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Assessment of genetic diversity in late flowering almond varieties using ISSR molecular markers aimed to select genotypes tolerant to early spring frost in Yazd province

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ABSTRACT

The genetic diversity of 19 late flowering almond genotypes in Yazd province were assessed using ISSR markers. 10 selected ISSR primers revealed 101 polymorphic bands among which the 5`-G(AG)7ASG-3` with 17 and 5`-A(GA)7GSC-3` with 3 bands had the most and the least polymorphic bands respectively. In principal component analysis the explanation of a minor part of the total diversity by few prior components as well as the distribution of total variance among different components, indicate the relevant scattering of the ISSR primers through the genome and the validity of ISSR data for the genetic analysis in almond germplasm. The most genetic similarity in cluster analysis was observed between the genotypes 88 and 191. The high genetic similarity between some genotypes may be caused by their common origin or the geographical similarity between their regions of cultivation and improvement. The transfer and translocation of these genotypes among different studied regions have been carried out frequently. The results of molecular analysis showed that almond varieties and genotypes that are collected from close geographical regions all over the Yazd province are of large genetic homogeneity and the overall polymorphism content in studied genomes is rather low. Considering the self-incompatible nature of the almond plants, it may be concluded that the domesticated genotypes and those cultivated in studied regions, have had little mixture with alien almond germplasm.

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Introduction

Almond occupies a very peculiar place among fruit trees (Miller et al., 1989). Because of almond's tolerance to cold, drought and salinity, it is considered an important tree crop and is cultivated in different climatic regions of Iran. Breeding practices in Prunus face unique challenges resulting from the narrow genetic background of commercial cultivars (Scorza et al., 1985). Morphological traits such as seed and kernel size, kernel yield, and blooming time are usually used for cultivar identification in almond (Deiorgio and Polignano, 1999). However, morphological traits are limited because of their environmental fluctuations.

In recent years, molecular markers have been used to study genetic diversity and cultivar identification of peach and almond (Sanchez-Pérez et al., 2006). Methods based on knowledge provided by advances in molecular genetics, notably molecular markers, promise faster and more efficient approaches to cultivar improvement. In fact important tools such as molecular markers, maps, DNA sequences, and quantitative trait loci (QTLs) have been developed and made available to researchers, and applications at the breeding program level have already started (Dirlewanger et al., 2004).

Materials and Methods

In order to study the genetic diversity of the late flowering genotypes of almond in Yazd Province with use of molecular marker and also evaluation of the marker's efficiency in genetic fingerprinting and acquaintance of almond species, an experiment was done in the interval between Apr and Sept. 2008. The vegetative materials used in these studies include 19 different genotypes and species of almond collected from different points of the province. DNA extraction from leaf tissue was performed according to changed CTAB method (Tompson and Mory, 1997) and the method presented by Gredzil et al. (1995).The results showed that due to deletion of abundant amounts of polysaccharides and poly-phenols existing in almond leaf by PVP, the changed CTAB method with use of PVP, is a more suitable method for DNA extraction from almond. 14 starters were studied on different genotypes of almond and 10 of them with high polymorphism and distinct band pattern were selected for analysis of the data obtained from ISSR marker.

Results and Discussion

10 primers created 101 polymorphic bands totally. Among them the starter 5-G(AG)7 ASG-3 with 17 bands and starter 5-A(GA)7 GSC-3 with three bands had the most and the least number of produced polymorphic bands respectively. Analysis of the balanced components (PcoA) on the basis of the resemblance matrix obtained from Jacard coefficient was performed with use of NTSYS pc 2.02 software. (Rohlf, 1998). The three first factors explain about 50% and the first 8 factors about 80% of the whole changes. Explanation of a smaller part of the whole diversity by some of the first factors and variance distribution among these factors indicate proper distribution of markers in genome that show validity of ISSR data in almond genetic studies. Cluster analysis was performed with NTSYS pc 2.02 e software (Rohlf, 2000). Mantel's test (Mantel, 1967) was performed for Jacard resemblance coefficient, simple and Dais conformity on the basis of UPGMA method. The cophenotypic correlation coefficient obtained on the basis of Jacard Similarity coefficient was 0.82 and Mantel's t static was 6.39, which showed good fitting of dandrogram and matrix of the main resemblance.

On this basis Jacard coefficient was selected for cluster analysis of the studies genotypes. In electrophoresis patterns arising from ISSR markers no allocated place marker (band) was observed for the species related to an area (a band, which exists in all genotypes related to an area and does not exist in any of the genotypes related to the other areas).

