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Molecular Characterization of Turkish Onion Germplasm Using SSR Markers

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Abstract

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In this study, molecular markers were used for the first time to study the genetic diversity of Turkish onion (*Allium cepa* L.) accessions. We used 83 turkish landraces, 3 breeding lines and 10 commercial varieties, in total 96 accessions, to study the genetic diversity. Bulked samples of 10 seeds were used to generate a representative profile of the accessions. Variability was evaluated at 46 microsatellite loci. We identified 308 alleles with these markers, out of which 303 were polymorphic. A dendrogram based on the UPGMA analysis grouped the 96 accessions into five main clusters. Dice's similarity coefficient ranged from 0.407 to 0.767 with an average of 0.587. The results showed that 44 out of the 46 SSR markers were convenient and polymorphic enough to distinguish all the studied accessions.

Keywords: *Allium cepa* L.; diversity; genetic resources; microsatellites; polymorphism

Alliums are a diverse taxon encompassing nearly 500 species, including onion, leek, garlic, shallot etc., and belong to the family *Alliaceae*. The *Allium* species are not evenly distributed within the northern hemisphere since most of them occur in Asia and Europe. A belt stretches from the Mediterranean basin to Iran and Afghanistan and contains many of them. A region of especially high species diversity occurs in Turkey and in the Irano-Turanian floristic region, i.e. Iran, Northern Iraq, Afghanistan, Central Asia and West Pakistan. The number of species decreases away from this centre of diversity (Rabinowitch & Brewster 1990).

The onion ($2n = 16$) belongs to *Allium* section *cepa* (Mill.), which consists of twelve species, most of which are used as spices, vegetable or medicinal plants (Gurushidze *et al*. 2007). The onion (*Allium cepa* L.) is one of the most important vegetables grown with a world production of over 85.8 million tons in 2013. Turkey produces 1.9 million tons of onions annually, which is 2.2% of the world onion production, and it ranks as the 6th largest onion producer (FAO 2013).

The knowledge of onion genetic diversity and resources is limited mainly due to a paucity of public markers and germplasm resources and their outbreeding, and biennial habit (McCallum & Havey 2006). A comparison of plant phenotypes is the simplest approach to the characterization of genotypes and the assessment of genetic diversity; however, phenotypic evaluation is influenced by environment and may not distinguish between closely related accessions (Rodriguez *et al*. 1999). Molecular markers have proved as valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Russell *et al*. 1997). Among these marker systems, simple sequence repeats (SSR; Fischer & Bachmann 2000) and random amplified polymorphic DNA (RAPD; Bradeen & Havey 1995), which are polymerase chain reaction (PCR) based markers, are simple, fast, cheap, and easily automated (Jones *et al*. 1997). Co-dominant simple sequence repeat (SSR) markers have been successfully used to detect genetic diversity and relationships in onion germplasms (Mahajan *et al*. 2009; Anandhan *et al*. 2014; Mitrová *et al*. 2015). The characteristics of genomic microsatellites (gSSR) are abundant in

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plant genomes, with reproducibility, high level of polymorphism and codominant inheritance (Nicot *et al*. 2004). The EST-derived SSRs belong to the transcribed regions of DNA and have a higher rate of transferability across species than genomic SSR markers (Rudd 2003).

The Onion Germplasm Bank of Atatürk Central Horticultural Research Institute (ACHRI) holds an important *A. cepa* L. collection, which was collected from different regions of Turkey. The majority of these landraces have already been characterized using morphological descriptors (Hanci & Gökçe 2015).

Despite their great economic importance, the genetic resource of the Turkish onion remains uncharacterized at the molecular level, with respect to parameters such as molecular-genetic diversity. The objectives of this study were to evaluate the population-level genetic diversity of these Turkish onion accessions based on molecular markers for the first time and to assess the implications of the study for future breeding and genebank management programs.

MATERIAL AND METHODS

Plant material. Eighty-three locally grown onion bulbs, which were collected from different regions of Turkey as part of the 'Onion Breeding Program', three breeding lines and ten commercial varieties, a total of ninety-six accessions, were used in the study.

