67	0.63	0.75	1.50	0.47
OPC-03	5’-GGGGGTCTTT-3’	11	4	36.36	0.30	0.53	2.13	0.33
OPC-04	5’-CCGCATCTAC-3’	5	1	20.00	0.06	0.13	0.13	0.12
OPC-05	5’-GATGACCGCC-3’	1	0	-	-	-	-	-
OPC-06	5’-GAACGGACTC-3’	7	0	-	-	-	-	-
OPC-07	5’-GTCCCGACGA-3’	11	9	81.82	0.54	0.47	4.25	0.31
OPC-08	5’-TGGACCGGTG-3’	3	0	-	-	-	-	-
OPF-08	5’-GGGATATCGG-3’	7	5	71.43	0.63	0.75	3.75	0.42
OPF-12	5’-ACGGTACCAG-3’	16	11	68.75	0.60	0.42	4.63	0.28
OPF-19	5’-CCTCTAGACC-3’	7	4	57.14	0.69	0.19	0.75	0.16
Total		145	70					
Average		6.90^{MR}	3.33^{EMR}	41.28		0.41	1.94	0.28

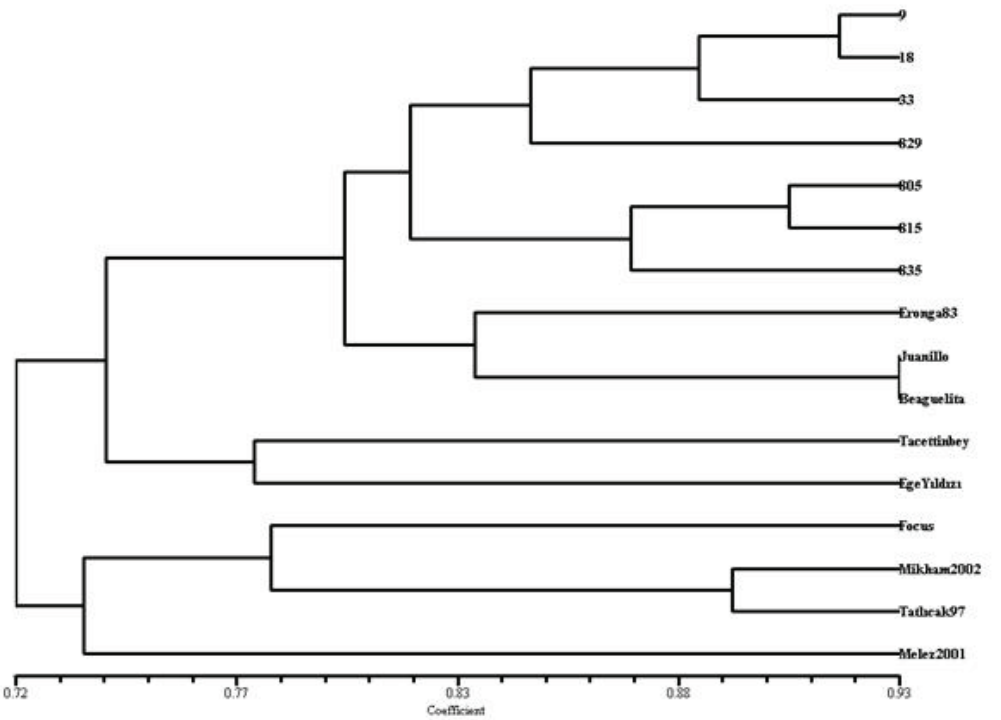

Fig. 1. The UPGMA dendrogram computed using genetic distance matrix based on ISSR data

