Coalescence, structure and reticulation analysis of genetic diversity in *Prunus scoparia* populations

Zahra Noormohammadi[1], Zahra Mahdavipoor[2], Masoud Sheidai[2], Kazem Mehdigholi[3], Yeganeh Hasheminejad-Ahangarani-Farahani[2]

[1] Biology Department, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran, e-mail: marjannm@yahoo.com, z-nouri@srbiau.ac.ir (corresponding author)

[2] Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran

[3] Botany Department, University of Tehran, Tehran, Iran

Received: June 26, 2013 ▷ Accepted: December 17, 2013

Abstract. Almond is an important food resource plant in Iran and over 30 *Amygdalus* (*Prunus*) species, subspecies or ecotypes have been reported in the country. Wild almond species can provide a gene pool of valuable characteristics for breeding the cultivated almond. The present study deals with a genetic diversity analysis of three *P. scoparia* populations by using nuclear ISSR markers, and tries to reveal genetic distinctness versus admixture of these populations. The three studied populations differed significantly in their molecular characteristics, allele polymorphism and expected heterozygosity and also showed isolation by distance. AMOVA test revealed significant genetic differences among the three populations ($p<0.01$). The Mantel test showed that genetic distance between the populations correlates with their geographical distance. Furthermore, Bayesian model-based clustering manifested a good separation of the populations at $k = 3$. However, according to the reticulation analysis, these populations were not completely isolated and some degree of gene exchange has occurred among them. The coalescence analysis also revealed horizontal gene transfer and gene exchange among these populations.

Key words: coalescence, genetic diversity, ISSR, wild almond

Introduction

The genus *Prunus* L. (family *Rosaceae*) forms a monophyletic group descended from some Eurasian ancestors (Bortiri & al. 2001). It contains about 200 economically important species, used as sources of fruit, nuts, oil, timber, and for ornamental purposes (Reynders & Salesses 1990). Members of this genus have been divided into five or six subgenera by various authors (Lee & Wen 2001; Bortiri & al. 2002; Potter & al. 2007). These genera are: 1 – subgenus *Amygdalus* L., almonds and peaches, with the type species *Prunus dulcis* L. (Almond); 2 – subgenus *Prunus* L., plums and apricots, with the type species *P. domestica* (Plum); 3 – subgenus *Cerasus* Mill., cherries, with the type species *P. cerasus* (Sour Cherry); 4 – subgenus *Lithocerasus*, with the type species *P. pumila* (Sand Cherry); 5 – subgenus *Padus* Mill., bird cherries, axillary buds single, with the type species *P. padus* (European Bird Cherry); and 6 – subgenus *Laurocerasus* M. Roem., cherry-laurels, mostly evergreen (all other subgenera are deciduous), with the type species *P. laurocerasus* (European Cherry-Laurel) (Lee & Wen 2001; Bortiri & al. 2002; Potter & al. 2007). Almond originates from Central to Southwest Asia. These plants show a wide range of distribution, including in the northeastern-most parts of Asia (Das & al. 2011). The almond tree *(Prunus dulcis*, syn. *P. amygdalus*, or *Amygdalus communis*) is a small deciduous tree of the genus *Prunus*, of subfamily *Prunoideae* of *Rosaceae*. The tree is native

to Southwest Asia, and grows in Iran and Afghanistan, and across the Turano-Iranian area. Native species or wild almonds are generally bitter kernelled due to the higher levels of cyanogenic glycoside amygdalin. Mutation and seedling segregation resulted in the sweet-kernelled types (Das & al. 2011). Iran and Anatolia are the centers of origin of several *Amygdalus* species considered by some (Browicz & Zohary1996; Kester & al. 1991; Kester & Gradziel 1996; Etemadi & Asadi 1999; Ghahreman & Attar 1999; Sorkheh & al. 2009) as potential subspecies or ecotypes diverging from the parental species and adapted to different environmental conditions. The endemic forms occur in Iran either in the form of small, thorny bushes (subgenus *Lycioides*), or of taller, tree-like shrubs which grow in colonies (subgenus *Spartioides* and the colonies of *Amygdalus scoparia* on the southwestern flank of the Zagros) (Zohary 1963; Ladizinsky 1999).

Besides taxonomic interest in the wild almond species (Khatamsaz 1983), they have breeding values too. They can provide an enlarged gene pool of important characteristics, such as late blooming, self-fertilization and resistance to drought, salinity and low winter temperatures, as well as resistance to abiotic and biotic stresses (Sorkheh & al. 2009).

