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DETERMINATION OF THE GENETIC VARIATION AMONG SOME TURKISH RICE (*ORYZA SATIVA* L.) CULTIVARS USING ISSR TECHNIQUE

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ABSTRACT

In this study, the genetic diversity levels of 28 different rice varieties developed in Turkey were determined by using ISSR markers. Twenty different ISSR primers amplified 268 bands of which 217 were polymorphic. Band number for each primer ranged from 5-22 with an average number of 13.4. Polymorphism percentages varied between 40.00% and 94.12% with an average of 80.97%. Polymorphism Information Content (PIC) values were between 0.274 and 0.374 with an average value of 0.347. Similarity values indicated that the most similar varieties were Efe and Hamzadere (0.856) and the least similar varieties were Demir and Bafra Yıldızı (0.395). UPGMA analysis grouped 28 rice varieties into two main clusters and showed that genotypes obtained from same institute tend to cluster together. This implies the possibility of natural cross pollination between rice varieties grown in close fields. Our results indicated that ISSR technique is effective to study genetic variation in Turkish rice cultivars. Data obtained from this study could be used in the rice improvement programs.

Key words: Rice; Genetic variability; Inter Simple Sequence Repeats.

INTRODUCTION

Oryza sativa L. belongs to Oryzeae tribe in Poaceae family. Genus *Oryza* contains 20 wild and 2 cultivated different species (2n=24, or 48) (Aggarwal *et al*., 1997). Rice is known as one of the most essentialcereal crops along with wheat and corn. A great majority of rice has been produced and consumed in Asia (Khush, 2005). China takes the first place in rice production with 205.201.696 tons whereas Turkey produced 900.000 tones (FAO, 2014). Turkey has been importing rice since 1923 which creates an important economic loss. The majority of people on the world hinge on rice for survival (Kanawapee *et al*., 2011). It is estimated that there will be 5 billion rice consumers in 2025 and therefore efforts should be made to increase rice yield and stability (Khush, 2005).

Genetic diversity is essential for breeding purposes to develop new varieties and to protect the germplasm. Definite knowledge of genetic diversity between any crop varieties becomes very important specifically when genetic relevance among the parental lines are narrow (Choudhary *et al*., 2013). Although cultivated rice is remarkably rich in diversity, its productivity is negatively affected by multiple biotic and abiotic stresses (Joshi *et al*., 2000).

The estimation of genetic diversity is performed using various techniques, one of which employes molecular markers. These markers are beneficial over conventional, morphological markers since they are stable and not influenced by environment and can increase the speed and precision of breeding programs (Govindaraj *et al.*, 2015). Among them, inter simple sequence repeat markers (ISSR) were developed by Zietkiewicz *et al.* (1994) and since then they have been employed in various genetic studies. Although ISSRs are dominant markers, they have several advantages such as high reproducibility, low cost, no prior DNA sequence data and require little amounts of DNA (Bornet and Branchard, 2001). ISSR technique has been used for estimating genetic variation in many cereals such as wheat (Nagaoka and Ogihara, 1997), barley (Tanyolaç, 2003), oat (Boczkowska *et al*., 2014), rye (Fernandez *et al*., 2002), triticale (Sözen, 2010) and rice (Singh and Sengar, 2015; Kshirsagar *et al*., 2014; Nagaraju *et al*., 2002).

Genetic variation in some rice cultivars of Turkish origin were previously studied by using seed storage proteins and RAPD markers (Büyükünal Bal and Bay, 2010). However, SDS-PAGE analysis of proteins showed no differences among cultivars studied and RAPD analysis revealed narrow genetic variation. Some studies using seed storage proteins demonstrated that cultivars may not be distinguished easily based on their seed protein profiles (Marchylo and LaBerge, 1981, Weiss *et al*., 1991). Also, Costa *et al.* (2016) showed that one type of DNA marker system would not be enough to study genetic variation in closely related genotypes. Due to the low discrimination power of seed storage proteins and RAPD markers among Turkish rice cultivars, the aim of this study was to test the efficiency of ISSR markers and determine the levels of genetic diversity among 28 Turkish rice varieties.

MATERIALS AND METHODS

Plant material: Seeds belonging to 28 rice varieties were obtained from Trakya Agricultural Research Institute and Karadeniz Agricultural Research Institute (Table 1). Rice seeds were germinated in petri dishes at 25 °C with 13 hours light and 11 hours dark period and 60% humidity in enviromental chamber (Sanyo, MLR-350H). Distilled water was used for germination. Leaves were collected from 15-day old plants and stored at -20 °C until DNA isolation.

DNA Isolation: Genomic DNA was isolated from 1 g plant material using 2X CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1987). Quality and quantity of DNA samples were determined by using Nanodrop ® ND-100 spectrophotometer (Wilmington, Delaware, USA) and also by gel electrophoresis.

