Biodiversity Journal, 2013, 4 (1): 243-252

Molecular variation of Iranian local and exotic strawberry (*Fragaria* × *ananassa* Duch.) varieties using SSR markers

Seyed Arman Hosseini[1], Ezzat Karami[2] & Reza Talebi[2*]

[1]College of Agriculture, Islamic Azad University, Kermanshah Branch, Kermanshah, Iran
[2]College of Agriculture, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran
[*]Corresponding author: srtalebi@yahoo.com

ABSTRACT In this study, we report the genetic diversity in a collection of 17 local strawberry cultivars collected from 'Kurdistan' province north-west of Iran with 13 exotic cultivars, using 20 SSR markers. The 20 SSR loci analysed produced 118 alleles with an average 5.9 alleles per marker. The number of alleles ranged from 3 to 16, whereas the maximum was observed in UAFv7648. The effective allelic number (ne) was 3.03 on average of and ranged from 1.22 to 6.98. The genetic diversity (GD) and PIC values ranged from 0.22 to 0.88 and 0.19 to 0.87, with an average of 0.63 and 0.60, respectively. Cluster analysis grouped genotypes in four clusters. Local cultivars grouped in two distinct clusters and other exotic cultivars grouped in other two clusters. Results of cluster analysis suggested that the local cultivars may originated from Queen, Missionary, Ventana and Fresho or may share a same pedigree sources with these cultivars. This is first information on commercially grown local and exotic strawberry cultivars from different origins in Iran using SSR markers.

KEY WORDS Strawberry; Genetic diversity; SSR markers; population structure.

Received 18.01.2013; accepted 20.2.2013; printed 30.03.2013

INTRODUCTION

Assessment of genetic diversity is important in plant breeding to foresee improvement by selection. The commercially important strawberries (*Fragaria* × *ananassa* Duch.) belong to the family Rosaceae and the genus *Fragaria* L., which comprises 23 species (Rousseau-Gueutin et al., 2009). With its high nutritional value, the strawberry is one of the most popular berry fruits in the world. All of these strawberries have seven basic types of chromosomes. However, they exhibit a series of ploidy levels, ranging from diploid species such as *Fragaria vesca* (2n = 2x = 14), to decaploid species, such as some accessions of *F. iturupensis* (2n = 10x = 70). The cultivated strawberry, *F.* × *ananassa*, is an octoploid (2n = 8x = 56) (Nathewet et al., 2010).

Because of this, it is difficult to breed it successfully to develop new varieties with differing genetic characteristics. The verification of strawberry cultivar identity is essential for farmers and plant breeders, as part of certification programs, to prevent mixtures or misidentification of cultivars and to protect breeder's rights. Because of the large number of strawberry cultivars grown today, there is a pressing need for the development of reliable methods for identifying/distinguishing strawberry cultivars and for assessing genetic diversity in strawberry germplasm for breeding purposes. The cultivated strawberry, *F.* × *ananassa* has a narrow genetic pool (Sjulin & Dale, 1987). Efforts to increase the germplasm diversity of the cultivated strawberry have focused on wild species and adapted local germplasm, as parents (Luby et al., 2008).

Strawberry was imported into Iran about 100 years ago from France. Its cultivation area in Iran is about 3000 ha and the total production averages about 21 Kt yearly. Strawberry can be grown successfully in different parts of Iran. Strawberry cultivation and production in Iran has doubled in the last two decades. At present the two main regions of the country producing strawberries are Kurdistan (North-west) and Golestan (North) provinces (Eshghi et al., 2007). Kurdistan province grows about 80% of the total strawberry production in the Iran (Fig. 1).

Currently, morphological traits are used to certify the identity of strawberry cultivars, but this information often does not yield clear answers concerning discrimination of a plant variety, due to ambiguous differences or phenotypic modifications caused by environmental factors (Garcia et al., 2002). For these reasons, as molecular marker technologies become available, they are being evaluated for their usefulness in strawberry cultivar identification and assessing genetic diversity. With respect to genetic diversity, the overriding question is how well do estimates based on molecular marker data correspond to actual levels of genetic diversity and/or to assessments of diversity based on pedigree. Microsatellites, or simple sequence repeats (SSRs) have proved to be locus-specific, codominant, highly reproducible and usually highly polymorphic molecular markers (Powell et al., 1996). Many simple sequence repeat (SSR) markers were developed for *Fragaria* from *F. vesca* (James et al., 2003; Cipriani & Testolin, 2004; Hadonou et al., 2004; Bassil et al., 2006a; Davis et al., 2006; Monfort et al., 2006), *F.* × *ananassa* (Lewers et al. 2005; Bassil et al., 2006b; Gil-Ariza et al., 2006), *F. virginiana* (Ashley et al., 2003), *F. bucharica* Losinsk. (Sargent et al., 2006), and *F. viridis* Weston (Sargent et al., 2003). A major drawback of SSRs, the high cost of microsatellite development (Gupta & Varshney, 2000), was eliminated by high levels of cross-species transferability within *Fragaria* (Ashley et al., 2003; Sargent et al., 2003; Lewers et al., 2005; Bassil et al., 2006a; Davis et al., 2006; Monfort et al., 2006). This has facilitated the use of SSRs in *Fragaria* for fingerprinting (Govan et al., 2008), linkage mapping (Sargent et al., 2006; 2009), and genetic diversity analysis (Hadonou et al., 2004; Gil-Ariza et al., 2006). Expressed sequence tag (EST) projects have generated a vast amount of

