Molecular Biology Research Communications 2018;7(3):125-132 DOI: 10.22099/mbrc.2018.29924.1327 MBRC
Original Article Open Access

IRAP and REMAP based genetic diversity among varieties of *Lallemantia iberica*

Arezoo Cheraghi[1], Fatemeh Rahmani[1,*], Abdollah Hassanzadeh-Ghorttapeh[2]

1) Biology Department, Faculty of Sciences, Urmia University, Urmia, Iran
2) Horticulture Crop Research Department, West Agricultural and Natural Resources Research and Education Center, Urmia, Iran

ABSTRACT

This study describes the genetic relationships among 34 varieties of *Lallemantia iberica* using inter-retrotransposon amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP). Samples were collected from Agriculture Research Center of Urmia city (northwest Iran). Ten IRAP and REMAP primers generated 76 scorable electrophoretic bands with 78.94% polymorphism. The pair-wise Jacquard genetic similarity varied from 0.48 to 0.94 for IRAP and REMAP data combined. Average PIC values for IRAP and REMAP markers were 0.38. The retro-elements marker system produced 76 alleles in range of 100- 3000 bp. The cophenetic correlation coefficient between Jaccard's similarity matrix and the plotted dendrogram was 0.66. A dendrogram constructed based on COMPLETE LINKAGE. Cluster analysis of IRAP and REMAP data using the NTSYSpc 2.02 resulted in five clusters. The present study represents high genetic distance at genotype level suggesting that IRAP and REMAP markers are useful for *Lallemantia iberica* genetic diversity analysis.

Keywords: Genetic diversity; IRAP; REMAP; *Lallemantia iberica*; Dendrogram

INTRODUCTION

Dragon's head (*Lallemantia iberica* Fish. et Mey.) is an annual herb and belongs to the tribe Stachyoideae-Nepeteae, family Lamiaceae [1]. *Lallemantia iberca* is introduced with popular name Balangu and traditional name Balangue shahri. The *Lallemantia sulphurea* and *Dracocephalum ibericum* are other synonyms (Bieb.) [2, 3]. The seed of the plant contains about 30% oil and high content of omega 3 fatty acid [4]. The major essential oil constituents include *i.e.* p-cymene (22.1%), isophytol (19.8%), T-cadinol (11.1%), 3-octanol (8.1%), caryophyllene oxide (7.4%) and terpinen-4-ol (5.7%) [5]. The oil is used in food, dye and varnish industry [6, 7]. The seeds are used for treatment of various disorders and as expectorant remedies in Iranian Folk medicine [2]. The wood preservative, ingredient of oil-based paints, furniture polishes, printing inks, soap making, and manufacture of linoleum are other applications of this plant [7]. The plant is ornamentally cultivated in arid landscaping and urban horticulture in Turkey [8].

*Corresponding Author: Biology Department, Faculty of Sciences, Urmia University, Urmia, Iran
Tel: +98-44-32753172
Fax: +98-44-32752117
E. mail: f.rahmani@urmia.ac.ir

pISSN 2322-181X eISSN 2345-2005

Genetic diversity within and between crop plant species is necessity for plant survival in nature and crop improvement. Variation in genetic resources permits selection of superior genotypes and developing new and improved cultivars with desirable characteristics for breeders [9]. Genetic markers can be classified into morphological markers, protein markers, and DNA markers. Assessment of genetic variability based on morphological traits is time-consuming [10] and traits are influenced by environmental factors. However, DNA markers are independent of age, physiological conditions and environmental factors and determine variations at nucleotide sequence [11]. Furthermore, PCR based molecular markers require low amount of DNA and are relatively simple and inexpensive to assess genetic diversity [12].