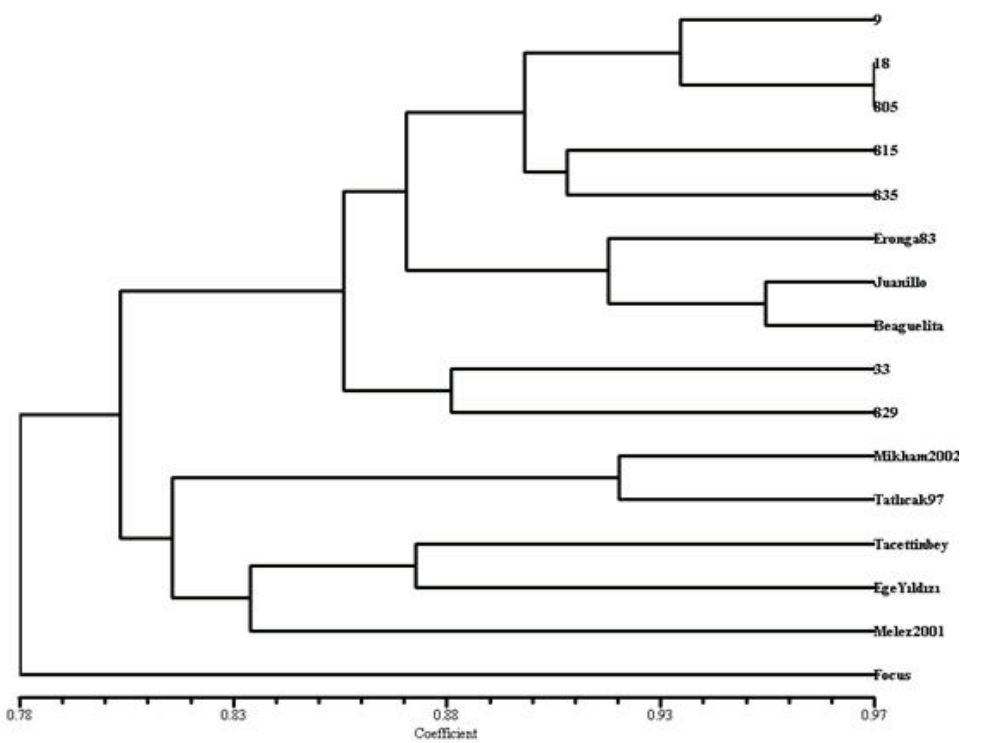

Fig. 2. The UPGMA dendrogram computed using genetic distance matrix based on RAPD data

This is the first research used ISSR and RAPD markers together to analyse the genetic diversity in triticale. There are a great number of studies used ISSR and RAPD techniques in different species and some of them reported efficiency of ISSR, while the others recommended RAPD markers. Hou et al. (2005) analyzed ISSR and RAPD diversity in a barley germplasm from western China and compared genetic diversity between selected accessions of the barley landraces and its wild relatives. In another study, efficiency of ISSR and RAPD were measured to access genetic variation in *Vigna umbellate* (Muthusamy et al., 2008).

In comparison of ISSR and RAPD marker efficiency in terms of multiplex ratio (MR) or the average number of fragments amplified per assay it was revealed that MR of ISSR was higher than that of RAPD. The percentage of polymorphism in ISSR was more than that of RAPD (Table 1 and Table 2). Similar results were reported by Patra et al. (2011) who recommended ISSR in respect to higher polymorphism and number of band in *Piper betle*. However, Guasmi et al. (2012) found that RAPD markers were superior to ISSR markers in the capacity of revealing more informative bands in a single amplification in *Hordeum vulgare*.

The effective multiplex ratio (EMR), which is an important comparison scale, found to be higher in ISSR system. The marker index (MI) may be used to evaluate overall utility of a marker system (Powell et al., 1996). The results have shown that ISSR have higher MI in comparison to RAPD in triticale genotypes. This is result from the higher EMR and average heterozygosity (Hav) of ISSR system. Our results corroborates those from other studies of *Bombyx mori* (Nagaraju et al., 2001), *Mangifera indica* (Srivastava et al., 2012) and *Moringa oleifera* (Saini et al., 2013) in which the EMR and MI values of ISSR was higher than those of the RAPD. The mp correlates strongly with the ability to distinguish between genotypes and mI_b represents the measurement of closeness of a band to be present in 50% of the genotypes under the study. ISSR markers indicated higher values of mp and mI_b compared to RAPD markers which indicates more effective of them in triticale genotypes.