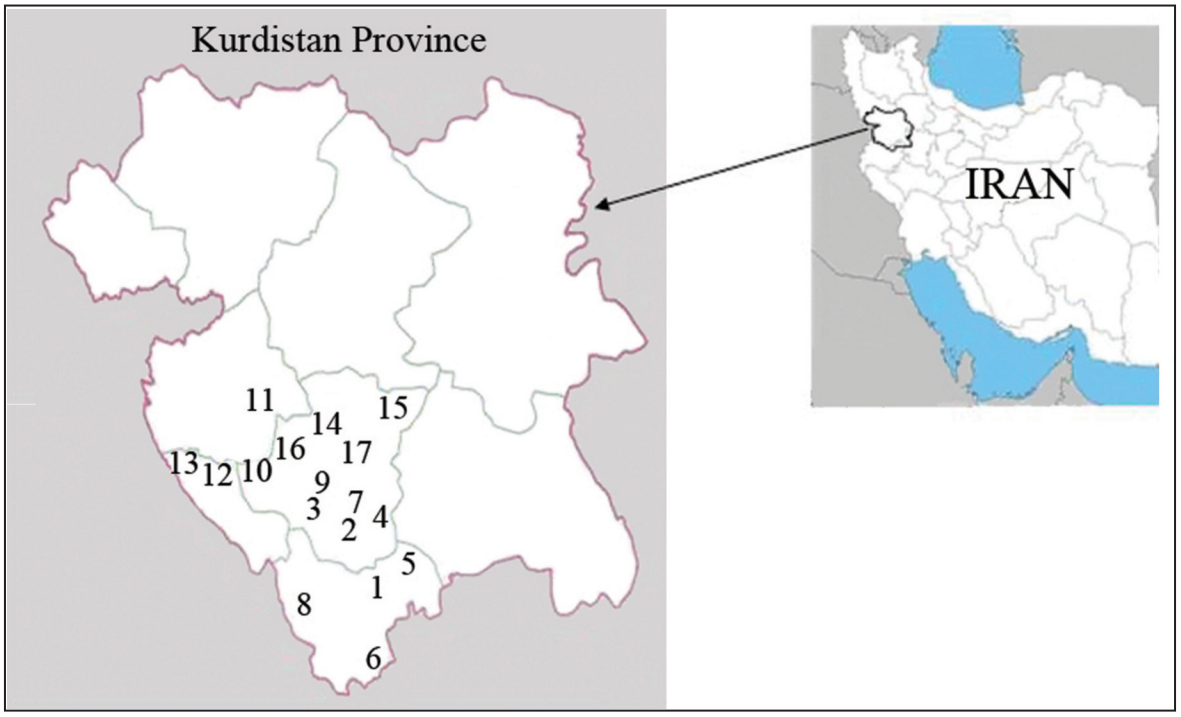

Figure 1. Map of Iran showing the approximate areas of collection site of the 17 local strawberry varieties used in this study.

No.	Genotype	Source	No.	Genotype	Source
1	NashurVasat	Local (Kurdistan province-Iran)	**16**	Sanandaj2	Local (Kurdistan province)
2	Barkulan	Local (Kurdistan province)	**17**	Sanandaj1	Local (Kurdistan province)
3	Yaminan	Local (Kurdistan province)	**18**	Missionary	Exotic (Europe)
4	NashurSafli	Local (Kurdistan province)	**19**	Ventana	Exotic (Europe)
5	Yaminan2	Local (Kurdistan province)	**20**	Fresho	Exotic (Europe)
6	Toriver1	Local (Kurdistan province)	**21**	Karcy	Exotic (Europe)
7	Karabad	Local (Kurdistan province)	**22**	Marak	Exotic (Europe)
8	Toriver2	Local (Kurdistan province)	**23**	Gaviota	Exotic (Europe)
9	Qalaji	Local (Kurdistan province)	**24**	Camarosa	Exotic (Europe)
10	Danikesh	Local (Kurdistan province)	**25**	Diamant	Exotic (Europe)
11	Sanandaj3	Local (Kurdistan province)	**26**	Paroos	Exotic (Europe)
12	Sanandaj4	Local (Kurdistan province)	**27**	Classica	Exotic (Europe)
13	Drileh	Local (Kurdistan province)	**28**	Pajaro	Exotic (Europe)
14	Dorood	Local (Kurdistan province)	**29**	Selva	Exotic (Europe)
15	Shian	Local (Kurdistan province)	**30**	Queen	Exotic (Europe)

Table 1. List of 30 strawberry (*Fragaria* x *ananassa* Duch.) varieties used for SSR fingerprinting.

publicly available sequence data from plant species; these data can be mined for simple sequence repeats (SSRs). These SSRs are useful as molecular markers because their development is inexpensive, they represent transcribed genes and a putative function can often be deduced by a homology search. Because they are derived from transcripts, they are useful for assaying the functional diversity in natural populations or germplasm collections (Varshney et al., 2005).