Among DNA markers, retro-elements and their derivatives are ubiquitous and abundant elements in plant genomes. Inter-retrotransposon amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP) retrotransposon have been successfully employed in fingerprinting [13, 14], linkage analysis, mapping [15], analysis of genome evaluation [16] and genetic diversity [17]. Both markers were first described by Kalendar et al. [18]. IRAP is generated based on the PCR amplification of genomic DNA fragments which lie between two retrotransposon insertion sites while REMAP is produced based on amplification of fragments lying between a retrotransposon insertion site and a microsatellite site. IRAP and REMAP describe the profile of a population [19, 20], discriminate between species or genotypes [21] and analyze population diversity [22]. They are easy to assess and could be established at low cost [23].

Up to our knowledge, based on literature reviews, no report has been published on genetic diversity of *Lallemantia iberica* using IRAP and REMAP markers. In this study, IRAP and REMAP were employed to study genetic diversity in *L. iberica* from different geographical locations in Iran.

MATERIALS AND METHODS

Plant materials and DNA extraction: Thirty-four plant specimens were collected from different growing habitats of Iran. Fresh leaves were randomly collected from 3-5 plants for each specimens and stored at -80°C prior to DNA extraction. Genomic DNA was isolated from fresh leaves tissue based on a modified CTAB procedure [24]. The quantity and quality of DNA were assessed using Biophotometer (Eppendorf, Germany) and 1% agarose gel electrophoresis, respectively. Geographic locations of *Lallemantia iberica* genotypes studied were indicated (Table 1 and Fig. 1).

Figure 1: Location of 34 varieties of *Lallemantia iberica* used in this study

Table 1: Name and geographic locations *Lallemantia iberica* varieties studied

Plant code	Collection site	Longitude	Latitude	Height
78-2	Miandoab (Qermezi Bolagh)	4625	3703	1550
78-4	Miandoab (NaderGoli)	4624	3658	1470
78-6	Shahin Dezh (Hulasu)	4638	3650	1550
78-8	Shahin Dezh (Obablagi)	4553	3645	2010
78-9	Shahin Dezh(Saeid Kandi)	4718	3648	1950
78-11	Naqadeh (kahriza ajam)	4623	3704	1450
78-12	Takab (Qarah Bolagh)	4714	3633	2000
78-13	Urmia (Dobra)	4547	3737	1700
78-14	Urmia (Dol)	4522	3710	1400
78-15	Urmia (Neychalan)	4445	3741	1700
78-16	Shahin Dezh (Doldolblagy)	4550	3633	1750
78-20	Sarab (Qarah Qayah)	4641	3642	1600
78-22	Heris (Abbasabad)	4634	3817	1722
78-23	Varzaqan (Chay Kandi)	4642	3822	1831
78-24	Varzaqan (Gholanbar)	4638	3825	1923
78-26	Ahar (Dopiyeh abad)	4646	3830	1684
78-30	Varzaqan (Mazraeh shadi)	4629	3836	1817
78-32	Bostanabad (Dahnab)	4644	3738	2008
78-36	Varzaqan (Kharvana)	4610	3841	1450
78-37	Shabestar (Sufian)	4608	3831	1668
78-41	Kaleybar	4702	3851	1138
78-42	Tabriz (Nosratabad)	4622	3826	2051
78-46	Varzaqan (Homaye sofla)	4624	3830	1986
78-49	Charuymaq (KhorramDaraq)	4656	3710	1932
78-50	Varzaghan (Ijan)	4628	3828	2016
78-52	Kermanshah(Eslamabad-e Gharb)	4625	3401	1700
78-54	Kermanshah (Kolehjoob)	4603	3404	1080
78-56	Kermanshah (kahriz olia)	4737	3418	1694
78-58	Kermanshah (MountFarrokhshad)	4706	3424	2250
78-62	Kermanshah (Ghiasabad)	4734	3445	1688
78-64	Lorestan (Chub Tarash)	4802	3352	1600
78-65	Lorestan (Qaleh-yeRahim)	4815	3328	1045
78-66	Lorestan (Aleshtar)	4818	3332	1175
78-67	Ardabil (Khalkhal)	4740	3820	1950