ISSR-PCR Analysis: Twenty different ISSR primers containing two- three nucleotide repeats were used in this study (Table 2). PCR reactions were prepared in 25 µL volume consisting of 1X Taq Buffer (Fermentas), 2 µM $MgCl_2$ (Fermentas), 2.5 µM dNTP, 2.5 µM primer, 6 ng of genomic DNA, 1U of Taq Polymerase (Fermentas). Amplifications were carried out in thermal cycler (Techne, Progene) with following programme; initial denaturation at 94 ºC 4 min, 45 cycles of denaturation at 94 ºC 45 sec, annealing at 40-64 ºC 45 sec, extension at 72 ºC 90 sec and final extension at 72 ºC for 7 min. PCR products were electrophoretically separated by using 1.4% agarose gels containing ethidium bromide (0.5 µg/mL).

Data Analysis: Amplified products of each ISSR PCR reaction were scored as present (1) or absent (0) to build a binary matrix. Genetic similarity index was calculated with Jaccard's coefficient (Jaccard, 1912) and cluster analysis was performed using UPGMA (Unweighted Pair Group Method with Arithmetic Average) method in NTSYS Pc. 2.02 software (Rohlf, 1998). A formula suggested by Anderson *et al*. (1993) was used to calculate Polymorphic information content (PIC).

RESULTS

All of the tested ISSR primers worked in PCR (Figure 1). The twenty ISSR primers amplified 268 bands of which 217 were polymorphic. Band numbers for each primer changed between 5-22 with an average number of 13.4 (Table 2). Band sizes ranged between 250 bp and 2500 bp. Percentage of polymorphic bands (PPB) were calculated between 40% [$(AGC)_6T$] and 94.12% [$(AC)_8G$], the overall polymorphism rate was found as 80.97%. The polymorphic information content (PIC) value ranged from 0.274 and 0.374 with an average value of 0.347.

The genetic similarity among the 28 rice cultivars ranged from 0.395 to 0.856 (Table 3). The highest similarity (0.856) was observed between cultivars Efe and Hamzadere and the lowest similarity (0.395) was between Demir and Bafra Yıldızı. UPGMA dendrogram grouped 28 rice cultivars into two main clusters (Figure 2). Cluster I included rice cultivars obtained from Trakya Agricultural Research Institute and cluster II included the cultivars obtained from Karadeniz Agricultural Research Institute.

Cluster I was further divided into two sub clusters. First sub cluster consisted Osmancık-97, Demir and Kıral. Second sub cluster included other twenty rice varieties obtained from Trakya Agricultural Institute. Cluster II consisted only varieties developed at Karadeniz Agricultural Institute and divided into two sub-clusters. One sub cluster contained only Bafra Yıldızı other sub cluster contained the restfour rice varieties. Mevlütbey and Osmancık (0.784) were most similar among varieties developed at Karadeniz Agricultural Institute.