The objective of this study was to use genomic SSR and EST-derived SSR markers for genetic diversity analysis in some local and exotic strawberry genotypes available in north-west of Iran.

MATERIALS AND METHODS

Thirty genotypes of strawberry, 17 of which collected from different villages of Kurdistan province, north-west of Iran, were examined. All of these genotypes are named as 'Kurdistan' variety, while huge morphological differences exist between them. The additional thirteen genotypes under study are famous exotic varieties that grow in most strawberry production zones of Iran (Table 1).

Genomic DNAs were extracted from approximately 2 g of young leaves tissue. Leaf tissue was ground to a fine powder in liquid nitrogen and stored at -70 °C until total genomic DNA was isolated. DNA was extracted using the CTAB method according to Lassner et al. (1989) with the modification described by Torres et al. (1993). The DNA final concentration was determined by agarose-gel electrophoresis using a known concentration of uncut λ DNA as a standard. Twenty-eight of 82 previously published SSR primer pairs (Lewers et al., 2005; Bassil et al., 2006a, b) were selected based on amplification of expected size fragments and allelic diversity reported by previous studies.

Primer pair	SSR type	sequence		Repeat motif	Reference
		Forward	reverse		
FAC-001	Genomic	AAATCCTGTTCCTGCCAGTG	TGGTGACGTATTGGGTGATG	(AAAAT)7	Lewers et al., 2005
FAC-008	Genomic	TACTGTGCACGCAACAACAG	CTCTCCAATCCTTCATTGAT	(CT)5A(TC)4	Lewers et al., 2005
FAC-011	Genomic	GTTTTCAGGCGGTCAATTCTA	GCTTCAAGCAAAATGCATCATC	(TA)7A(AT)6	Lewers et al., 2005
FAC-012	Genomic	TACACGTGTCCTAGGGTTTTCA	AGCGGAGAATGAGTGACGATAG	(CCT)6	Lewers et al., 2005
UAFv7648	EST	AACCAGAGCCAGAGCCAG	CGACAGTGATGTAGAGGAAGA	(CT)12	Bassil et al., 2006a
UAFv8204	EST	CTCTGCCTTTCTCTACCC	CCCAAGTCTATGAGTGGAAC	(CT)11	Bassil et al., 2006a
UAFv8936	EST	GTGACTTTGACGCTGACC	TGAGAGTGGTTCTGTTCCTC	(TA)7	Bassil et al., 2006a
UFFa01H05	EST	GGGAGCTTGCTAGCTAGATTTG	AGATCCAAGTGTGGAAGATGCT	(CT)8	Bassil et al., 2006a
UFFa02A03	EST	GAGCTACACAATGCCATCAAAA	GCGCATTCGACTCTGTAACTCT	(AG)12	Bassil et al., 2006a
UFFa02G01	EST	ACGAGGTGGGTTTTGTGTTGT	CCCAGATGAAGAAACCGATCTA	(AG)6	Bassil et al., 2006a
UFFa03D11	EST	TACCTTCTGCATTCACCATGAC	GCCTTGATGTCTCGTTGAGTAG	(AGA)5	Bassil et al., 2006a
UFFa09B11	EST	CTTGGGAGAGAACCAGAAAAAC	TCAGAACCAACTCCAGAGAAGC	(AG)6	Bassil et al., 2006a
UFFa14F08	EST	GTTTCTCTCAGGGCCAAAAT	CTTGAGTAGTCCTCTCACCATTG	(TC)9, (TA)5	Bassil et al., 2006a
UFFa19B10	EST	ATTTCTGTTGTCTCCCTCCTTC	GCTCGATCTCTAGCTTTCTCTCT	(CT)10, (TC)6	Bassil et al., 2006a
UFFa20G06	EST	ACTCAACCACCACATTTCACAC	GAGAAGTTGTCAATAGTCCAGGTG	(CT)11	Bassil et al., 2006a
UFFa16H07	EST	CTCTACCACCATTCAAAACCTC	CACTGGAGACATCTAGCTCAAAC	(CT)11	Bassil et al., 2006a
SF- 2H12	EST	CCTGCATATCTTCTGCAACAAC	AAGCAGCACCACCTTCAGTAGT	(TC)15	Bassil et al., 2006b
SF-1B07	EST	GGAGAGACAGACCTCAAAGGTG	GAGGGGTTCTGTTTTTGACAAG	CTs (AG)7	Bassil et al., 2006b
SF-4B12	EST	GCAAAGTCGGAGAGAGATAGA	CTGAAGAAGGTGTTGAGGAA	(CT)8	Bassil et al., 2006b
SF-5G02	EST	CTTTTGCTGCTAGCTCTTTGTG	TACGTACTCCACATCCCATTTG	(TC)11	Bassil et al., 2006b

Table 2. List of 20 SSR primer pairs, including the type of SSR (EST or genomic), primer sequences (F-forward, R-reverse), and repeat motifs, used for genetic diversity assessment of strawberry varieties.