IRAP Reaction: Four IRAP primer combinations including TP1IRAP [25], TP2IRAP [25], NIKTA [26] and LTR6150 [27] (Table 2) were used to study genetic diversity in 34 *Lallemantia iberica* genotypes. PCR was performed in a final volume of 25µL contained 3µL of template DNA (10 ng μl^{-1}), 3µL of each forward and reverse primers (10 µM), 6.5µL deionized water and 12.5µL 2X PCR Master Mix (Cinnagen, Tehran) including dNTP, $MgCl_2$, 10X PCR buffer and Taq DNA polymerase. PCR amplification was performed under the following conditions: 3 min of denaturing at 94°C, followed by 35 cycles of 30 s of denaturing at 94°C, 45 s of annealing at 55°C and 1:30 min of elongation at 72°C. The reaction was completed by final extension step of 10 min at 72°C.

REMAP Reaction: Six REMAP primer combinations (Table 2), derived from three single IRAP primers (TP1IRAP, TP2IRAP, NIKITA) with two ISSR primers (UBC807, UBC808) [28 and 29] were tested on 34 *L. iberica* genotypes. PCR amplifications were performed in a volume of 25µl containing 6µl primer (ISSR+IRAP), 3 µl of template DNA (10 ng μL^{-1}), 3.5 µL of ddH_2O and 12.5 µL PCR master mix including PCR buffer, MgCl2, dNTP and Taq DNA polymerase. Initial denaturation was carried out for 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 45 s at 55°C, 1: 30 min at 72°C, and a final 10 min extension at 72°C. All PCR

products were analyzed on 1 % agarose gel with 0.5X TBE buffer. By staining with ethidium bromide, the gels were visualized under UV transilluminator (Ingenius3, Syngene, UK) and photographed.

Table 2: Genetic diversity of *Lallemantia iberica* revealed by IRAP and REMAP

Primer	Total loci	Polymorphic loci	Polymorphism(%)	Allele size(bp)	PIC
TP1IRAP	3	2	66.67	300-700	0.18
TP2IRAP	7	4	57.14	100-700	0.27
LTR6150	15	15	100	400-3000	0.90
NIKITA	9	7	77.78	100-1000	0.46
TP1IRAP-UBC807	6	4	66.67	150-800	0.35
TP1IRAP-UBC808	4	2	50	150-800	0.07
TP2IRAP-UBC807	5	3	60	200-1000	0.17
TP2IRAP-UBC808	10	10	100	150-1000	0.44
NIKITA- UBC807	9	7	77.78	100-1000	0.69
NIKITA- UBC808	8	6	75	150-750	0.30
IRAP+REMAP	76	60	78.94	-	0.38

Data analysis: The reproducibility of the DNA bands profile was tested by repeating the PCR with each of the selected primers. The presence and absence of each amplified fragment was scored as a binary code, 1 and 0, and each band regarded as a locus. Data were set in a binary matrix and analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) 2.02 version software package [30]. Polymorphic information content (PIC) value was obtained using formula, PIC = $1 - \Sigma pi^2$ (where pi is the frequency of each allele per primer). The matrix data was used to obtain the Jacquard genetic similarity [31] and to construct dendrogram [30] using the SAHN module (part to the NTSYS package). A cluster analysis, using a COMPLETE LINKAGE was performed. All computations were carried out using the NTSYSpc, version 2.02 package [30].