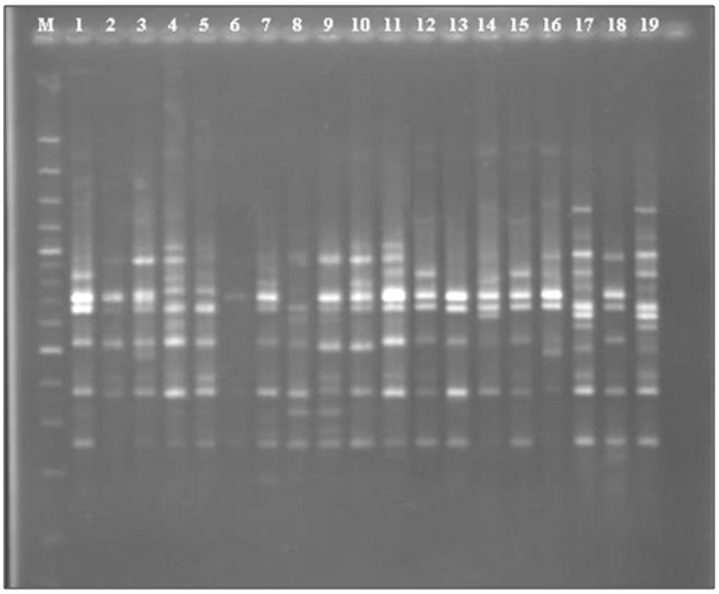

Fig 1. The electerophoresis pattern resulted by (AC)8YG primer combination in 19 almond genotype. Column M indicates size marker (Ladder 100 bp) and columns 1-19 are studied genotypes

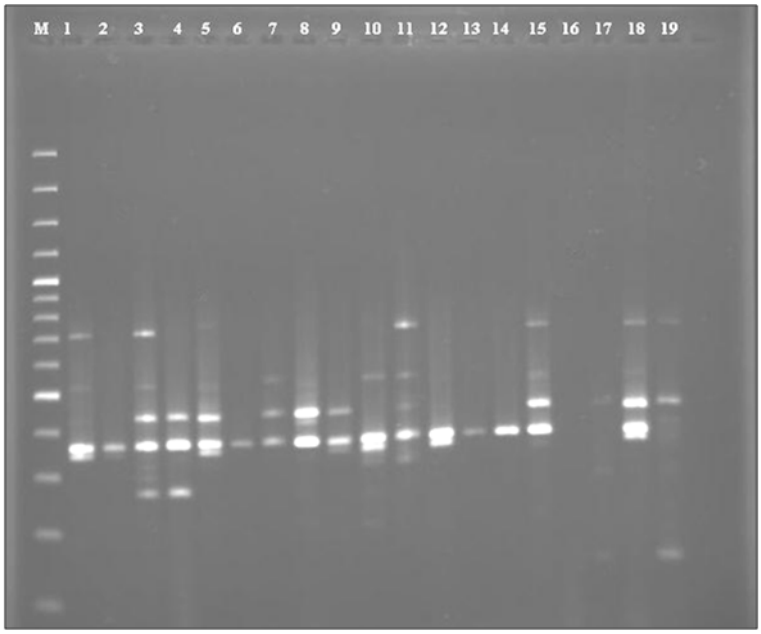

Fig 2. The electerophoresis pattern resulted by (AG)8T primer combination in 19 almond genotype. Column M indicates size marker (Ladder 100 bp) and columns 1-19 are studied genotypes

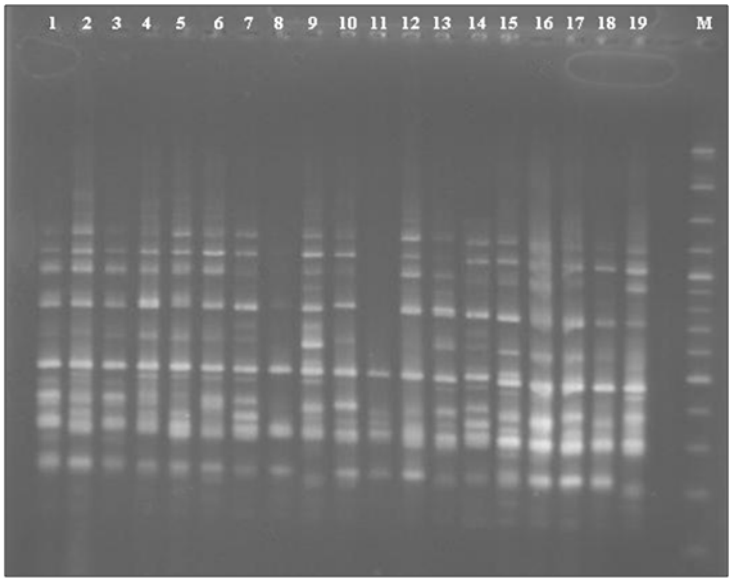

Fig 3. The electerophoresis pattern resulted by DBD(AC)7 primer combination in 19 almond genotype. Column M indicates size marker (Ladder 100 bp) and columns 1-19 are studied genotypes