These twenty-eight SSRs were tested for amplification by 3% agarose gel electrophoresis in 10 genotypes and identified 20 well-amplified SSR primer pairs (Table 2). These SSR primer pairs were chosen for this study based on polymorphism and ease of scoring after fragment analysis.

These SSRs consisted of 16 expressed sequence tag (EST) SSRs and four genomic SSRs. PCRs were performed in 20 µl total reaction volumes containing: 1× PCR buffer, 2 mM MgCl2, 0.2 mM each dNTP, 0.3 µM of each primer, 1 U of Taq polymerase enzyme (Cinagen, Iran) and 15 ng of DNA

Microsatellite	Expected size	N_A	N_e	GD	C_j	D_j	PIC
FAC-001	212	4	1.95	0.53	0.22	0.78	0.50
FAC-008	138	3	1.22	0.22	0.39	0.61	0.19
FAC-011	298	4	3.36	0.74	0.15	0.85	0.71
FAC-012	169	6	2.99	0.69	0.16	0.84	0.68
UAFv7648	235	16	6.98	0.88	0.06	0.94	0.87
UAFv8204	238	6	2.17	0.59	0.27	0.77	0.55
UAFv8936	310	3	1.41	0.34	0.35	0.65	0.30
UFFa01H05	246	5	2.18	0.60	0.20	0.80	0.55
UFFa02A03	168	5	4.12	0.79	0.13	0.77	0.77
UFFa02G01	159	4	2.16	0.57	0.28	0.72	0.55
UFFa03D11	189	4	2.11	0.58	0.19	0.71	0.54
UFFa09B11	197	6	3.46	0.74	0.14	0.86	0.72
UFFa14F08	137	6	2.96	0.69	0.17	0.73	0.67
UFFa19B10	183	7	3.09	0.72	0.18	0.82	0.69
UFFa20G06	154	5	1.9	0.50	0.25	0.75	0.53
UFFa16H07	248	3	2.54	0.66	0.18	0.82	0.62
SF- 2H12	240	8	5.44	0.88	0.09	0.91	0.83
SF-1B07	163	9	3.8	0.77	0.14	0.86	0.75
SF-4B12	355	5	1.62	0.42	0.31	0.69	0.39
SF-5G02	229	9	5.17	0.85	0.09	0.91	0.82

Table 3. List of 20 SSR primer pairs, including the type of SSR (EST or genomic), primer sequences (F-forward, R-reverse), and repeat motifs, used for genetic diversity assessment of strawberry varieties.

template. PCR products were analyzed using 3% methaphor agarose electrophoresis gels stained with ethidium bromide. Alleles were scored as present (1) or absent (0) for each marker and a binary data matrix was created. DARwin version 5.0 was used for calculating pair-wise genetic distances and for constructing the dissimilarity matrix (Perrier et al., 2003). The dissimilarity matrix thus obtained was subjected to cluster analysis using the un-weighted neighbour-joining (UNJ) method (Gascuel, 1997), followed by bootstrap analysis with 1,000 permutations to obtain a dendrogram for all the 30 accessions (Perrier et al., 2003). Frequencies of incidence of all polymorphic alleles for each SSR markers were calculated and used for determining statistical parameters. Confusion probability (Cj) and discri-

minating power (Dj) of each SSR were estimated according to Tessier et al. (1999) and polymorphic information content (PIC) following Botstein et al. (1980).

RESULTS

SSR polymorphisms

In the present study, a total of 20 SSR loci (4 genomic and 16 EST-derived SSRs) (Table 2) were used to analyze genetic diversity among 17 local varieties collected from 'Kurdistan' province (North-west of Iran) and 13 exotic cultivars. All SSR markers showed a high level of polymorphisms, display a total of 118 different alleles with fragment size ≈ 130 to 300 bp (Table 3). The number of alleles per locus varied from 3 to 16, with an average value of 6 alleles. FAC-008, UAFv8936 and UFFa16H07 amplified minimum number of alleles (3), whereas the maximum was observed at UAFv7648, with 16 different alleles. The effective allelic number (ne) was 3.03 on average and ranged from 1.22 to 6.98 (Table 3). The gene diversity (GD) and PIC values ranged from 0.22 to 0.88 and 0.19 to 0.87, with an average of 0.63 and 0.60, respectively (Table 3). High value of discriminating power (Dj≥ 0.70) and PIC≥0.50, and low values of confusion probability (Cj≤0.30) were obtained for all SSR markers evaluated except for FAC-008, UAFv8936 and SF-4B12 (Table 3), thus indicating the usefulness and power of these SSR markers for genetic identification in strawberries.