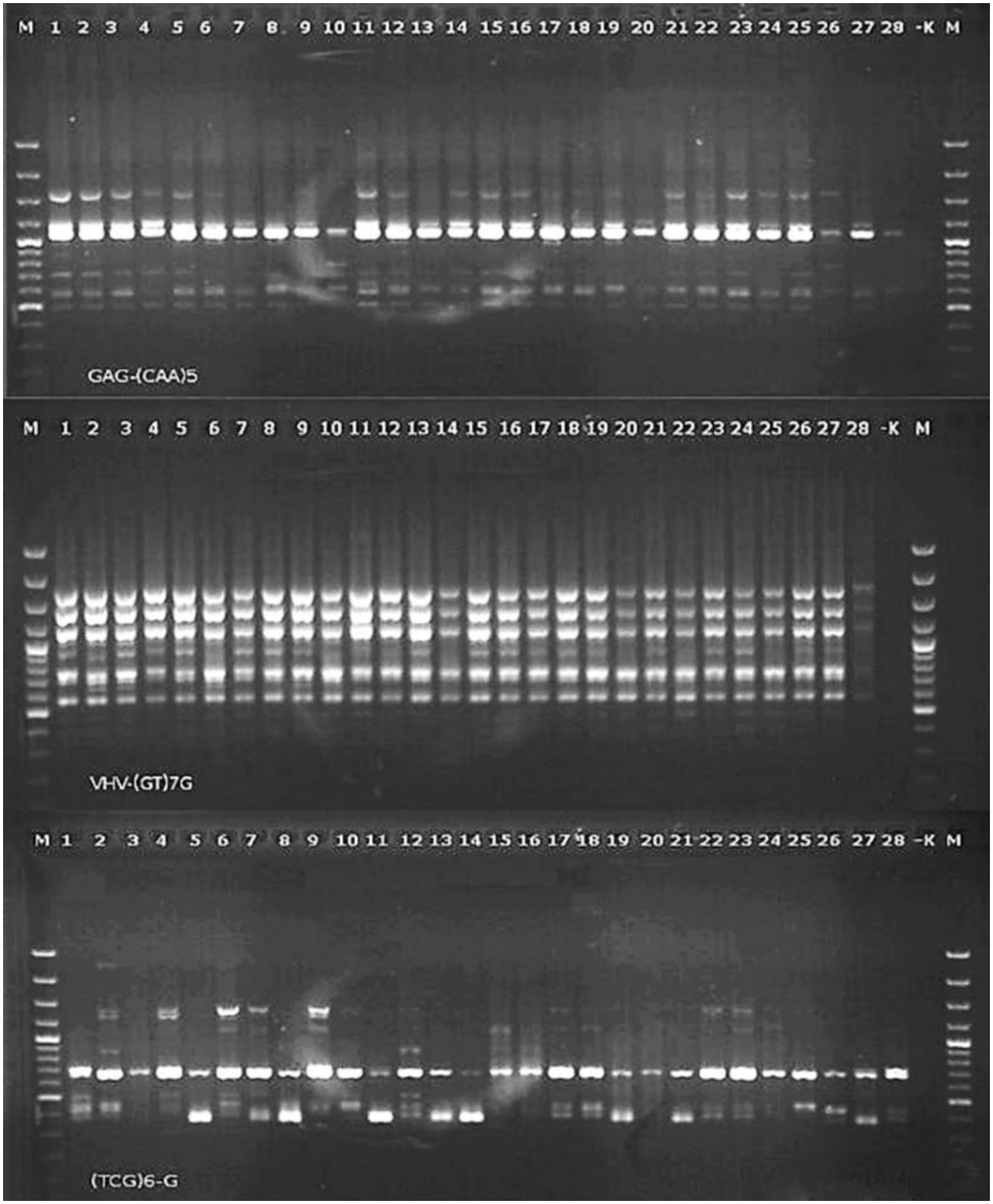

Figure 1.ISSR profiles of rice varieties with ISSR primers GAG-$(CAA)_5$ (a), VHV-$(GT)_7$G (b), and $(TCG)_6$G (c). M, 100bp DNA ladder (Fermentas), (1:Osmancık97, 2:Demir, 3:Kıral, 4:Gönen, 5:Halilbey, 6:Ece, 7:Kırkpınar, 8:Şumnu, 9:Beşer, 10:Kızıltan, 11:Aromatik-I, 12:Tunca, 13:Paşalı, 14:Çakmak, 15:Efe, 16:Hamzadere, 17:Sürek95, 18:Serhat92, 19:Trakya, 20:Ergene, 21:Meriç, 22:İpsala, 23:Altınyazı, 24:Mevlütbey, 25:Kızılırmak, 26:Osmancık, 27:Karadeniz, 28:Bafra Yıldızı)