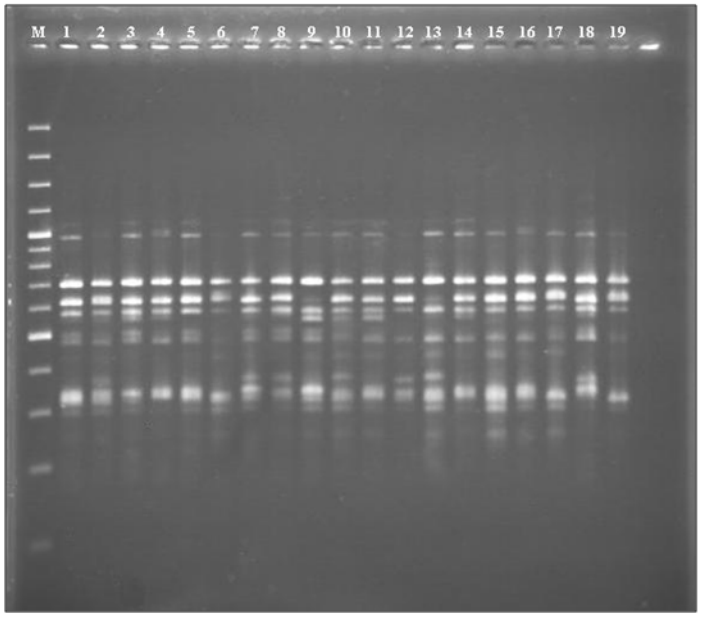

Fig 4. The electerophoresis pattern resulted by HVH(TCC)7 primer combination in 19 almond genotype. Column M indicates size marker (Ladder 100 bp) and columns 1-19 are studied genotypes

Among the studied genotypes, the most genetic similarity is seen between genotypes 191 and 88. Similarity and genetic closeness of some studied genotypes may arise from having the same origin or geographic similarity of their cultivation and breeding areas. Absence of full separation of the species existing in an area from the other species shows that transfer and replacement of these genotypes between the studied areas, which are close with a view to situation has occurred very much. Separation method of genotype 5-Z arises from protection of the attributes of wild almond in this genotype or its difference with the other genotypes in species level. This genotype may also be inter-species or inter-genus hybrid of almond. The results show that there is species diversity in almond genotypes cultivated in different areas of Yazd Province. A number of the species, which were very different with a view to morphologic specifications, were placed in one group in cluster analysis. This may be due to the genetic nature of ISSR markers used in this study, in such a way that the DNA zones propagated and sampled by ISSR markers are related to a part of the genome that have not been translated and have no role in morphologic attributes control. On the other hand some attributes, has extra-nuclear inheritance and their controlling genes are located in cytoplasmic organelles. Early flowering species (5-Z, 4-Z, 21-Z) in this group have not been separated from the late flowering species and scattered in dandrogram. Therefore ISSR could not succeed in separation of the late flowering species from the early flowering ones. ISSR markers are proper tools for recognition of genotypes and wild species of almond. It is advised to be used for increment of the amendatory plans efficiency for investigation of genetic diversity and determination of relationships in almond and the close genera and species. The results arising from molecular studies show that the genotypes and species collected from the areas close to each other at Yazd Province are even genetically and the results indicate relative low percentage of

polymorphism in the genome of the studied individuals and their resemblance. With a view to the self discrepant nature of almond it is cleared that the domesticated species and genotypes of almond cultivated in the studied areas, there is a very low mixing with external germ plasm and a great part of the genetic diversity between them arises from diversity among the individuals existing in the collection. In other words, absence of full separation among the genotypes collected from the studied areas show that diversity between the germ plasm existing in these areas have a small role in the whole computed diversity and for exploitation of the genetic diversity existing in almond germ plasm, we cannot take specimens only from the adjacent areas and more various and richer collections from vaster areas of the state should be studied so that with recognition of the new resources of diversity and the main centers of almond species diversity in the state, exploitation of it in agronomic attributes breeding becomes possible.

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