Cultivar identification

The genetic similarity matrix was analysed using un-weighted neighbor joining (UNJ) clustering algorithm by software programme DARwin 5.0 (Fig. 2). The radial branching clearly delineated the genotypes in four major clusters (Fig. 2). Group 1 was further divided in two subgroups. This cluster contained eight genotypes three of which (Fresho, Ventana and Missionary) were exotic cultivars and the remaining ones were local cultivars collected from southern parts of 'Kurdistan' province. In group 2, only two exotic cultivars (Karcy and Marak) were placed with two local cultivars originated from central zone of 'Kurdistan' province.

Eight cultivars grouped in cluster 3 including all exotic cultivars. Cluster 4 contained ten genotypes all of which were local cultivars mostly collected from the southern part of 'Kurdistan' province (Fig. 2). Most of the local cultivars grouped in clusters 1 and 4, and four exotic cultivars (Queen, Missionary, Ventana and Fresho) were the closest cultivars to these local varieties. In cluster 3 were grouped most of the remained exotic cultivars, significantly distant from local varieties. These results suggested that the local cultivars in Kurdistan province may be originated from Queen, Missionary, Ventana and Fresho or may share the pedigree sources with these cultivars. In order to determine the ability of SSR markers to display genetic relationships among strawberry cultivars, principle co-ordinate analysis (PCoA) was carried out and cultivars were plotted in the coordinate system for the first two coordinates which accounted for 45% and 30% of the variation, respectively (Fig. 3). Most of the cultivars are separated by the first or second PCoA (Fig. 3) which demonstrated distinct groups of cultivars corresponding to cluster analysis (Fig. 2).

DISCUSSION AND CONCLUSIONS

Whereas phenotypic descriptors may vary with climate, geography or cultural practices, forensic tools based on genetic material allow cultivar authentication with great certainty. DNA markers provide a robust, rapid, and relatively inexpensive means to differentiate closely related plant materials. Strawberry plants are propagated vegetatively and can be easily misidentified based on phenotype (Bassil et al., 2006b). Being a vegetative propagated species, collected strawberry accessions show considerable levels of genetic divergence. Despite its economic value, the polyploid constitution of the strawberry has been a major barrier to the genetic characterization of the cultivated species and limited information on the genome structure has been published.

In this study, 20 SSR primers that bracket well-conserved and readily amplifiable regions of strawberry genomic DNA were used to assay the strawberry germplasm typically grown by farmers in 'Kurdistan' province (North-west of Iran) and the exotic cultivars available in the collection of agricultural research station of Kurdistan. SSR markers have the advan-