The resolving power (Rp) is another method used to measure the ability of primers or techniques to distinguish between genotypes (Prevost and Wilkinson, 1999). The Rp values of ISSR primers were observed to be greater than that of RAPD primers of which evidence could be showed the average Rp values of two marker systems. Similar results were obtained in comparative analysis of ISSR and RAPD markers reported by Gupta et al. (2008) in *Jatropha curcas* L. and Safavi et al. (2010) in *Carthamus tinctorius* L. The polymorphic information content (PIC) value ranges from zero for monomorphic markers to 0.5 for markers that are present in 50% of the plants and absent in the other 50% (Roldán-Ruiz et al., 2000). It was understood clearly from average PIC values of ISSR and RAPD, ISSR markers exhibited higher level of polymorphism. This result was in agreement with Hou et al. (2005), Safavi et al. (2010) and Saini et al. (2013) who found higher average PIC value in ISSR compared to RAPD. However, Muthusamy et al. (2008) stated higher average PIC value in RAPD in comparison with ISSR.

Conclusion

In this study, spring and winter growth habit of triticale genotypes were used and they were exactly discriminated in ISSR dendrograms. In RAPD dendrogram, the genotypes were separated according to their origins. This difference is very important result of our study and it is more likely to result from different target regions of ISSR and RAPD markers in the genome. Different marker systems, which survey different region of genome, exhibit polymorphism only of DNA region targeted and this could lead to clustering of the genotypes differently. Different clustering of genotypes using ISSR and RAPD polymorphism were previously reported from Gupta et al. (2008), Patra et al. (2011), Srivastava et al. (2012) in several plant species.

In conclusion, the ISSR technique seems to be convenient tool for genetic diversity of triticale genotypes compared to RAPD technique. It differs not only in amount of polymorphism detected but also in underlying comparison scales. However, the results of both techniques are slightly different in comparison of efficiency of markers. Here, the triticale genotypes were clustered from two different points of view and both of clustering provides consistent results of their qualifications. On the basis of results of this study, it can be concluded that in future study of genetic diversity like here especially in breeding programs of triticale genotypes, more than one marker systems should be used for higher genetic resolution of the genome.

References

Doyle, J. J. and J. L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bulletin*, **19:** 11-15.

FAOSTAT, 2012. FAO Statistical Databases. http://faostat.fao.org/ Accessed 17 November 2013.

Guasmi, F., W. Elfalleh, H. Hannachi, K. Feres, L. Touil, N. Marzougui, T. Triki and A. Ferchichi, 2012. The use of ISSR and RAPD markers for genetic diversity among South Tunisian Barley. *ISRN Agronomy,* pp. 1-10.

Gupta, S., M. Srivastava, G. P. Mishra, P. K. Naik, R. S. Chauhan, S. K. Tiwari, M. Kumar and R. Singh, 2008. Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *American Journal of Botany,* **7:** 4230-4243.

Hou, Y., Z. Yan, Y. Wei and Y. Zheng, 2005. Genetic diversity in barley from west China based on RAPD and ISSR analysis. *Barley Genetics Newsletter*, **35:** 9-22.

Jaccard, P., 1908. Novelles recherches sur la distribution florale. *Bulletin de la Société vaudoise des sciences naturelles***, 44:** 223-270.

Kuleung, C., P. S. Baenziger, S. D. Kachman and I. Dweikat, 2006. Evaluating the genetic diversity of triticale with wheat and rye SSR markers. *Crop Science,* **46:** 1692-1700.

Liu, Z. Q., Y. Pei and Z. J. Pu, 1999. Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat (*Triticum aestivum* L.). *Plant Breeding*, **118:** 119-123.

Mantel, N. A., 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research,* **27:** 209-220.

Mergoum, M., W. H. Pfeiffer, R. J. Pena, K. Ammar and S. Rajaram, 2004. Triticale Crop Improvement: The CIMMYT Programme. In: M. Mergoum and H. Gómez-Macpherson (Ed.) Triticale Improvement and Production. *FAO Plant Production and Protection*, **179**: 11-27.