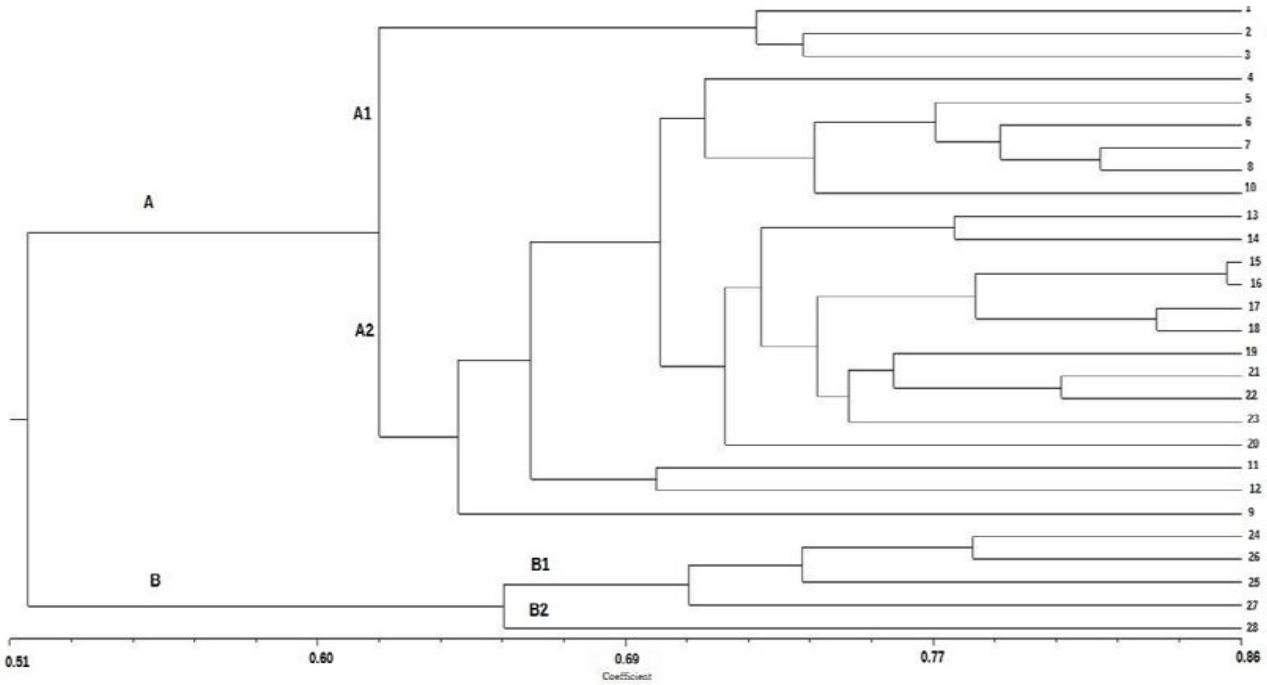

Figure 2.UPGMA dendrogram of 28 rice cultivars based on ISSR data. See Fig 1. for variety names.

Table 1.Rice varieties used in this study.

NUMBER	VARIETY	MOTHER	FATHER
1	Osmancık 97	Rocca (I)	Europa (I)
2	Demir	Plovdiv (BG)	Lido (I)
3	Kıral	Gritna (I)	Balilla-28 (F)
4	Gönen	Bonni (I)	Shinei
5	Halilbey	İpsala	Veneria (I)
6	Ece	8203- TR413-6-1-1	8260- TR470-6-1-1
7	Kırkpınar	İpsala	80110-TR253-4-1-1
8	Şumnu	Rialto (I)	Koral
9	Beşer	İpsala Mutation	
10	Kızıltan	Veneria (I)	Thainato
11	Aromatik-I	Introduction	
12	Tunca	Rocca (I)	Thainato
13	Paşalı	Osmancık-97	820700-TR480-1-1-1-1
14	Çakmak	Trakya	N1-41T-1T-0T
15	Efe	Baldo (I)	Demir
16	Hamzadere	Demir	83013-TR631-4-1-2
17	Sürek 95	Rocca (I)	Rodina (BG)
18	Serhat 92	Rocca (I)	Krasnodarsky-424 (RUS)
19	Trakya	Baldo (I)	Komsomolsky (RUS)
20	Ergene	Delta (F)	Zoria (BG)
21	Meriç	Delta (F)	Akçeltik (TR)
22	İpsala	Rodina (BG)	Delta (F)
23	Altınyazı	Baldo (I)	Ribe (I)
24	Mevlütbey	Drago (BG)	Kral
25	Kızılırmak	N1,41 T-1T-0T	8317-TR 635-1-2
26	Osmancık	-	-
27	Karadeniz	Roma (I)	Silla
28	Bafra Yıldızı	Ballilla-28 (F)	TR666-8-1-1-1

BG=Bulgaria, F=France, I=Italy, RUS=Russia, TR=Turkey

Table 2.Band profile and polymorphism generated in 28 rice varieties using ISSR primers.

Primer	Annealing temperature	Total No. of Bands	No. of Polymorphic bands	Polymorphism (%)	PIC*
GAG-$(CAA)_5$	50	17	15	80.24	0.352
$(CAG)_5$	50	19	16	84.22	0.373
VHV-$(GT)_7$G	55	17	15	88.24	0.364
$(AG)_8$-G	52	11	9	81.82	0.370
$(GA)_8$-T	50	22	19	83.37	0.374
$(GA)_8$-A	50	13	12	92.31	0.366
$(AG)_8$-T	50	13	9	69.24	0.364
$(AG)_8$-C	52	13	8	61.54	0.374
$(AC)_8$-C	52	21	19	90.84	0.320
$(TCG)_6$-G	64	15	13	86.67	0.340
$(AGC)_6$-T	62	5	2	40.00	0.300
$(AGC)_6$-G	64	14	13	92.86	0.274
$(AGC)_6$-C	64	6	4	66.66	0.360
$(AC)_8$-G	52	17	16	94.12	0.304
$(GT)_8$-C	52	12	9	72.73	0.347
BDB-$(ACA)_5$	52	14	12	85.72	0.372
DD-$(CGA)_5$	55	7	5	71.43	0.363
$(AG)_8$-YT	52	10	6	60.00	0.309
$(GT)_8$-YC	55	11	10	90.91	0.371
$(CAA)_5$	40	11	5	45.46	0.350
Average		**13.4**	**10.85**	**80.97**	**0.347**

(B= C,G,T; D= A,G,T; H= A,C,T; V= A,G,C; Y= C,T), *PIC: Polymorphic Information Content

Table 3. Jaccard's similarity matrix of rice varieties.