RESULTS

In this study, the ability of four IRAP and six REMAP primers were investigated to generate polymorphic DNA for similarity and cluster analyses. Ten primers generated 76 clear and scorable bands with considerable polymorphism. The presence and absence of bands were manually scored for each *Lallemantia iberica* (Fig. 2). The number of bands varied from 3 (TP1IRAP) to 15 (LTR 6150) with an average of 7.6 markers per primer (Table 2). The most polymorphic primer was LTR 6150, which generated 15 polymorphic bands, followed by TP2IRAP-UBC808 producing 10 bands (Table 2). The size of the amplified products ranged from 100-3000 bp. The lowest (0.07) and highest (0.90) PIC values were estimated for TP1IRAP-UBC808 and LTR6150 loci, respectively, with an average of 0.38 (Table 2). Genetic similarity coefficient ranged from 0.48, between Kermanshah (Kahriz olia) and Ardabil (Khalkhal) genotypes to 0.94, between Sarab (Qarah Qayah) and Heris (Abbasabad) genotypes for combined IRAP and REMAP markers. The average similarity value was 0.71.

The matrix band sharing data of both markers was used to construct cluster based on COMPLETE LINKAGE which divided 34 genotypes into five main clusters considering an abrupt point of change at a distance of 0.66 (Fig. 3). Cluster I was further divided into two sub clusters. Genotypes Urmia (Dobra), Naqadeh (kahriza ajam), Shahin Dezh (Obablagi), Miandoab (Qermezi Bolagh) and Varzaqan (Mazraeh shadi) were grouped in the first sub cluster and second sub cluster consisted of Miandoab (NaderGoli) and Takab (Qarah Bolagh) genotypes. Cluster II also subdivided in two sub clusters. The first sub cluster manifested of Shahin Dezh (Hulasu), Shahin Dezh (Doldolblagy), Tabriz (Nosratabad), Bostanabad (Dahnab), Sarab (Qarah Qayah), Heris (Abbasabad), Lorestan (Chub Tarash), Lorestan (Aleshtar) and Kermanshah (MountFarrokhshad) genotypes and second sub cluster included. The cophenetic correlation coefficient between Jaccard's similarity matrix and the plotted dendrogram was

0.66. The genetic distances based on IRAP and REMAP Jacquard similarity indexes did not correlate with geographic distance, demonstrating that the genetic variability is not distance-related. This observation could be due to the exchange of the same genotypes between neighboring farmers under different names.