Molecular markers such as isozyems (Vezvaei 1994), Amplified Fragments Length Polymorphism (AFLPs) (Shiran & al. 2009), Inter Simple Sequence Repeats (ISSR) (Shahi-Gharahlara & al. 2011), EST and genomic SSRs (Tahana & al. 2009; Liu & al. 2012), a combination of nuclear and chloroplast SSRs (Zeinalabedini & al. 2008), RAPD (Random Amplified Polymorphic DNA), and SSR markers (Nikoumanesh & al. 2011; Martínez-Gómez & al. 2003. Shiran & al. 2007) and RAPD and ISSR markers (Martins & al. 2003) have been used to study the species relationship and diversity in *Prunus*. In the present study though we have used ISSR nuclear markers to investigate for the first time the intra- and inter-population genetic diversity in three *P. scoparia* populations, and have tried to present the populations' genetic structure as well as the possible gene exchange among them.

Different phylogenetic analyses have been used to show the species/populations genetic affinity and to group the accessions (species, populations, individual trees) (for example, Shiran & al. 2007; Zeinalabedini & al. 2008; Shiran & al. 2009, Liu & al. 2009). A phylogenetic tree relating to the sequences of a single locus, known as gene tree, which usually shows great discordance (both in topologies and branch lengths) has been compared with a phylogenetic tree obtained from multilocus sequence due to deep coalescence (Maddison 1997; Maddison & Knowles 2006). Techniques have been developed to show horizontal gene transfer (HGT) and gene duplication/loss (Legendre & Makarenkov 2002), as well as coalescence. Therefore, in the present study we use a combination of genetic diversity analysis, population structure, reticulation and coalescence analyses to deal with the obtained data and for better understanding of genetic diversity in *P. scoparia* plants in Iran.

Material and methods

Plant material

Seventy-five wild almond trees (*P. scoparia*) growing in three different geographical regions of Iran were used in present study. Trees were collected from Khorasan-Jonoobi (Birjand), Fars (Firoozabad), and Tehran (Koohdasht), respectively. Ten leaves were randomly taken from each tree and used for DNA extraction. The total genomic DNA was extracted by the CTAB method of Murray & Thompson (1980), with a modification described by de la Rosa & al. (2002). The quality of extracted DNA was examined by running on 0.8 % agarose gel.

ISSR assay

Ten ISSR primers – (AGC)5GT, (CA)7GT, (AGC)5GG, UBC 810, (CA)7AT, (GA)9C, UBC 807, UBC 811, (GA)9A, and (GT)7CA commercialized by UBC (the University of British Columbia) – were used. PCR reactions were performed in a 25 µl volume containing 10 mM Tris – HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). Amplification reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94 °C, 30 s at 94 °C; 1 min at 50 °C and 1 min at 72 °C. The reaction was completed by final extension step of 7 min at 72 °C. Amplification products were visualized by running on 2 % agarose gel, followed by ethidium bromide staining. Fragment size was estimated by a 100 bp molecular size ladder (Fermentas, Germany). The experiment was triplicated and constant ISSR bands were used for further analyses.

Data analysis

The obtained ISSR bands were treated as binary characters and coded accordingly (presence = 1, absence = 0). Dice, as well as Nei's genetic distance (Weising 2005; Freeland & al. 2011), was determined among the trees and used for grouping of the genotypes by Unweighted Paired Group Method with arithmetic average (UPGMA) and Neighbor Joining (NJ) clustering methods, after 100 times of bootstrapping (Freeland & al. 2011). Similarly, an ordination plot was made, based on Principal Coordinate Analysis (PCoA), and Multidimentional Scaling (MDS) (Podani 2000), by using PAST ver. 2.17 (Hammer & al. 2001) and DARwin ver. 5.

Genetic diversity parameters were determined in each population. These parameters represented percentage of the allelic polymorphism, allele diversity (Weising 2005), Nei's gene diversity (H), Shannon information index (I) (Weising 2005; Freeland & al. 2011), number of effective alleles, and percentage of polymorphism. AMOVA (Analysis of Molecular Variance) test (with 1000 permutations), as performed in GenAlex 6.4 (Peakall & Smouse 2006), was used to show molecular difference among the populations. Mantel test (Podani 2000) was applied to study the association between molecular distance and geographical distance of the populations.

Model-based clustering was carried out to elucidate the genetic structure of the populations by using STRUCTURE ver. 2.3 (Pritchard & al. 2000). We resorted to an admixture ancestry model under the correlated allele frequency model. The Markov chain Monte Carlo simulation was run 20 times for each value of *K* (2–6) for 10^6 iterations, after a burn-in period of 10^5. All other parameters were set at their default values. Data were scored with dominant markers and analysis following the method suggested by Falush & al. (2007). STRUCTURE Harvester web site (Earl & von Holdt 2012) was used to visualize the STRUCTURE results, and also to apply the Evanno method (Evanno & al. 2005) for identifying the proper number of K (Evanno & al. 2005).