	Osmancık 97	Demir	Kıral	Gönen	Halilbey	Ece	Kırkpınar	Şumnu	Beşer	Kızıltan	Aromatik-I	Tunca	Paşalı	Çakmak	Efe	Hamzadere	Sürek-95	Serhat-92	Trakya	Ergene	Meriç	İpsala	Altınyaza	Mevlütbey	Kızılırmak	Osmancık	Karadeniz	Bafra Yıldıza
Osmancık 97	1,000																											
Demir	0,730	1,000																										
Kıral	0,714	0,736	1,000																									
Gönen	0,635	0,653	0,716	1,000																								
Halilbey	0,665	0,719	0,737	0,769	1,000																							
Ece	0,667	0,667	0,695	0,737	0,783	1,000																						
Kırkpınar	0,680	0,616	0,720	0,728	0,762	0,818	1,000																					
Şumnu	0,671	0,640	0,688	0,662	0,774	0,766	0,820	1,000																				
Beşer	0,616	0,588	0,644	0,619	0,627	0,716	0,743	0,675	1,000																			
Kızıltan	0,579	0,571	0,627	0,643	0,696	0,759	0,763	0,738	0,678	1,000																		
Aromatik-I	0,623	0,564	0,641	0,636	0,701	0,680	0,706	0,673	0,607	0,664	1,000																	
Tunca	0,606	0,560	0,624	0,609	0,658	0,671	0,651	0,665	0,580	0,656	0,694	1,000																
Paşalı	0,612	0,603	0,688	0,648	0,730	0,733	0,763	0,763	0,662	0,742	0,684	0,674	1,000															
Çakmak	0,536	0,509	0,616	0,622	0,630	0,655	0,706	0,671	0,612	0,674	0,643	0,623	0,779	1,000														
Efe	0,573	0,555	0,613	0,641	0,686	0,701	0,716	0,705	0,643	0,684	0,689	0,667	0,792	0,756	1,000													
Hamzadere	0,607	0,567	0,647	0,676	0,697	0,725	0,754	0,704	0,655	0,708	0,726	0,667	0,760	0,740	0,856	1,000												
Sürek 95	0,595	0,547	0,592	0,629	0,603	0,759	0,737	0,713	0,653	0,693	0,606	0,664	0,729	0,697	0,790	0,831	1,000											
Serhat 92	0,607	0,558	0,624	0,589	0,682	0,697	0,711	0,748	0,590	0,669	0,640	0,653	0,727	0,709	0,760	0,758	0,836	1,000										
Trakya	0,571	0,563	0,621	0,683	0,716	0,683	0,735	0,761	0,628	0,691	0,660	0,628	0,740	0,695	0,732	0,731	0,741	0,752	1,000									
Ergene	0,568	0,539	0,641	0,625	0,634	0,658	0,712	0,688	0,604	0,705	0,635	0,627	0,702	0,696	0,694	0,720	0,664	0,702	0,756	1,000								
Meriç	0,601	0,603	0,660	0,678	0,733	0,689	0,752	0,776	0,595	0,697	0,667	0,658	0,731	0,701	0,750	0,761	0,719	0,712	0,782	0,760	1,000							
İpsala	0,589	0,552	0,606	0,643	0,673	0,699	0,701	0,726	0,613	0,694	0,609	0,645	0,691	0,674	0,748	0,721	0,743	0,729	0,741	0,718	0,809	1,000						
Altınyazı	0,573	0,555	0,613	0,653	0,650	0,688	0,716	0,693	0,610	0,684	0,629	0,632	0,734	0,701	0,769	0,766	0,748	0,707	0,732	0,721	0,724	0,789	1,000					
Mevlütbey	0,455	0,451	0,513	0,524	0,554	0,576	0,589	0,571	0,517	0,549	0,552	0,517	0,608	0,603	0,651	0,651	0,612	0,601	0,574	0,594	0,596	0,616	0,664	1,000				
Kızılırmak	0,447	0,451	0,485	0,460	0,533	0,554	0,566	0,550	0,516	0,559	0,541	0,497	0,571	0,590	0,587	0,600	0,599	0,589	0,550	0,580	0,551	0,569	0,587	0,723	1,000			
Osmancık	0,421	0,417	0,450	0,471	0,487	0,527	0,549	0,524	0,480	0,532	0,493	0,471	0,565	0,585	0,606	0,595	0,599	0,550	0,556	0,587	0,535	0,553	0,619	0,784	0,748	1,000		
Karadeniz	0,405	0,409	0,399	0,417	0,466	0,465	0,503	0,491	0,442	0,468	0,471	0,424	0,493	0,500	0,529	0,521	0,500	0,493	0,507	0,533	0,500	0,507	0,540	0,677	0,688	0,744	1,000	
Bafra Yıldızı	0,399	0,395	0,410	0,402	0,434	0,452	0,461	0,431	0,428	0,454	0,447	0,446	0,500	0,486	0,504	0,485	0,476	0,450	0,462	0,496	0,448	0,464	0,515	0,613	0,624	0,690	0,675	1,000