Genomic DNA isolation. The fresh young leaves from 20-day old onion seedlings were used for DNA extraction. Bulked samples of 10 seeds were used for DNA isolation using a QIAGEN DNeasy plant mini kit (Qiagen GmbH., Hilden, Germany). Frozen leaf samples were ground into fine powder using a tissuelyser (TissueLyser II Retsch, Qiagen Retsch GmbH., Hannover, Germany). DNA was extracted following the procedures described by the manufacturers. DNA quality was analysed on agarose gel and quantified using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). After quantification, DNA samples were diluted to 10 ng/l in double-distilled water and stored at –20°C until PCR amplification.

SSR analysis. SSR markers were chosen for their high polymorphism, according to the results of previous studies. A set of 46 SSR markers, 19 EST-SSRs (expressed sequence tag-simple sequence repeat microsatellites; McCallum *et al*. 2008) and 27 gSSRs (genomic microsatellites; Fischer & Bachmann 2000) were used. The PCR reaction was conducted in a reaction volume of 25 µl containing 1× PCR buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.3 µM of each primer, 1.25 unit Taq DNA polymerase and 25 ng template DNA. PCR amplification was performed with initial denaturation at 94°C for 3 min, followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and the final extension at 72°C for 10 min before cooling at 4°C. After the amplifications, PCR products were checked on 3% agarose gel containing ethidium bromide and separated by capillary electrophoresis in a QSEP100 DNA fragment analyser (BiOptic, Inc., New Taipei City, Taiwan).

Data analysis. For 46 SSR loci, the presence or absence of alleles in each size category through all accessions were scored as either present (1) or absent (0). The informativeness and the discriminatory power of each SSR marker was determined by calculating the polymorphic information content (PIC) and heterozygosity (*H*) using the PICcalc program (Nagy *et al*. 2012). PIC was calculated according to the formula (Hildebrand *et al*. 1992):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2$$

where:

p_i, p_j – population frequency of the i^{th} and j^{th} allele

H value was calculated according to the formula (Hildebrand *et al*. 1992):

$$H = 1 - \sum_{i=1}^{n} p_i^2$$

where:

p_i – frequency for the i^{th} allele among a total of l alleles

Similarity matrices were calculated using Dice's coefficient. The resulting matrix was computed by the UPGMA algorithm (unweighted pair group method with arithmetic mean) using the XLSTAT program (Garcia-Vallve *et al*. 1999).

RESULTS AND DISCUSSION

For the 96 onion accessions, 19 EST-SSRs (expressed sequence tag-simple sequence repeat microsatellites) and 27 gSSRs (genomic microsatellites), a total of 46 markers, were amplified for a total of 308 bands, among which 303 bands were polymorphic (98%) across all the accessions. The size of the amplified DNA fragments ranged from 90 bp to 390 bp and the amount of amplified DNA fragments ranged from 1 to 15 in all accessions, and the average number of bands per marker was 6.69.

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The EST-SSRs markers produced 110 bands, out of which 105 were polymorphic (95%). The number of bands ranged from 1 to 14, with an average of 5.79 amplicons per marker. The size of the alleles was observed to be between 137 bp and 350 bp. The ACM238 produced the highest number of bands (14). The EST-SSRs markers showed a higher percentage of amplification than in previous reports (Jakše *et al*. 2005; Khar *et al*. 2011; Mallor *et al*. 2014; Mitrová *et al*. 2015). The bands of ACM315 (290 bp), ACM238 (153 bp) and ACM300 (184 bp, 207 bp, 216 bp) were excluded from further evaluation because of being monomorphic across the studied set of 96 accessions. Similarly, ACM300 produced monomorphic bands in Spanish onions (Mallor *et al*. 2014). The observed allele sizes in this study for the EST-SSR markers were similar to expected values (McCallum *et al*. 2008) but not exactly the same. Other authors reported different allele sizes compared to those described in the original publications. For example, ACM004 gave bands between 201 bp and 212 bp in Czech onion varieties (Mitrová *et al*. 2015); 220 bp and 230 bp in Spanish onion accessions (Mallor *et al*. 2014); and 201 bp and 210 bp in Indian onion accessions (Khar *et al*. 2011). We observed 224 bp and 256 bp bands in Turkish onion accessions using the same marker. Such different values may result from the reading sensitivity of different devices. In addition, this reflects the diversity and genetic variation of varieties originating from different regions of the world (Jakše *et al*. 2005; McCallum *et al*. 2008; Mitrová *et al*. 2015). The gSSRs produced 198 bands, and all of them were polymorphic. The number of bands ranged from 1 to 15, with an average of 7.33 amplicons per marker. The size of the alleles was observed to be between 90 bp and 390 bp. The AMS014 produced the highest number of bands (15) while AMS002, AMS005 and AMS030 produced the lowest number of bands (1). The band sizes of the gSSRs were similar to previous reports but not exactly the same. This difference is thought to arise for similar reasons to those causing variation in EST-SSRs. In this study, the polymorphism ratio of gSSRs was higher than that of EST-SSRs (i.e. 100% vs. 95%). This result is consistent with previous studies revealing higher polymorphism levels in gSSR than in EST-SSR markers for different plants (La Rota *et al*. 2005; Liu *et al*. 2012). This is because gSSRs mostly reside in nongenic regions (Varshney *et al.* 2005).