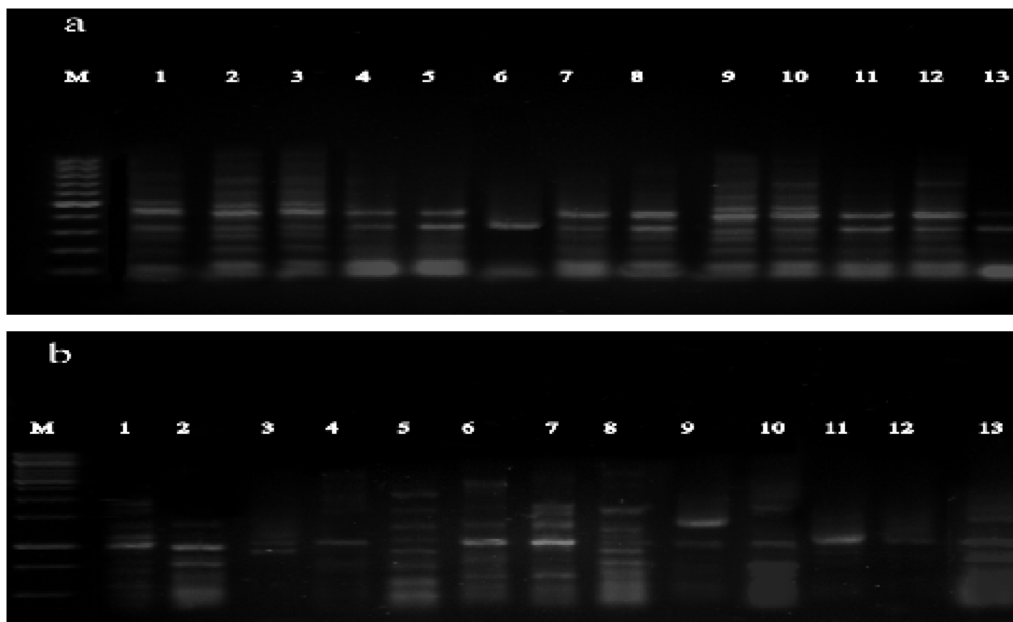

Figure 2: (a) REMAP marker profiles of NIKITA- UBC807, M: Ladder 100-1000 bp, 1: Shahin Dezh (Saeid Kandi), 2: Urmia (Dol), 3: Urmia (Neychalan), 4: Miandoab (Qermezi Bolagh), 5: Heris (Abbasabad), 6: Urmia (Dobra), 7: Tabriz (Nosratabad), 8: Varzaqan (Homaye sofla), 9: Varzaqan (Chay Kandi), 10: Ahar (Dopiyeh abad , 11: Kermanshah (kahriz olia), 12: Kermanshah (MountFarrokhshad), 13: Kermanshah (Eslamabad-e Gharb), (b) IRAP marker profiles of LTR6150, M: Ladder 250-1000 bp, 1: Miandoab (Qermezi Bolagh), 2: Varzaqan (Chay Kandi), 3: Shahin Dezh (Hulasu), 4: Sarab (Qarah Qayah), 5: Shahin Dezh (Saeid Kandi), 6: Naqadeh (kahriza ajam), 7: Takab (Qarah Bolagh), 8: Urmia (Dobra), 9: Varzaqan (Mazraeh shadi), 10: Ahar (Dopiyeh abad), 11: Bostanabad (Dahnab), 12: Ardabil (Khalkhal)**,** 13: Varzaqan (Kharvana).

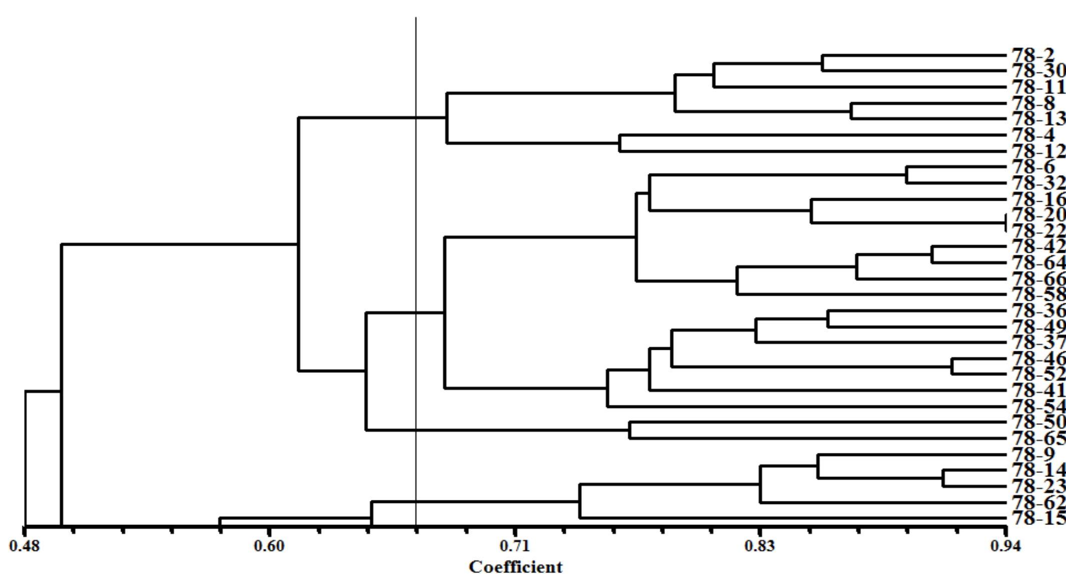

Figure 3: Dandrogram generated using COMPLETE LINKAGE, showing relationships between 34 *Lallemantia iberica* genotypes using IRAP and REMAP data combined.