DISCUSSION

Knowledge of genetic diversity is essential for breeding programs and satisfactory genetic variation level is a prerequisite for selection of parents carrying wider variability for different characters. To our knowledge this work represents the first report on the estimation of genetic variation between Turkish rice varieties by using ISSR technique. In this study, twenty ISSR primers produced 10.85 polymorphic bands per primer with an average polymorphism of 80.97%, which indicated a high level of genetic variation among rice varieties studied. The polymorphism ratio detected in our study was similar in the studies of Wan *et al*. (2008), Rajani *et al*. (2013) and Al-Turki and Basahi (2015).

Primers which has tri or dinucleotide repeats produced different polymorphism ratios. Primers with dinucleotide repeats showed 79.04% polymorphism ratio while trinucleotide repeats showed 84.10%. Reddy *et al*. (2002) reported that ISSR primers with (AG), (CT) and (AC) repeats were supposed to show higher polymorphism than primers with other di-, tri- or tetra-nucleotide repeats. However, parallel to our findings Galvan *et al*. (2003) showed that trinucleotide motif primers amplified a higher percentage of polymorphic bands in comparison with dinucleotide motifs in common bean. Al-Turki and Basahi (2015) found high polymorphism rates with tri nucleotide repeat primers in Hassawi rice. Kantety *et al*. (1995) also found similar results in maize.

The primers with different anchor base amplified different number of bands. For example $(AC)_8G$ primer produced 14 bands while $(AC)_8C$ produced 15 bands. Likewise $(AGC)_6C$ primer produced 12 bands while $(AGC)_6G$ primer produced 17 bands. This result indicates that the ISSR primers with same repeat sequences and different anchor bases, raises the possibility of binding different parts of the genome. Primers with (GA) and (AG) repeat sequences amplified clear and high number of bands in our study. The primers with (AG) and (GA) repeats have also been found to produce clear band profiles in rice (Joshi *et al*., 2000; Sarla *et al*., 2005), trifoliate orange (Fang *et al*., 1997) and chickpea (Ratnaparkhe *et al*., 1998).

The polymorphism information content (PIC) values have been employed in numerous genetic diversity studies (Najaphy *et al*., 2011; Thudi *et al*., 2010; Singh and Sengar, 2015). PIC generally used as a measurement for polymorphism of the marker loci and this value shows which allelles will be inherited to the next generation. PIC value varies between 0.5–1.0 for codominant markers while it takes maximum 0.5 for dominant markers like ISSR (Boopathi, 2013). In this study, the PIC value for 20 ISSR primers varied from 0.274 to 0.374 with a mean of 0.347 (Table 2).It was reported that moderate PIC values for ISSR primers imply diverse nature of accessions studied and/or informative feature of ISSR markers (Najaphy *et al*., 2011).

Genetic diversity levels among rice genotypes from different country origin were reported in several studies in which a close relationship between cultivars was obtained (Rangel *et al*., 1996; Fuentes *et al*., 1999). In our study, Jacquard's similarity coefficient values ranged from 0.395 to 0.856, average genetic similarity value among 28 rice varieties was 0.637 (Table 3). However, genetic similarity among some variety pairs are relatively low and they could be exploited as parents in breeding programs. Previously, narrow genetic diversity was reported for some Turkish rice cultivars by using RAPD and seed storage proteins (Büyükünal and Bay, 2010). This indicates that the patterns of variation detected in Turkish rice varieties are clearly influenced by the type of genetic marker.

The dendrogram generated using UPGMA analysis grouped 28 varieties into two major clusters. Cluster I was further divided into two sub-clusters. First sub-cluster consisted Osmancık-97, Demir and Kıral. These three varieties are similar in maturity duration but different in length and yield. In the second sub-cluster, one variety, Beşer, was placed separately from other varieties. Beşer was developed from a mutation of İpsala but these two varieties were placed separately on the same sub-cluster. This could be due to the consequence of mutations e.g. large deletions in the genome affecting primer binding sites. In the second sub-cluster Sürek-95 and Serhat-92 were grouped together. Similarly, Büyükünal and Bay (2010) found Sürek-95 and Serhat-92 on the same nod in their RAPD study. However, unlike our results they grouped Osmancık-97, Altınyazı varieties and Demir, İpsala varieties under the same group. Application of different marker system could be the reason of different groupings.