The markers with PIC values equal to 0.7 or higher are highly informative for genetic studies and are very useful in distinguishing the polymorphic rate of a marker at a specific locus (Hildebrand *et al*. 1992). PIC values of all studied primers ranged from 0.18 to 0.92 with a mean of 0.7. Seventeen primers had a PIC value lower than 0.7. The highest PIC value (0.92) was observed in AMS004 marker. The average PIC value of gSSRs (0.73) was higher than that of EST-SSRs microsatellites (0.65). The average *H* value of the whole marker set was 0.74. These values ranged from 0.20 to 0.92. The lowest *H* value (0.20) was observed in AMS002, with the highest observed in AMS004 (0.92). The average *H* value of gSSRs (0.77) was higher than that of EST-SSRs microsatellites (0.70). Compared with the PIC values reported by other researchers, higher results were obtained in this study. Khar *et al*. (2011) obtained the maximum PIC value of 0.89 in 34 onion accessions while Mallor *et al*. (2014) obtained the maximum PIC value of 0.77 in 85 Spanish onion genotypes. In this study, 38 SSRs markers (82%) had a PIC value higher than 0.5. This result was higher than the result published by Khar *et al*. (2011) but lower than the result published by Mallor *et al*. (2014), which were 37% and 92%, respectively.

Genetic distance between Turkish onion accessions varied in the range of Dice's similarity coefficient from 0.407 to 0.767, average 0.587. The highest genetic similarity coefficient (0.767) was found between genotypes 035AC003 and 033AC003, which were collected from the Afyonkarahisar province. The lowest coefficient was obtained between 001AC014 (collected from Bolu) and Metan-88 (a commercial variety). Based on the UPGMA cluster analysis, five main clusters were obtained (Figure 1). Clusters of analysed accessions did not reveal any specific features based on bulb colour or bulb shape. Fischer and Bachmann (2000) and McCallum *et al*. (2008) reported that genetic clustering in onion germplasm was related to geographical origin.

The smallest group was Group-I and consisted of only one local variety (001AC014). Group-II consisted of two local varieties: 034AC003 and 069AC067. The genetic similarity coefficient of these varieties was found to be 0.595. The largest group was Group-III consisting of 84 onion accessions. Members of this group did not correspond in terms of any morphological features. There were four commercial cultivars in this group (Kaf-38, Güntan, Akgün-12 and Yalova-15). Compared with other accessions, Güntan and Akgün-12, which were developed by different breeding companies, were close to each other with