Reticulation was carried out by T-REX (Tree and Reticulogram Reconstruction) ver. 3, and DARwin ver. 5, which infer reticulogram from a distance matrix (Legendre & Makarenkov 2002). Coalescence analysis was performed for molecular data after 1000 times reiteration (Liu & al. 2009), as suggested for SSR polymorphic data by Wilson & Balding (1998) and performed in Mesquite (Maddison & Madison 2011), and deep coalescence, gene duplication and extinction were determined.

Results

Genetic diversity analysis

All 10 SSR primers produced 145 bands in the studied 72 trees. Therefore a data matrix of 72 × 145 was formed for further analysis. The number of alleles varied from 1.08 (Khorasan-Jonoobi, Birjand) to 1.56 (Tehran, Koohdasht) (see Table 1), while the number of effective alleles varied from 1.26 to 1.39. Although we did not see any common allele in these populations, they should have specific (private) alleles, ranging from six in Pop. 2 to 18 in Pop. 3. This latest population also showed the highest percentage of allelic polymorphism (74.48 %) and expected heterozygosity (0.20), while the lowest values occurred in Pop. 1 (46.90 % and 0.15, respectively). The AMOVA test showed some significant genetic differences among the three populations ($p<0.01$) and revealed that 48 % of the total genetic variation was among the populations variation, while 52 % was within the population variation. This result clearly shows the occurrence of intra- as well as inter-population genetic differences in *Prunus scoparia,* which will be further supported by the following tree analyses.

Results of UPGMA and NJ clustering after bootstrapping were alike and, therefore, the NJ tree is discussed bellow (Fig. 1). In general, three distinct clusters are formed, with high bootstrap values supporting their

Table 1. Genetic diversity parameters in the studied *P. scoparia* populations.

Population		Na	Ne	I	He	UHe	PP	NDB	NPB
Birjand	Mean	1.083	1.262	0.236	0.157	0.160	46.90 %	89	16
Firoozabad	Mean	1.352	1.368	0.327	0.217	0.222	65.52 %	101	6
Koohdasht	Mean	1.566	1.394	0.351	0.232	0.237	74.48 %	119	18
Total	Mean	1.333	1.341	0.305	0.202	0.206	62.30 %	103	13.33

Na = No. of different alleles, **Ne** = No. of effective alleles = 1 / (p^2 + q^2), **I** = Shannon's Information Index = –1* (p* Ln (p) + q* Ln(q)), **He** = expected heterozygosity = 2* p* q, **UHe** = unbiased expected heterozygosity = (2N / (2N-1))* He, **PP** = Polymorphism Percentage, **NDB** = No. of different bands, and **NPB** = No. of private bands.

genetic distinctness, as also suggested by the AMOVA test. Intra-population genetic diversity is evident in each population (major clusters); for example, in the first cluster of the population from Khorasan-Jonoobi (Birjand), three subclusters are formed (indicated by arrows 1–3) indicating the genetic differences among 24 studied trees in this population. The other two populations also reveal subclusters due to genetic diversity in the studied trees.

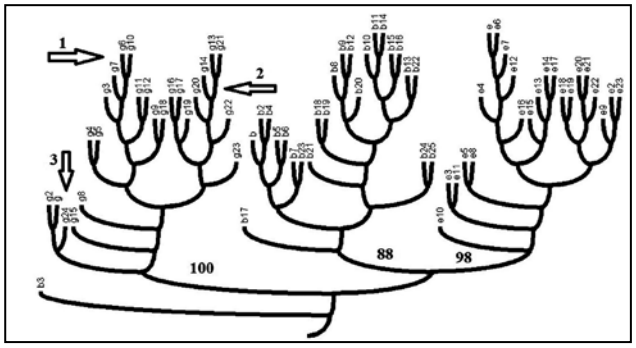

Fig. 1. NJ tree of *P. scoparia* trees (numbers above branches indicate bootstrap values; arrows indicate subclusters formed in one population). Populations' abbreviations: **g** = Khorasan-Jonoobi (Birjand), **b** =Fars (Firoozabad), **e** = Tehran (Koohdasht). Numbers indicate population individuals.

Both MDS PCoA plots have grouped the *Prunus scoparia* trees into three distinct groups, so we present here only the PCoA plot (Fig. 2). Separation of trees of three populations supports the above-mentioned clustering result, but also offers a better visualization of the three populations. In NJ tree, the populations of Tehran (Koohdasht) and Fars (Firoozabad) are placed close to each other, which suggests certain genetic similarity, but in the PCoA plot, although these two populations are placed on the left side of the plot and far from the population of Khorasan-Jonoobi (Birjand), they are positioned at the top and bottom parts of the plot, which indicates their genetic difference. The Mantel test showed a positive significant correlation between the genetic distance and geographical distance among the populations ($p<0.01$).