DISCUSSION

The study of genetic relationship is important in the primary gene pool collection to improve efficiency of germplasm management for both breeding and conservation programs in the process of facing the environmental changes and adaption [32, 33]. Molecular markers are independent of being influenced by environmental factors and provide a good estimate of genetic diversity as a prerequisite for breeding programme. In order to determine the extent of genetic diversity and pattern of genetic relationships, application of PCR based fingerprinting techniques are informative. Retro-transposons are mobile, ubiquitous, and abundant genetic elements. They influence plant genome structure and evolution by genetic alteration at the point of insertion. Therefore, they provide excellent sources of polymorphism due to the insertion of variable number and size in the host genome. The IRAP and REMAP molecular markers are retrotransposon based markers which are extensively employed in plant breeding including phylogenetics, genotyping and gene mapping studies [34-37]. Difference in DNA sequence leads to polymorphism and higher polymorphism indicates greater genetic diversity. In the present study, two PCR-based markers including IRAP and REMAP were applied to evaluate genetic relationships among 34 *Lallemantia iberica* genotypes. The combined banding patterns from two marker systems exhibited high polymorphism allowing the identification of different genotypes of *L. iberica.* Abedinpour et al., [38] generated a total of 56 bands, with 86% polymorphism rate, when analyzing 29 genotypes of *Citrus* using IRAP markers. These researchers also reported a polymorphism range between 63 and 100%. Kalendar et al., [18] obtained a polymorphism range between 52 and 83% with a polymorphism rate of 61% in a genetic assessment study of *Hordeum vulgare* using 5 selected REMAP primers. Khaleghi et al. [39] found 88% polymorphism among 200 accessions of *O. europaea* using eight IRAP primers. Similarity results have been already observed in *Medicago sativa* when using IRAP markers [40]. Markers with high PIC value are more informative due to production of more alleles [41]. Hence, LTR6150 and NIKITA- UBC807 primers with high PIC are suggested for analysis of other *L. iberica* germplasms. Large values of polymorphic loci and PIC revealed that IRAP and REMAP markers are efficient for estimation of genetic diversity of different *L. iberica* genotypes. Based on the result of IRAP+ REMAP dendrogram and matrix similarity, genotypes with lowest similarity

including Kermanshah (Kahriz olia) and Ardabil (Khalkhal) genotypes (0.48), Shahin Dezh (Doldolblagy) and Ardabil (Khalkhal) genotypes (0.49) as the most divergent ones are recommended in breeding programs as parents. COMPLETE LINKAGE cluster analysis (Fig. 3) was also performed to examine relationships between different genotypes. In the present study, the COMPLETE LINKAGE dendrogram (Fig. 3) did not reveal any geographic tendency.

Our data suggests that both DNA markers are reliable and effective tools for assessment of genetic variation with high accuracy. However, the relative genetic distances among the studied genotypes were not correlated with geographical distances of their origins. In the present study, based on IRAP and REMAP markers, the similarity among genotypes ranged from 0.48 to 0.94. Our data based on two marker systems revealed the existence of low genetic similarity among studied genotypes. The Sarab (Qarah Qayah) and Heris (Abbasabad) genotypes were the most similar ones (0.94). The average similarity value of 0.71, indicates the high variability in genetic resources of *L. iberica* in Iran. The observation of high genetic diversity in this study is due to specific and codominant nature of these markers.