Muthusamy, S., S. Kanagarajan and S. Ponnusamy, 2008. Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. *Electronic Journal of Biotechnology,* **11:** 1-10.

Myer, R. and A. J. Lozano del Rio, 2004. Triticale as Animal Feed. In: M. Mergoum and H. Gómez-Macpherson (Eds.) Triticale Improvement and Production. *FAO Plant Production and Protection*, **179**: 49-58.

Nagaraju, J., K. D. Reddy, G. M. Nagaraja and B. N. Sethurama, 2001. Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm. *Bombyx mori. Heredity*, **86:** 58-597.

Patra, A. P., A. K. Mukherjee and L. Acharya, 2011. Comparative study of RAPD and ISSR markers to assess the genetic diversity of betel vine (*Piper betle* L.) in Orissia, India. *American Journal of Biochemistry and Molecular Biology,* **1:** 200-211.

Pena, R. J., 2004. Food Uses of Triticale. In: M. Mergoum and H. Gómez-Macpherson (Eds.) Triticale Improvement and Production. *FAO Plant Production and Protection*, **179:** 37-48.

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.

Prevost, A. and M. J. Wilkinson, 1999. A new system of comparing PCRprimers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics***, 98:** 107-112.

Rana, M. K., S. Singh and K. V. Bhat, 2006. RAPD, STMS and ISSR markers for genetic diversity and hybrid seed purity testing in cotton. *Seed Science and Technology***, 35:** 709-721.

Rohlf, F. J., 1998. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.0, Exeter Software, *Applied Biostatics Inc.,* New York.

Roldan-Ruiz, I., J. Dendauw, E. Van Bockstaele, A. Depicker and M. De Loose, 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding,* **6:** 125-134.

Safavi, S. A., S. S. Pourdad, M. Taeb and M. Khosroshahli, 2010. Assessment of genetic variation among safflower (*Carthamus tinctorius* L.) accessions using agro-morphological traits and molecular markers. *Journal of Food, Agriculture and Environment*, **8:** 616-625.

Saini, R. K., K. R. Saad, G. A. Ravishankar, P. Giridhar and N. P. Shetty, 2013. Genetic diversity of commercially grown *Moringa oleifera* Lam. cultivars from India by RAPD, ISSR and cytochrome P450-based markers. *Plant Systematics and Evolution*, doi: 10.1007/s00606-013-0789-7.

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 Agriculture and Biology,* **10:** 466-468.

Sözen, E., 2010. Evaluation of ISSR markers to assess genetic variability and relationship among winter triticale (x *Triticosecale wittmack*) cultivars. *Pakistan Journal Botany*, **42** (4)**:** 2755-2763.

Srivastava, N., A. Bajpai, R. Chandra, S. Rajan, M. Muthukumar and M. K. Srivastava, 2012. Comparison of PCR based marker systems for genetic analysis in different cultivars of mango. *Journal of Environmental Biology*, **33:** 159-166.

Tanyolac, B., 2003. Inter-Simple Sequence Repeat (ISSR) and RAPD variation among wild barley (*Hordeum. vulgare* subsp. *spontaneum*) populations from west Turkey. *Genetic Resources and Crop Evolution*, **50:** 611-614.

Tonk, F. A., S. Yüce, E. Bayram, R. R. A. Giachino, Ç. Sönmez, I. Telci and M. A. Furan, 2010. Chemical and genetic variability of selected Turkish oregano (*Origanum onites* L.) clones. *Plant Systematics and Evolution,* **288:** 157-165.

Tonk, F. A., R. R. A. Giachino, Ç. Sonmez, S. Yuce, E. Bayram, I. Telci and M. A. Furan, 2011. Characterization of Various *Hypericum perforatum* Clones by Hypericin and RAPD Analyses. *International Journal Agriculture and Biology,* **13** (1)**:** 31-37.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research,* **18:** 6531-6535.

Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics,* **20:** 176-183.

Received December, 13, 2013; accepted for printing June, 2, 2014.