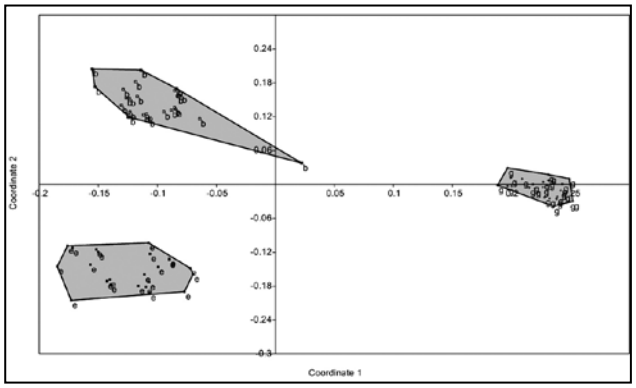

Fig. 2. PCoA plot of the *P. scoparia* trees. Populations' abbreviations: **g** = Khorasan-Jonoobi (Birjand), **b** =Fars (Firoozabad), **e** = Tehran (Koohdasht).

Population structure

MCMC structure reconstruction of ISSR loci showed a moderate genetic structure. When Evanno's *ad hoc* estimator of the actual number of clusters was used, ΔK indicated modes at $K = 3$. The percentages of membership for individuals in each of the three clusters was >66 %.

STRUCTURE analysis showed the highest value of allele frequency divergence (0.14) between the Populations 1 (Khorasan-Jonoobi, Birjand) and 2 (Tehran, Koohdasht), followed by a difference between Khorasan-Jonoobi (Birjand) and Fars (Firoozabad (0.12). However, the populations of Fars (Firoozabad) and Tehran (Koohdasht) showed only 0.09 allele frequency divergence.

The mean value of Fst determined for these populations was 0.44, 0.52 and 0.32, respectively, which means moderate values of population differentiation supporting our AMOVA and clustering results. A STRUCTURE plot of 75 *P. scoparia* trees clearly showed three kinds of allelic composition in them (colored differently in Fig. 3). This indicates that the trees growing in these geographical regions have got specific combinations of alleles, possibly due to their local adaptation. Another point revealed in this plot is a very low degree of ad-

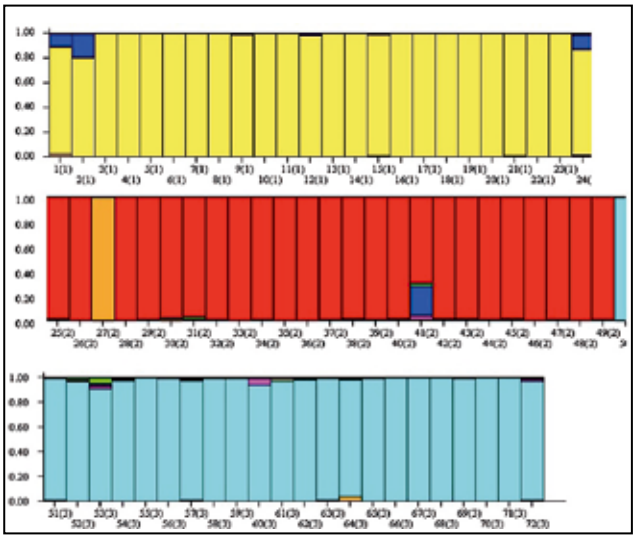

Fig. 3. STRUCTURE plot of the *P. scoparia* trees (each color shows a separate population, the numbers in brackets indicate the respective population of each tree). Population code: **1** = Khorasan-Jonoobi (Birjand), **2** =Fars (Firoozabad), **3** = Tehran (Koohdasht).

mixture among the studied trees and only few occasions of gene exchange determined among these populations. However, mention deserves the fact that in each population some alien alleles exist, which are not from the other two studied populations (small coloring segments not present in other populations). For example, blue colored segments in the populations of Khorasan-Jonoobi (Birjand) and Fars (Firoozabad), and green colored segments in Population 3 (Tehran, Koohdasht). The source of these allelic forms is not known to us but they might have come from other neighboring almond populations, or species growing in the vicinity which are not studied by us, or could be some ancestral alleles still present in small amount in these populations.

Reticulation and coalescence analyses

A reticulogram of *P. scoparia* trees also showed gene exchange among two populations only (Fig. 4). This clearly testifies to the fact that gene exchange is limited among the studied wild almond populations, but the reason for that is yet unknown to us. Also, mention deserves the fact that although two populations (Fars, Firoozabad) & Tehran (Koohdasht) show close genetic affinity in the NJ tree, they do not have allelic exchange. There is some gene exchange among the trees of each population.