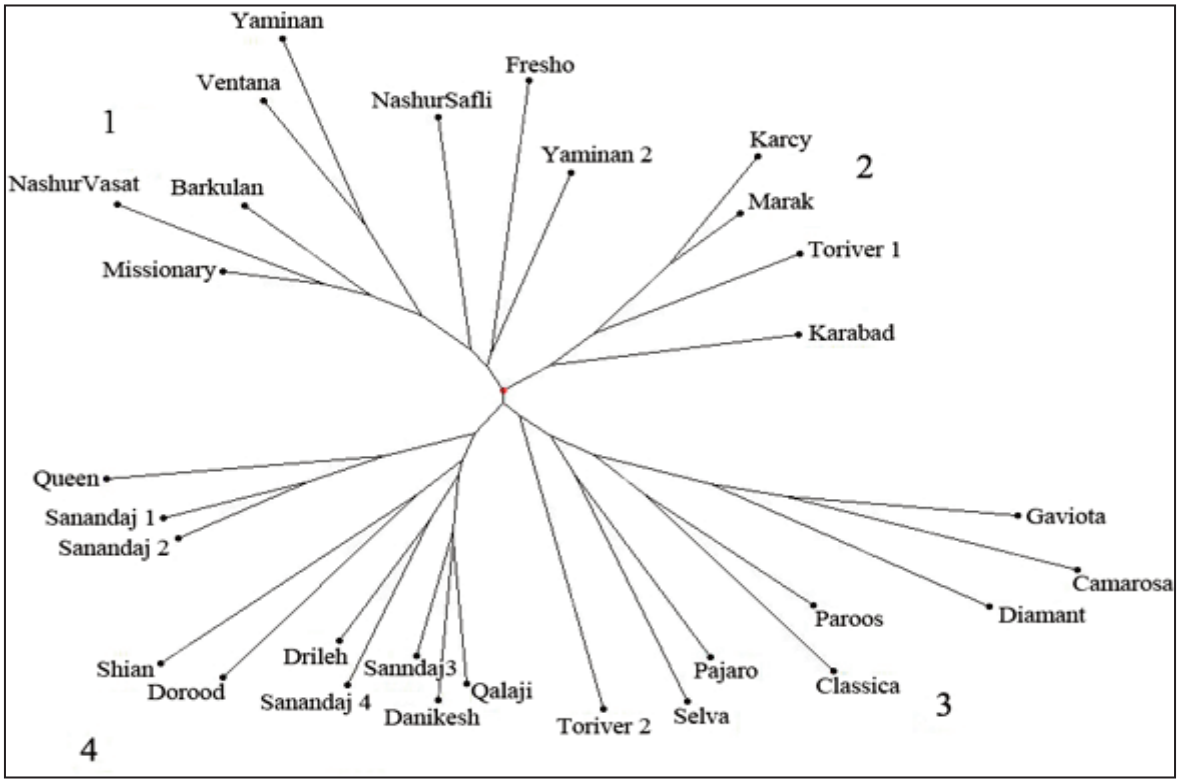

Figure 2. Un-weighted pair grouping method of arithmetic averages dendrogram of 30 strawberry varieties based on genetic distances computed from SSR markers.

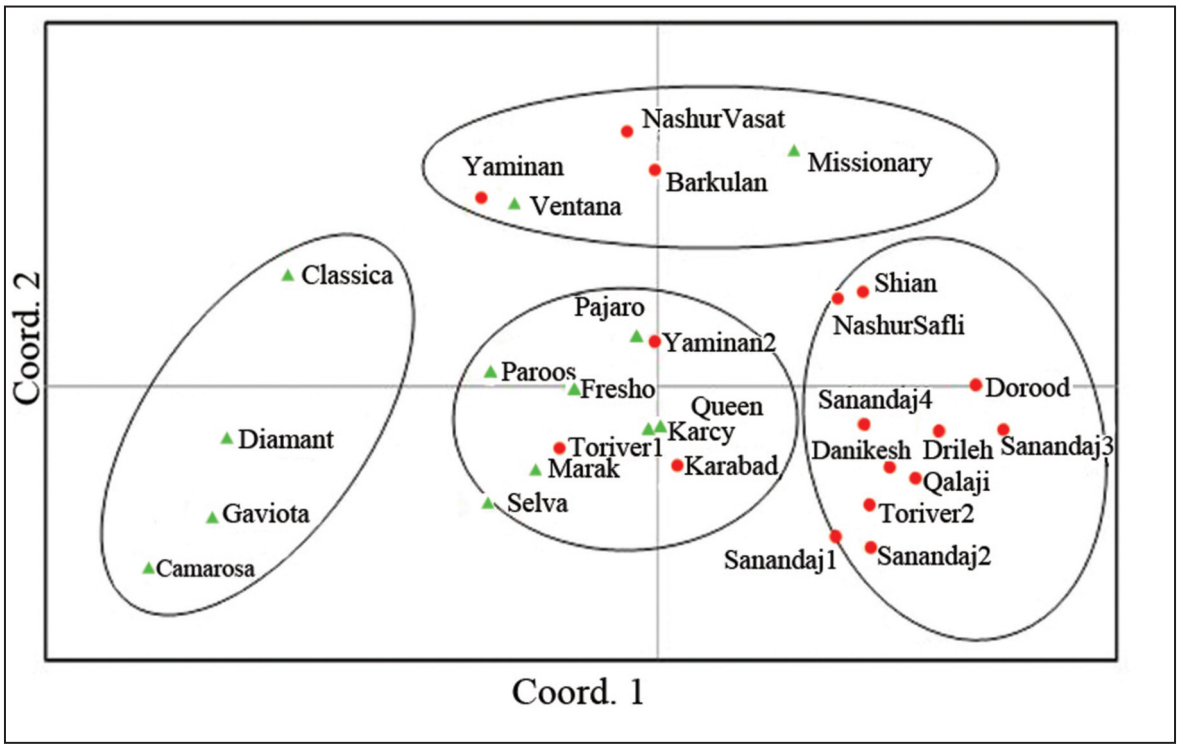

Figure 3. Two-dimensional representation of genetic relations among 30 strawberry varieties using SSR data.

tage over arbitrary marker systems in using primer pairs of a defined sequence, and are therefore highly reproducible and in the case of EST-SSRs, are developed directly from the sequence of genes of known function. Thus, SSR markers could correspond to candidate sequences relevant to horticultural traits. In addition, SSRs can be genotyped with a high degree of precision, permitting the standardization of fingerprint data between laboratories providing a reference cultivar set which can be used in all experiments. This is the first known report on the use of SSR markers for creating genetic fingerprints and determining genetic relationships among Iranian strawberry genotypes.