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Naghibi F, Mosaddegh M, Mohammadi Motamed S, Ghorbani A. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iran J Pharm Res 2010;20:63-79.
2. Amin GH. Popular medicinal plants of Iran. Ministry of health, 1991.
3. Mozaffarian V. A dictionary of Iranian plant names: Latin, English, Persian. Farhang Moaser; 1996,1452 p.
4. Gunstone FD, Harwood JL, Dijkstra AJ. Occurrence and characterisation of oils and fats. The lipid handbook; 2007,1472 p.
5. Morteza-Semnani K. Essential oil composition of *Lallemantia iberica* Fisch. et CA Mey. J Essent Oil Res 2006; 18:164-165.
6. Shafiee SS, Motlagh AM, Minaee S, Haidarbigi K. Moisture dependent physical properties of dragon's head seeds (*Lallemantia iberica*). Agric Eng Int: GIGR Journal 2016;16:99-106.
7. Ion V, Basa AG, Sandoiu DI, Obrisca M. Results regarding biological characteristics of the species *Lallemantia iberica* in the specific conditions from south Romania. UASVM Bucharest, Series A 2011;54:275-280.
8. Ozdemir FA, Yildirim MU, Pourali Kahriz M. Efficient micropropagation of highly economyic, medicinal and ornamental plant *Lallemantia iberica* (Bieb.) Fisch. and C.A Mey. Biomed Res Int 2014;2014:476346.
9. Bhandari HR, Nishant Bhanu A, Srivastava K, Singh MN, Shreya, Hemantaranjan A. Assessment of genetic diversity in crop plants. An overview. Adv Plants Agric Res 2017;7: 00255.
10. Fang JG, Zhang Z, Zhou LH, Chen CS, Wang SH. The possibility of using RAPD marker for the identification of fruit sport. J Fruit Sci 2001;18:182-185.
11. Joshi K, Chavan P, Warude D, Patwardhan B. Molecular markers in herbal drug technology. Curr Sci 2004;25:159-165.
12. Liu L, Yang Z, Wei S, Ouyang Z, Wu K, Peng Z. ISSR and SRAP markers in the genetic relationship analysis among Pinellia in China. J Med Plant Res 2012;6:3596-3602.
13. Bretó MP, Ruiz C, Pina JA, Asins MJ. The diversification of *Citrus clementina* Hort. ex Tan., a vegetatively propagated crop species. Mol Phylogenet Evol 2001 21:285-293.
14. Bernet GP, Mestre PF, Pina JA, Asins MJ. Molecular discrimination of *lemon* cultivars. Hort Science 2004;39:165-169.
15. Manninen O, Kalendar R, Robinson J, Schulman AH. Application of *BARE*-1 retrotrans-poson markers to the mapping of a major resistance gene for net blotch in barley. Mol Gen Genet 2000;264:325-334.
16. Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH. Genome evolution of wild barley (*Hordeum spontaneum*) by *BARE*-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc Natl Acad Sci USA 2000;97:6603-6607.
17. Yannic G, Baumel A, Ainouche M. Uniformity of the nuclear and chloroplast genomes of *Spartina maritima* (Poaceae), a salt-marsh species in decline along the Western European Coast. Heredity 2004;93:182.
18. Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A. IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 1999;98:704-711.
19. Murata H, Babasaki K, Saegusa T, Takemoto K, Yamada A, Ohta A. Traceability of Asian *Matsutake*, specialty mushrooms produced by the ectomycorrhizal basidiomycete *Tricholoma matsutake*, on the basis of retroelement-based DNA markers. Appl Environ Microbiol 2008;74:2023-2031.
20. Mateus Ferreira S, Elza Fernandes A, Jorge Teodoro S, Eduardo Seiti Gomide M, Marisa Q. Development of molecular markers based on retrotransposons for the analysis of genetic variability in *Moniliophthora perniciosa*. Eur J Plant Pathol 2012;134:497-507.
21. Biswas MK, Xu Q, Deng XX. Utility of RAPD, ISSR, IRAP and REMAP markers for the genetic analysis of *Citrus* spp. Sci Hort 2010;124:254-261.