Coalescence analysis of the molecular data was carried out to obtain different gene trees later on retained in the species tree. The different tree topologies produced the best tree with 0 deep coalescence and 0 gene duplication/extinction, which shows that complete agreement between the gene tree and the species/population tree was achieved (Fig. 5). Tree topology is

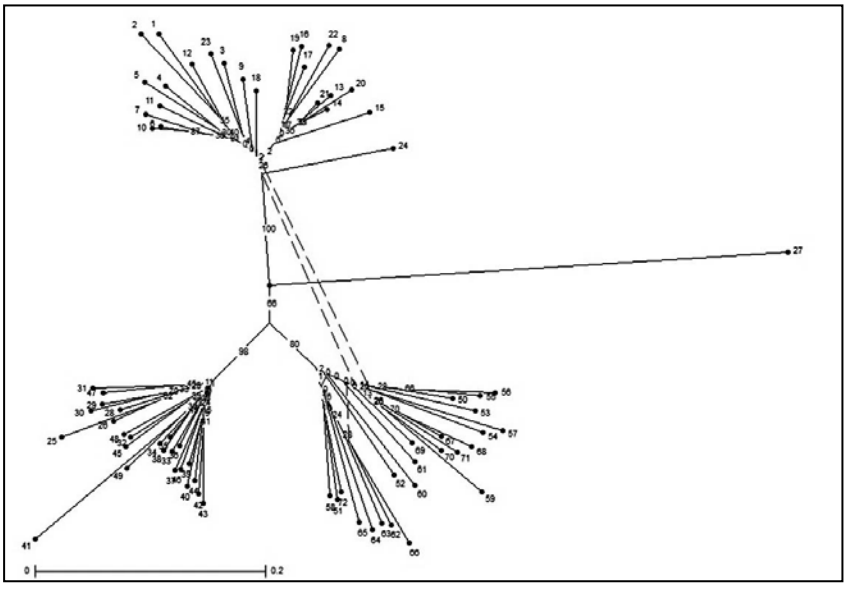

Fig. 4. Reticulogram of the *P. scoparia* trees (dashed lines indicate gene exchange/common alleles). Taxon number: **1-24** = population Khorasan- Jonoobi (Birjand), **25-48** = Fars (Firoozabad), **49-72** = Tehran (Koohdasht)

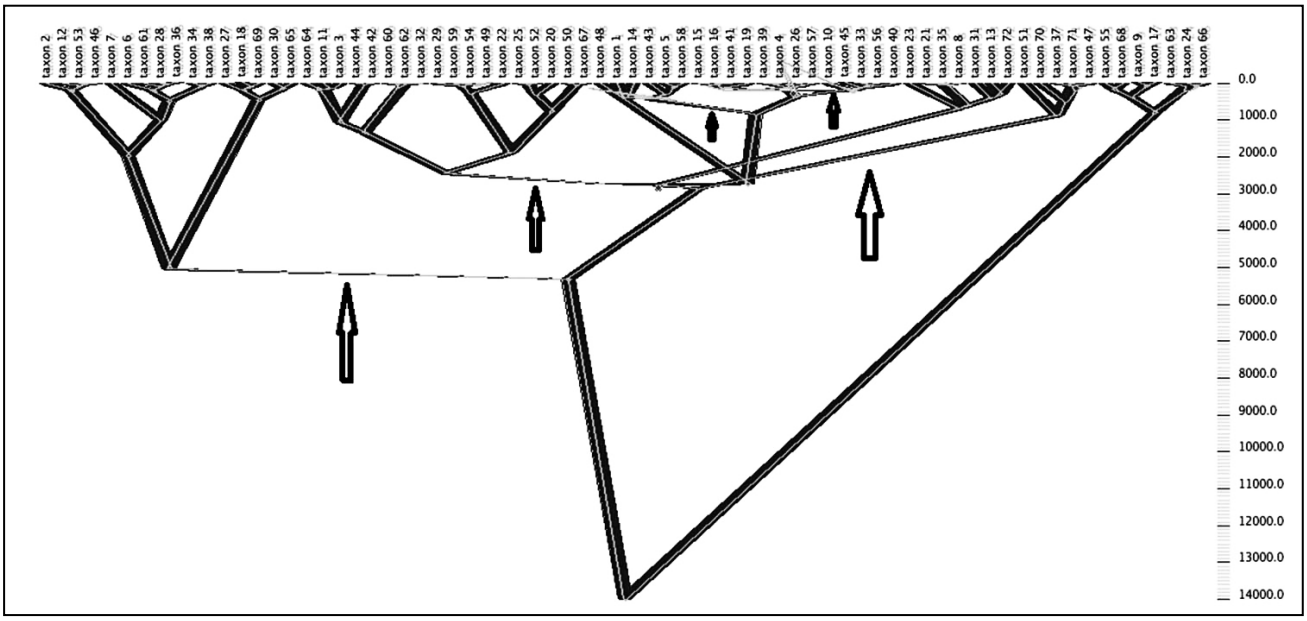

Fig. 5. Gene tree inside the species tree (arrows indicate the horizontal gene transfers).
Taxon numbers: **1–24** = population Khorasan-Jonoobi (Birjand), **25–48** = Fars (Firoozabad), **49–72** = Tehran (Koohdasht).