The results clearly demonstrate that SSR markers can be used in a genetic diversity study as well as in genotypic identification of strawberries, as already noted by Brunings et al. (2010) and Njuguna et al. (2011). In the current study, a total number of 118 alleles, detected using 20 SSR selective markers, with an average of 5.9 allele per locus, suggested the presence of a considerable polymorphism at studied microsatellite loci and revealed a high level of genetic diversity in the existing strawberry germplasm (Table 3) which is close to results obtained by Brunings et al. (2010) and Njuguna et al. (2011). Moreover we demonstrate that a low number of polymorphic SSR markers could be applied to differentiate a large number of strawberry genotypes and that SSR markers potentially detect a higher level of DNA polymorphism than do other DNA markers in strawberries. The effective number of allele (ne) was taken into account for both the number of alleles and their frequencies. It allowed us to compare populations where the number and distributions of alleles differ drastically. We also tried to assess the effective number of alleles as a corollary to the expected heterozygosity. PIC values estimate the discriminatory power of a marker. The mean PIC value for markers used in present study was 0.60 and it ranged from 0.19 for locus FAC-008 to 0.87 for locus UAFv7648 (Table 3).

Markers with high PIC values could be effectively used in genetic diversity studies in strawberry. Despite the high number of alleles observed in most SSR loci, a high number of alleles was observed in exotic cultivars in comparison to local cultivars. There are fewer alleles present in the local cultivars, suggesting that the resource was constructed from a narrower genetic set than that which comprises the bulk of the exotic cultivars. This finding is somewhat anticipated as the exotic cultivars assayed represent plant materials arising from many different origins and breeding programs. Still, future breeding efforts might benefit from a parallel introgression of additional diversity from ranging sources. SSR-based fingerprinting can guide the increase in genetic diversity by selection of genotypes with different alleles than those present in existing breeding programs.

The existing genetic diversity observed in studied cultivar, indicated the efforts underway to widen the genetic base of strawberry for various traits. Information about current genetic diversity permits the classification of our available germplasm into various/heterotic groups, which is particularly important to hybrid/cross-breeding programs in strawberry. Even though the genetic mechanisms that explain heterosis are not fully understood, it is well documented that crosses between unrelated and genetically distant parents, show a greater hybrid-vigor than crosses between closely related parents (Stuber 1994; Hallauer, 1999). There could be a possibility to exploit hybrid in strawberry. The previous crossing programs in most research centers of Iran were based on only phenotypic characters.

The current study confirmed the importance of molecular studies beside the morphological data in detecting genetic variation among genotypes in selecting diverse parents to carry out a new crossing program successfully.

REFERENCES

Ashley M.V., Wilk J.A., Styan S.M.N., Craft K.J., Jones K.L., Feldheim K.A., Lewers K.S. & Ashman T.L., 2003. High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill (Rosaceae). Theoretical and Applied Genetics, 107: 1201-1207.

Bassil N.V., Njuguna W. & Slovin J.P., 2006a. EST-SSR markers from *Fragaria vesca* L. cv. Yellow Wonder. Molecular Ecology Notes, 6: 806-809.

Bassil N.V., Gunn M., Folta K. & Lewers K., 2006b. Microsatellite markers for *Fragaria* from 'Strawberry Festival' expressed sequence tags. Molecular Ecology Notes, 6: 473-476.

Botstein D., Withe R.L., Skolnick M. & Davis R.W., 1980. Construction of genetix linkage map in man

using restriction fragment length polymorphisms. American Journal of Human Genetics, 32: 314-331.

Brunings A.M., Moyer C., Peres N. & Folta K.M., 2010. Implementation of simple sequence repeat markers to genotype Florida strawberry varieties. Euphytica, 173: 63-75.

Cipriani G. & Testolin R., 2004. Isolation and characterization of microsatellite loci in *Fragaria*. Molecular Ecology Notes, 4: 366-368.

Davis T.M., Dimeglio L.M., Yang R., Styan S.M.N. & Lewers K.S., 2006. Assessment of SSR marker transfer from the cultivated strawberry to diploid strawberry species: functionality, linkage group assignment, and use in diversity analysis. Journal of American Society of Horticultural Science, 131: 506-512.

Eshghi S., Abdi G., Tafazoli E. & Yavari S., 2007. Strawberry Research and Biotechnology in Iran. Middle Eastern and Russian Journal of Plant Science and Biotechnology, 1: 39-41.

Garcìa M.G., Ontivero M., Diaz Ricci J.C. & Castagnaro A., 2002. Morphological traits and high resolution RAPD markers for the identification of the main strawberry varieties cultivated in Argentina. Plant Breeding, 121: 76-80.

Gascuel O., 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Molecular Biology and Evolution, 14: 685-695.

Gil-Ariza D.J., Amaya I., Botella M.A., Muñoz Blanc J., Caballero J.L., López-Aranda J.M., Valpuesta V. & Sánchez-Sevilla J.F., 2006. EST-derived polymorphic microsatellites from cultivated strawberry (*Fragaria ×ananassa*) are useful for diversity studies and varietal identification among *Fragaria* species. Molecular Ecology Notes, 6:1195-1197.