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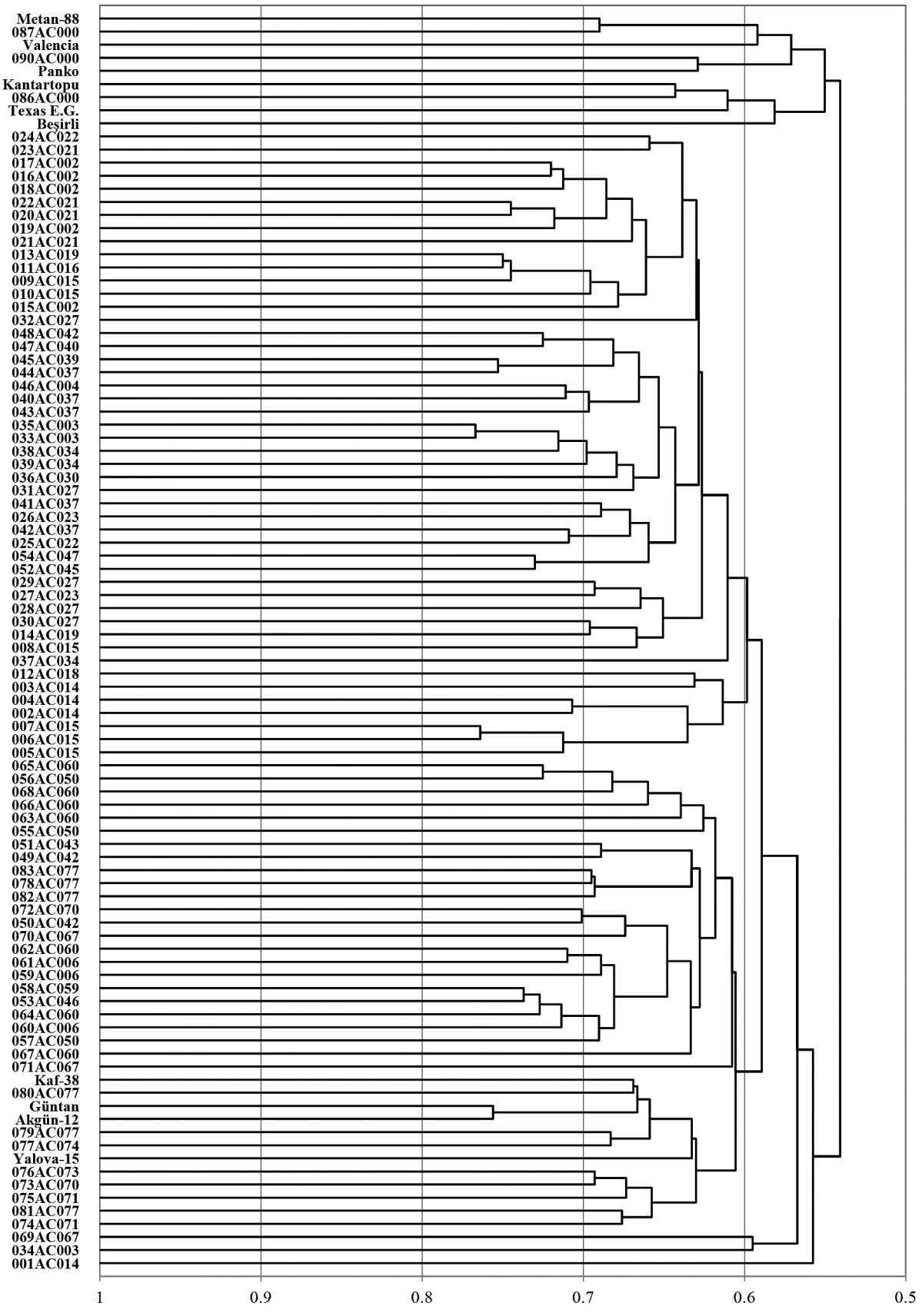

Figure 1. UPGMA dendrogram of Turkish onion accessions based on Dice's similarity coefficients

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0.756 similarity coefficient. Group-IV consisted of three commercial cultivars and two breeding lines (Panko, Valencia, Metan-88, 087AC000 and 090AC000). Group-V consisted of four commercial varieties (Beşirli, Kantartopu, Teksas Early Grano) and a breeding line (087AC000).

Considering all genetic dissimilarity results, the cluster analysis of Turkish onion accessions based on molecular data showed no relationship to bulb skin colour or bulb shape. Similar results have been reported by Khar *et al*. (2011) for Indian onions, Mallor *et al*. (2014) for Spanish onions and Mitrová *et al*. (2015) for Czech onions.

CONCLUSIONS

This study is the first report on the molecular-based genetic diversity analysis of Turkish onion landraces, all of them maintained in the Ataturk Central Horticultural Research Institute, Germplasm Bank of Yalova. The SSR method (EST-SSRs and gSSRs) was used for this aim because of its high efficiency. According to results, the polymorphism ratio of gSSRs is higher than that of EST-SSRs. These results demonstrate the potential value of gSSR markers for the assessment of genetic diversity. The average PIC and *H* values of gSSRs (0.73; 0.77) were higher than those of EST-SSRs microsatellites (0.65; 0.70). Considering the results obtained in the present report, the genetic diversity of the Turkish onion accessions used in this study was at a high level, as reflected by average Dice's similarity coefficient (0.587) and average number of alleles per locus (6.69). The results in this study demonstrate that all the studied accessions should be considered different, and maintained separately in the germplasm collection. The molecular analysis performed in this study provides valuable information to researchers and plant breeders for future studies.

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