much more complicated than the one we observed in the NJ tree; there are several subclusters formed not by individuals of the same populations (in contrast to subclusters formed in NJ tree). For example, taxons Nos 2 and 13 of the first population are grouped with taxons Nos 46 (pop 2) and 53 (pop 3); similarly, taxons Nos 6 and 7 (pop 1) are placed close to taxons Nos 28, 34, 36 and 38 (pop 2), and taxon Nos 61 of pop 3. On the other side of the tree, taxons Nos 55, 68, 9, 17, 63, 24, and 66 from different populations are separated from the others with presence of more ancestral form of allelic combinations. Furthermore, a high level of horizontal gene transfer (indicated by arrows in Fig. 5) occurred during the genealogical events in *P. scoparia* which is possible due to hybridization and gene exchange among the trees.

Discussion

The presence of different ISSR allelic forms in the studied *P. scoparia* populations indicates genetic polymorphism in these genotypes. The occurrence of specific alleles only in some of the plants/genotypes illustrates the occurrence of unique insertion/deletion in the DNA material of these genotypes. The high level of genetic diversity and expected heterozygosity reported here is in agreement with the reports of Fathi & al. (2008). These authors reported a high level of heterozygosity and genetic variation among the almond genotypes and attributed it to cross pollination and self-incompatibility of the almond.

Nikoumanesh & al. (2011) used RAPD dominant molecular markers to study the genetic diversity and relationships among 55 Iranian almond genotypes and seven related *Prunus* species. They also observed great genetic diversity among the genotypes. Similar results were obtained by Shahi-Gharahlara & al. (2011) with ISSR molecular markers. These authors studied the genetic diversity in some Iranian wild *Prunus* taxa of subgenus Cerasus and observed high genetic diversity and genetic distance among the studied species and accessions.

Shiran & al. (2007) used both RAPDs and SSRs to study the genetic diversity of Iranian almond cultivars and their relationship to important foreign cultivars and three related species. The results demonstrated extensive genetic variability within the tested cultivars, and the value of SSR markers developed in peach for characterization of almond and related species of *Prunus*.

Bayesian model-based clustering though manifested notable separation of the presumed number of studied populations (k = 3), but some degree of admixture was observed in all three identified groups (populations). Geographical distance between these populations varied between 100-600 km which seems quite great; thus another interesting question is how the genetic exchange has occurred among the studied populations. Zeinalabedini & al. (2008) studied four wild almond species of Iran, namely *P. eleagnifolia*, *P. hauskenchtii*, *P. scoparia,* and *P. lycioides,* by nuclear and chloroplast SSRs and self-incompatibility (S) allele. They also noticed a genetic exchange between different wild almond species. Therefore another possible scenario could be the gene exchange between *P. scoparia* populations and other wild almond forms growing in the vicinity, which was not taken in consideration in the present study.

An AMOVA analysis showed significant difference across the three populations, suggesting that ISSR markers are useful in the genetic investigation of wild almond. Such a significant genetic difference among the populations of *P. scoparia* may indicate an occurrence of genetic changes in geographical populations, in order to adapt to local environmental conditions, as suggested by Liu & al. (2012), who reported infra-specific molecular (SSR) variations in the *Pinus* species due to habitat preferences. The Mantel test also showed that with the increase of geographical distance between populations, their genetic difference increased too. Therefore, we have isolation by distance in the *P. scoparia* populations, but the reticulation analysis has shown the occurrence of some gene exchange among these populations. Therefore, the studied *P. scoparia* populations are not completely isolated from each other.

Nikoumanesh & al. (2011) studied the genetic relationships among 55 Iranian almond genotypes and seven related *Prunus* species and observed that grouping of the genotypes correlated with their geographical origin. Similar results were obtained by Shahi-Gharahlara & al. (2011), who studied genetic relationship between some Iranian wild *Prunus* taxa of subgenus *Cerasus*. Shiran & al. (2007) used both RAPDs and SSRs to study the genetic diversity of Iranian almond cultivars and their relationship to important foreign cultivars and three related species. The

genotypes formed distinct groups based on their geographical origins or pedigree information. However, Martins & al. (2003) used a combination of RAPD and ISSR molecular markers to study some Portuguese *Prunus dulcis* cultivars and their relationship to important foreign cultivars, and have obtained a very heterogeneous grouping of plants which was not related to the geographical origin of the studied genotypes.