Interestingly, varieties obtained from Trakya and Karadeniz Agricultural Institutes grouped separately in the dendrogram. This could be explained to some extent by the occurrence of cross pollination. If the fields were close to each other, cross pollination may occur among different rice varieties so that similarity arised among them. In cultivated rice, usually autogamy is seen but natural cross pollination can also occur at low rates (Reona and Pahm, 1980). It was reported that physical contact and the wind can rise this possibility at close fields (Reona and Pahm, 1980). Windborne cross-pollination between fields was detected in maize, rice, sugar beet and oilseed rape varieties (Hoyles and Cresswell, 2007). Similar findings of separation of varieties on the dendrogram according to their breeding stations were also reported for cotton (Iqbal *et al*., 1997), basmati rice (Bligh *et al*., 1999), wheat (Bhutta, 2006) and triticale (Sözen, 2010).

Conclusion: To the best of our knowledge, this is the first report of estimating the genetic variability of Turkish rice cultivars using ISSR technique. This study showed that ISSR is a quick and reliable technique providing reproducible band profiles and adequate polymorphism for assessing genetic diversity among rice varieties. The levels of genetic variation detected among 28 Turkish rice cultivars could be used in rice breeding programs.

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REFERENCES

Aggarwal, P.K., M.J. Kropff, K.G. Cassman, and H.F.M. Ten Berge (1997). Simulating genotypic strategies for increasing rice yield potential in irrigated, tropical environments. Field Crop Res. 51: 5-17.

Al-Turki, T.A., and M.A. Basahi (2015). Assessment of ISSR based molecular genetic diversity of Hassawi rice in Saudi Arabia. Saudi J. Biol. Sci. 22(5): 591-599.

Anderson, J.A., G.A. Churchill, J.E. Sutrique, S.D. Tanksley, and M.E. Sorrells (1993). Optimizing parental selection for genetic linkage maps. Genome. 36(1): 181-186.

Bhutta, W.M. (2006). Biochemical and molecular characterization of wheat genotypes determined by RAPD analysis. Acta Agric. Scand. B-S P. 57(4): 335-341.

Bligh, H.F.J., N.W. Blackhall, K.J. Edwards, and A.M. McClung (1999). Using amplified fragment length polymorphisms and simple sequence length polymorphisms to identify cultivars of brown and white milled rice. Crop Sci. 39(6): 1715-1721.

Boczkowska, M., J. Nowosielski, D. Nowosielska, and W. Podyma (2014). Assessing genetic diversity in 23 early Polish oat cultivars based on molecular and morphological studies. Genet Resour Crop Evol. 61(5): 927-941.

Boopathi, N.M (2013). Genetic Mapping and Marker Asisted Seletion: Basics, Practice and Benefits. 1st Ed. Springer; India. 303 p

Bornet, B., and M. Branchard (2001). Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Int. S. Plant Mol. Biol. 19(3): 209-215.

Büyükünal Bal, E., and S. Bay (2010). Genetic analysis of Turkish rice varieties (*Oryza sativa*L.) using seed storage proteins and RAPD markers. Eur. Food Res. Technol. 230(4): 609-617.

Choudhary, G., N. Ranjitkumar, M. Surapaneni, D.A. Deborah, A. Vipparla, and G. Anuradha (2013). Molecular genetic diversity of major Indian rice cultivars over decadal periods. PLoS ONE. 8: e66197.

Costa, R., G. Pereira, I. Garrido, M.M. Tavares-de-Sousa, and F. Espinosa (2016). Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify Orchardgrass (*Dactylis glomerata* L.) germplasm variations. PLoS ONE. 11(4): e0152972.

Doyle, J.J., and J.L. Doyle (1987). Isolation of Plant DNA From Fresh Tissue. Focus. 12: 13-15.

Fang, D.Q., M.L. Roose, R.R. Krueger, and CT. Federici (1997). Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs and inter-simple sequence repeat markers. Theor. Appl. Genet. 95(1): 211-219.

Fernandez, M.E., A.M. Figueiras, and C. Benito (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet. 104(5): 845-851.

FAOSTAT (2014). FAO Statistical Databases. Available at: http://faostat3.fao.org/browse/Q/*/E (Accessed: 03 08. 2016)

Fuentes, J.L., F. Escobar, A. Álvarez, G. Gallego, M.C. Duque, M. Ferrer, J.E. Deus, and J. Tohme (1999). Analysis of genetic diversity in Cuban rice varieties using AFLP, RAPD and isozyme markers. Euphytica. 109(2): 107-115.

Galvan, M.Z., B. Bornet, P.A. Balatti, and M. Branchard (2003). Inter Simple Sequence Repeat (ISSR) Markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica. 132(3): 287-301.

Govindaraj, M., M. Vetriventhan, and M. Srinivasan (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Genet. Res. Int. Volume 2015: Article ID 431487, 14 pages

Hoyles, M., and J.E. Cresswell (2007). The effect of wind direction on cross-pollination in wind-pollinated GM crops. Ecol. Appl. 17(4): 1234-1243.

Iqbal, M. J., N. Aziz, N. A. Saeed, Y. Zafar, and K.A. Malik (1997). Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theor. Appl. Genet. 94(1): 139 -144.