22. Chadha S, Gopalakrishna T. Retrotransposon-microsatellite amplified polymorphism (REMAP) markers for genetic diversity assessment of the rice blast pathogen (*Magnaporthe grisea*). Genome 2005;48:943-945.
23. Kalendar R, Flavell AJ, Ellis TH, Sjakste T, Moisy C, Schulman AH. Analysis of plant diversity with retrotransposon-based molecular markers. Heredity 2011;106:520.
24. Doyle J. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 1987;19:11-15.
25. Žiarovská J, Ražná K, Senková S, Štefúnová V, Bežo M. Variability of *Linum usitatissimum* L. based on molecular markers. ARPN J Agri Biol Sci 2012;7:50-8.
26. Mamaghani RA, Mohammadi SA, Aharizad S. Transferability of barley retrotransposon primers to analyze genetic structure in Iranian *Hypericum perforatum* L. populations. Turk J Bot 2015;39:664-72.
27. Elframawy A, El-Bakatoushi R. Genetic divergence analysis among barley genotypes using inter-retrotransposon amplified polymorphism markers (IRAP). India J Agric Res 2017;51: 580-585.
28. Araújo FS, Pacheco MV, Vieir FA, Ferrari CS, Félix FC, Chagas KPT. ISSR molecular markers for the study of the genetic diversity of *Mimosa caesalpiniaefolia* Benth. Idesia (Chile) Junio 2016;34:47-52.
29. Henareh M, Dursun A, Abdollahi-Mandoulakani B, Haliloğlu K. Assessment of genetic diversity in tomato landraces using ISSR markers. Genetika 2016;48:25-35.
30. Rohlf J. NTSYS-pc. Numerical taxonomy and multivariate analysis: version 2.02. Exeter Software. Setauket, New York, 1998.
31. Jaccard P. Nouvelles researches sur la distribution florale. Bull Soc Vaud Sci Nat 1908;44: 223-270.
32. Sheidai M, Zanganeh S, Haji-Ramezanali R, Nouroozi M, Noormohammadi Z, Ghsemzadeh-Baraki S. Genetic diversity and population structure in four *Cirsium* (Asteraceae) species. Biologia 2013;68:384-397.
33. Choudhary P, Khanna SM, Jain P, Bharadwaj C, Kumar J, Lakhera P, Srinivasan R. Molecular characterization of primary gene pool of chickpea based on ISSR markers. Biochem Genet 2013; 51:306-322.
34. Nair AS, Teo CH, Schwarzacher *T*, Harrison PH. Genome classification of banana cultivars from South India using IRAP markers. Euphytica 2005; 144:285-290.
35. Queen RA, Gribbon BM, James C, Jack P, Flavell AJ. Retrotransposon-based molecular markers for linkage and genetic diversity analysis in wheat. Mol Genet Genomics 2004; 271:91-97.
36. Antonius-Klemola K, Kalendar R, Schulman AH. TRIM retrotransposons occur in apple and are polymorphic between varieties but not sports. Theor Appl Genet 2006; 112:999-1008.
37. Guo D, Zhang H, Luo Z. Genetic relationships of *Diospyros kaki* Thunb. and related species revealed by IRAP and REMAP analysis. Plant Sci 2006;170:528-533.
38. Abedinpour H, Ranjbar GA, Jelodar NB, Golein B. Evaluation of genetic diversity in *Citrus* genotypes by IRAP molecular marker. Int J Farm Alli Sci 2014;3:230-234.
39. Khaleghi E, Sorkheh K, Chaleshtori MH, Ercisli S. Elucidate genetic diversity and population structure of *Olea europaea* L. germplasm in Iran using AFLP and IRAP molecular markers. 3 Biotech 2017;7:71.
40. Abdollahi Mandoulakani B, Piri Y, Darvishzadeh R, Bernoosi I, Jafari M. Retroelement insertional polymorphism and genetic diversity in *Medicago sativa* populations revealed by IRAP and REMAP markers. Plant Mol Bio Rep 2012;30:286-296.
41. Ebrahimi A, Naghavi MR, Sabokdast M, Mardi M. Assessment of genetic diversity in two accessions of barely species (*H. vulgare* L. and *H. spontaneum* L.) using SSR markers. Iran J Crop Sci 2010;12:333-345.