An AMOVA test revealed a high degree of genetic variation within the population, which was also evident in the obtained NJ tree. We don't know yet if within the geographical boundaries of each population different microhabitats operate, causing such intra-population genetic differentiation.

The results of the reticulation and coalescence analyses show the role of the gene exchange among *P. scoparia* populations/individuals, which is clearly a genetic mechanism to improve the gene pool in this species. Furthermore, the complex picture we see in the gene tree contained within the species/population tree, suggests usage of different molecular markers, in order to understand the path of gene exchange among the wild almond trees and to illustrate genetic diversity of these plants in the country.

References

Bortiri, E., Oh, S., Gao, F.Y. & Potter, D. 2002. The phylogenetic utility of nucleotide sequences of sorbitol 6-phosphate dehydrogenase in *Prunus* (*Rosaceae*). – Amer. J. Bot., **89**(11): 1697-1708.

Bortiri, E., Oh, S., Jiang, J., Baggett, S., Granger, A., Weeks, C., Buckingham, M., Potter, D. & Parfitt, D.E. 2001. Phylogeny and systematics of *Prunus* (*Rosaceae*) as determined by Sequence Analysis of ITS and the Chloroplast trnL-trnF Spacer DNA. – Syst. Bot., **26**(4): 797-807.

Browicz, K. & Zohary, D. 1996. The genus *Amygdalus* L. (*Rosaceae*): Species relationships, distribution and evolution under domestication. – Genet. Resources Crop Evol., **43**: 229-247.

Das, B., Ahmed, N. & Singh P. 2011. *Prunus* diversity – early and present development: A Review. – Int. J. Biodiv. Conserv., **3**(14): 721-734.

De la Rosa, R., James, C. & Tobutt, K.R. 2002. Isolation and characterization of polymorphic microsatellite in olive (*Olea europaea* L.) and their transferability to other genera in the *Oleaceae*. Primer Note. – Molec. Ecol. Notes, **2**: 265-7.

Earl, D.A. & von Holdt B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. – Conserv. Genet. Resour., **4** (2): 359-361.

Etemadi, N. & Asadi, E. 1999. Study of the wild species of almond and their distribution in Chaharmahal Bakhtiari province. – In: Proc. First Natl. Conf. Almond Shahrekord (Iran), August, 24-27.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: A simulation study. – Molec. Ecol., **14**: 2611-2620.

Falush, D., Stephens, M. & Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. – Molec. Ecol. Notes, **7** (4): 574-578.

Fathi, A., Ghareyazi, B., Haghnazari, A., Ghaffari, M.R., Pirseyedi, S.M., Kadkhodaei, S., Naghavi, M.R. & Mardi, M. 2008. Assessment of the genetic diversity of almond (*Prunus dulcis)* using microsatellite markers and morphological traits. – Iranian J. Biotechnol., **6** (2): 98-106.

Freeland, J.R., Kirk, H. & Peterson, S.D. 2011. Molecular Ecology, 2nd ed. Wiley Blackwell, UK.

Ghahreman, A. & Attar, F. 1999. Biodiversity of Plant Species in Iran.Vol. 1, Publ. Tehran Univ.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. – Palaeontol. Electron., **4** (1): 9pp.

Kester, D.E. & Gradziel, T.M. 1996. Almonds. – In: **Janick, J. & Moore, J.N.** (eds). Fruit Breeding. Jonh Wiley and Sons, New York, USA.

Kester, D.E., Gradziel, T.M. & Grasselly, C. 1991. Almonds (*Prunus*). – In: **Moore, J.M. & Ballington, J.R**. (eds), Genetic Resources of Temperate Fruit and Nut Crops. Intl. Soc. Hort. Sci., Wageningen, Netherlands, pp. 701-758.

Khatamsaz, M. 1983. Flora Iranica, Number 6. *Rosaceae* Family. Institute of Forest and Rangeland Research publ., pp. 274-315.

Ladizinsky, G. 1999. On the origin of almond. – Genet. Resources Crop Evol., **46**:143-147.

Lee, S. & Wen, J. 2001. A phylogenetic analysis of *Prunus* and the *Amygdaloideae* (*Rosaceae*) using ITS sequences of nuclear ribosomal DNA". – Amer. J. Bot., **88** (1): 150-160.

Legendre, P. & Makarenkov, V. 2002. Reconstruction of biogeographic and evolutionary networks using reticulograms. – Syst. Biol., **51**(2): 199-216.

Liu, L., Yu, L., Kubatko, L., Pearl, D.K. & Edwards, S.V. 2009. Coalescent methods for estimating phylogenetic trees. – Molec. Phylogen. Evol., **53**: 320-328.

Liu, Z.L., Cheng, C. & Li, J. 2012. High genetic differentiation in natural populations of Pinushenryi and Pinustabuliformis, as revealed by nuclear microsatellites. – Biochem. Syst. Ecol., **42**: 1-9.