Jaccard, P (1912). The distribution of the flora in the alpine zone. New Phytol. 11(2): 37-50.

Joshi, S.P., V.S. Gupta, R.K. Aggarwal, P.K. Ranjekar, and D.S. Brar (2000). Genetic diversity and

phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza.* Theor. Appl. Genet. 100(8): 1311-1320.

Kanawapee, N., J. Sanitchon, P. Srihaban, and P. Theerakulpisut (2011). Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. Electron. J. of Biotechn. 14(6): 1-17.

Kantety, R.V., X.P. Zeng, J.L. Bennetzen, and B.E. Zehr (1995). Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. Mol. Breed. 1(4): 365-373.

Khush, G.S (2005). What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol. 59(1): 1-6.

Kshirsagar, S.S., K.C. Samal, R. Mihir, N.B. Debendra, and G.R. Rout (2014). Identification of variety diagnostic molecular marker of high yielding rice varieties. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 84: 389-396.

Marchylo, B.A., and D.E. Laberge (1981). Barley cultivar identification by electrophoretic analysis of hordein proteins II. Catalogue of electrophoregram formulae for Canadian grown barley cultivars. Can. J. Plant Sci. 61(4): 859-870.

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(5): 597-602.

Nagaraju, J., M. Kathirvel, R.R. Kumar, E.A. Siddiq, and S.E. Hasnain (2002). Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc. Natl. Acad. Sci. 99(9): 5836-5841.

Najaphy, A., R.A. Parchin, and E. Farshadfar (2011). Evaluation of genetic diversity in wheat cultivars and breeding lines using Inter Simple Sequence Repeat markers. Biotechnol. Biotech. Eq. 25(4): 2634-2638.

Rajani, J., V. Deepu, G.M. Nair, and A.J. Nair (2013). Molecular characterization of selected cultivars of rice, *Oryza sativa* L. using Random Amplified Polymorphic DNA (RAPD) markers. Int. Food Res. J. 20(2): 919-923.

Rangel, P.H.N., E.P. Guimaraes, and P.C.F. Neves (1996). The genetic base of Brazilian irrigated rice (*Oryza sativa* L) cultivars. Pesqui Agropecu. Bra. 31(5): 349-357.

Ratnaparkhe, M.B., M. Tekeoglu, and F.J. Muehlbauer (1998). Inter simple sequence repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theor. Appl. Genet. 97(4): 515-519.

Reddy, M.P., N. Sarla, and E.A. Siddiq (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica. 128(1): 9-17.

Reona, R., and J.L. Pham (1980). Does crosspollination between accessions occur during seed regeneration at the International Rice Genbank. Int. Rice Res. Notes. 23(3): 5-6.

Rohlf, M (1998). NTSYSPC. Numerical Taxonomy and Multivariate Analysis System, Version 2.02. Department of Ecology and Evaluation: StateUniversity of New York, NY, USA.

Sarla, N., C.N. Neeraja, and E.A. Siddiq (2005). Use of anchored (AG)n and (GA)n primers to assess genetic diversity of Indian landraces and varieties of rice. Curr. Sci. 89 (8): 1371-1381.

Singh, A., and R.S. Sengar (2015). DNA fingerprinting based decoding of Indica rice (*Oryza sativa* L) via molecular marker (SSR, ISSR, & RAPD) in aerobic condition. Adv. Crop Sci. Tech. 3: 167.

Sözen, E (2010). Evaluatıon of ISSR markers to assess genetic variability and relationship among winter *Triticale* (x *Tritico secale* Wıttmack) cultivars. Pakistan J. Bot. 42(4): 2755-2763.

Tanyolaç, B (2003). Inter simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum. vulgare* subsp. s*pontaneum*) populations from west Turkey. Genet. Res. Crop Evol. 50: 611-614.

Thudi, M., R. Manthena, S.P. Wani, L. Tatikonda, D.A. Hoisington, and R.A. Varshney (2010). Analysis of genetic diversity in Pongamia (*Pongamia pinnata* L. Pierrre) using AFLP markers. J. Plant Biochem. Biotechnol. 19: 209-216.

Wan, Y.T., A. Xin- Xiang, C.Z. Fan, F. Xu, T. Yu, C. Tang, and L. Dai (2008). ISSR analysis on genetic diversity of the 34 populations of *Oryza meyeriana* distributing in Yunnan Province, China. Rice Sci. 15(1): 13-20.

Weiss, W., W. Postel, and A. Görg (1991). Barley cultivar discrimination: II. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing with immobilized pH gradients. Electrophoresis. 12(5): 330-337.

Zietkiewicz, E., A.Rafalski, and D. Labuda (1994). Genome fingerprinting by simple sequence repeat (SSR) – anchored polymerase chain reaction amplification. Genomics. 20(2): 176-183.