Govan C.L., Simpson D.W., Johnson A.W., Tobutt KR.. & Sargent D.J., 2008. A reliable multiplexed microsatellite set for genotyping *Fragaria* and its use in a survey of 60 *F. ×ananassa* cultivars. Molecular Breeding, 22: 649-661.

Gupta P.K. & Varshney R.K., 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica, 113: 163-185.

Hadonou A.M., Sargent D.J., Wilson F., James C.M. & Simpson D.W., 2004. Development of microsatellite markers in *Fragaria*, their use in genetic diversity analysis, and their potential for genetic linkage mapping. Genome, 47: 429-438.

Hallauer A.R., 1999. Temperate maize and heterosis. In: Coors J. and Pandey S. (Eds.). The Genetics and exploitation of heterosis in crops, CIMMYT, Mexico City 17-22 Aug. 1997 ASA, Madison, WI. pp. 353-361.

James C.M., Wilson F., Hadonou A.M. & Tobutt K.R., 2003. Isolation and characterization of polymorphic microsatellites in diploid strawberry (*Fragaria vesca* L.) for mapping, diversity studies and clone identification. Molecular Ecology Notes, 3:171-173.

Lassner M.W., Peterson P. & Yoder J.I., 1989. Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. Plant Molecular Biology Reporter, 7: 116-128.

Lewers K.S., Styan S.M.N., Hokanson S.C. & Bassil N.V., 2005. Strawberry GenBank-derived and genomic simple sequence repeat (SSR) markers and their utility with strawberry, blackberry, and red and black raspberry. Journal of American Society of Horticultural Science, 130:102-115.

Luby J.J., Hancock J.F., Dale A. & Serçe S., 2008. Reconstructing *Fragaria ×ananassa* utilizing wild *F. virginiana* and *F. chiloensis*: inheritance of winter injury, photoperiod sensitivity, fruit size, female fertility and disease resistance in hybrid progenies. Euphytica, 163: 57-65.

Monfort A., Vilanova S., Davis T.M. & Arus P., 2006. A new set of polymorphic simple sequence repeat (SSR) markers from a wild strawberry (*Fragaria vesca*) are transferable to other diploid *Fragaria* species and to *Fragaria × ananassa*. Molecular Ecology Notes, 6: 197-200.

Nathewet P,, Hummer K.E., Yanagi T., Iwatsubo Y. & Sone K., 2010. Karyotype analysis in octoploid and decaploid wild strawberries in *Fragaria* (Rosaceae). Cytologia, 75: 277-288.

Njuguna W., Hummer K.E., Richards C.M., Davis T.M. & Bassil N.V., 2011. Genetic diversity of diploid Japanese strawberry species based on microsatellite markers. Genetic Resources & Crop Evolution, 58: 1187-1198.

Perrier X., Flori A. & Bonnot F., 2003. Data analysis methods. In: Hamon P., Seguin M., Perrier X. & Glaszmann J.C. (Eds.). Genetic diversity of cultivated tropical plants. Science Publishers, Montpellier, pp. 43-76.

Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S. & Rafalski A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Rousseau-Gueutin M., Gaston A., Aïnouche A., Aïnouche M.L., Olbricht K., Staudt G., Richard L. & Denoyes-Rothan B., 2009. Tracking the evolutionary history of polyploidy in *Fragaria* L. (strawberry): new insights from phylogenetic analyses of low-copy nuclear genes. Molecular Phylogenetics & Evolution, 51: 515-530.

Sargent D., Fernandez-Fernandez F., Ruiz-Roja J., Sutherland B., Passey A., Whitehouse A. & Simpson D., 2009. A genetic linkage map of the cultivated strawberry (*Fragaria × ananassa*) and its comparison to

the diploid *Fragaria* reference map. Molecular Breeding, 24: 293-303.

Sargent D.J., Clark J., Simpson D.W., Tobutt K.R., Arús P., Monfort A., Vilanova S., Denoyes-Rothan B., Rousseau M., Folta K.M., Bassil N.V. & Battey N.H., 2006. An enhanced microsatellite map of diploid *Fragaria*. Theoretical and Applied Genetics, 112: 1349-1359.

Sargent D.J., Hadonou A.M. & Simpson D.W., 2003. Development and characterization of polymorphic microsatellite markers from *Fragaria viridis*, a wild diploid strawberry. Molecular Ecology Notes, 3: 550-552.

Sjulin T. & Dale A., 1987. Genetic diversity of North American strawberry cultivars. Journal of American Society of Horticultural Science, 112: 375-385.

Stuber C.W., 1994. Heterosis In Plant Breeding. Plant Breeding Review, 12: 227-251.

Tessier C., David J., This P., Boursiquot J.M. & Charrier A., 1999. Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theoretical and Applied Genetics, 98: 171-177.

Torres A.M., Weeden N.F. & Marín A., 1993. Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. Theoretical and Applied Genetics, 85: 937-945.

Varshney R.K., Graner A. & Sorrels M.E., 2005. Genic microsatellite markers in plants: Features and applications. Trends in Biotechnology, 23: 48-55.