Maddison, W.P. 1997. Gene trees in species trees. – Syst. Biol., **46**: 523-536.

Maddison, W.P. & Knowles, L.L. 2006. Inferring Phylogeny despite Incomplete Lineage. – Syst. Biol. 55(1): 21-30.

Maddison, W.P. & Madison, D.R. 2011. Mesquite: a Modular System for Evolutionary Analysis. Version 2.75. http://mesquiteproject.org.

Martínez-Gómez, P., Arulsekar, S. Potter, D. & Gradziel, T.M. 2003. Relationships among Peach, Almond and related species as detected by simple sequence repeat markers. – J. Amer. Soc. Hort. Sci., **128**(5): 667-671.

Martins, M., Tenreiro, R. & Oliveira, M.M. 2003. Genetic relatedness of Portuguese almond cultivars assessed by RAPD and ISSR markers. – Pl. Cell Rep., **22** (1): 71-78.

Murray, M.G. & Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. – Nucl. Acids Res., **8**: 4321-4325.

Nikoumanesh, K., Ebadi, A., Zeinalabedini, M. & Gogorcena, Y. 2011. Morphological and molecular variability in some Iranian almond genotypes and related *Prunus* species and their potentials for rootstock breeding. – Sci. Hort., **129**(1): 108-118.

Peakall, R. & Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. – Molec. Ecol. Notes, **6**: 288-295.

Podani, J. 2000. Introduction to the Exploration of Multivariate Data [English translation]. Backhuyes, Leide., Ltd.

Potter, D., Eriksson, T., Evans, R.C., Oh, S., Smedmark, J.E.E., Morgan, D.R., Kerr, M., Robertson, K.R., Arsenault, M., Dickinson, T.A. & Campbell, C.S. 2007. Phylogeny and classification of *Rosaceae*. – Pl. Syst. Evol., **266** (1-2): 5-43.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype Data. – Genetics, **155**: 945-959.

Reynders, S. & Salesses, G. 1990. Study on the genetic relationships within the subgenus Prunophora. Restriction maps of the ribosomal genes in *P. cerasifera* and *P. spinosa*. – Acta Hort., **283**:13-26.

Shahi-Gharahlara, A., Zamania, Z., Fatahia, R. & Bouzarib, N. 2011. Estimation of genetic diversity in some Iranian wild *Prunus* subgenus *Cerasus* accessions using inter-simple sequence repeat (ISSR) markers. – Biochem. Syst. Ecol., **39** (4-6): 826-833.

Shiran, B., Amirbakhtiara, N., Kiania, S., Mohammadia, Sh., Sayed-Tabatabaeib, B.E. & Moradic, H. 2007. Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. – Sci. Hort., **111**(3): 280-292.

Shiran, B., Sorkheh, K., Rouhi, V., Gradziel, T.M. & Martínez-Gómez, P. 2009. Molecular characterization of Iranian almond cultivars and related wild species using amplified fragment-length polymorphisms (AFLPs). – Acta Hort., **814** (1): 137-142.

Sorkheh, K., Shiran, B., Rouhi, V., Asadi, E,. Jahanbazi, H., Moradi, H., Gradziel, T.M. & Martínez-Gómez, P. 2009. Phenotypic diversity within native Iranian almond (*Prunus* spp.) species and their breeding potential. – Genet. Resources Crop Evol., **56** (7): 947-961.

Tahana, O., Genga, Y., Zenga, L., Donga, S., Chena, F., Chena, J., Songa, Z. & Zhonga, Y. 2009. Assessment of genetic diversity and population structure of Chinese wild almond, *Amygdalus nana*, using EST- and genomic SSRs. Biochem. – Syst. Ecol., **37**(3): 146-153.

Vezvaei, A. 1994. Pollination studies in Almond. Ph. D. thesis, University of Adelaide, South Australia.

Weising, K., Nybom, H. Wolff, K. & Kahl, G. 2005. DNA Fingerprinting in Plants. Principles, Methods, and Applications, 2nd ed. CRC Press, Taylor & Francis Group, Boca Raton.

Wilson, I.J. & Balding, D.J. 1998. Genealogical Inference from Microsatellite Data. – Genetics, **150** (1): 499-510.

Zeinalabedini, M., Majourhat, K. Khayam-Nekoui, M. Grigoriana, V. Torchia, M. Dicentab, F. & Martínez-Gómez, P. 2008. Comparison of the use of morphological, protein and DNA markers in the genetic characterization of Iranian wild *Prunus* species. – Sci. Hort., **116** (1): 80-88.

Zohary, M. 1963. On the Geobotanical Structure of Iran. Bulletin of the Research Council of Israel, Section D, Botany, vol. II D, Supplement, Passim, esp. pp. 35-38.