African Journal of Biotechnology Vol. 11(43), pp. 10070-10078, 29 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2106
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

The study of genetic diversity in some Iranian accessions of *Hyoscyamus* sp. using amplified fragment length polymorphism (AFLP) and retrotransposon/AFLP markers

Alireza Etminan[1]*, Mansoor Omidi[2], Eslam Majidi Hervan[1], Mohammad reza Naghavi[2], Shamsali reza zadeh[3] and Mostafa Pirseyedi[4]

[1]Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Plant Breeding, Faculty of Agriculture and Natural Resources, University of Tehran, Iran.
[3]Institute of Medicinal Plants (IMP), Tehran, Iran.
[4]Agricultural Biotechnology Research Institute, Tehran, Iran.

Accepted 14 October, 2011

***Hyoscyamus* sp. is well known as a natural source of two main tropan alkaloids including hyoscyamine and scopolamine. The environmental conditions make a very wide diversity of this herb in Iran. This study was conducted to evaluate the genetic diversity within a set of 45 Iranian accessions *of Hyoscyamus* sp. using amplified fragment length polymorphism and retro/AFLP markers. 18 primer combinations of AFLP markers and five retro/AFLP primer combinations were also used to estimate genetic diversity among accessions. Analysis of banding patterns of 18 AFLP primer combinations revealed 264 polymorphic bands. A total of 264 polymorphic fragments were scored with an average of 14.7 fragments per primer combination. The five retro/AFLP primer combinations generated 42 clearly scorable polymorphic bands across 45 genotypes. The number of polymorphic fragments for each primer pair varied from 5 to 12 with an average of 8.4 polymorphic fragments per primer combination. The cluster analysis discriminated the accessions based on the species and the origin of accessions and demonstrated a high level of genetic diversity in *Hyoscyamus* sp. accessions. According to cluster analysis, almost all accessions of *Hyoscyamus niger* and all accessions of *Hyoscyamus reticulatus* were placed in the same group and the accessions of *Hyoscyamus puccillus* from the Yazd province were classified in the same group. These results provide important information with regard to future domestication and breeding programs for management of germplasm resources.**

Key words: Hyoscyamus, retrotransposon, amplified fragment length polymorphism (AFLP), genetic diversity.

INTRODUCTION

Hyoscyamus sp. is one of the most important medicinal plants belonging to the Solanaceae family. The plants of this family are well known as a natural source of tropan alkaloids including hyoscyamine, scopolamine and atropine (Suzuki et al., 1991; Kartle et al., 2003) and are cultivated for their medicinal compounds. The origin of *Hyoscyamus* species is Africa and South west Asia, and one of the most famous *Hyoscyamus* sp., *Hyoscyamus niger*, is widely distributed in Europe and Asia (Sajeli et al., 2006). The variation in environmental conditions in Iran, makes a very wide diversity of *Hyoscyamus* sp. such that in taxonomic studies, 13 *Hyoscyamus* sp. have been reported from Iran (Sheidai et al., 2000). The study of genetic diversity is the first step of a breeding program and genetic resources that are very valuable for breeders. Genetic variation might be evaluated by

*Corresponding author. E-mail: alietminan55@yahoo.com.

assessing morphological or biochemical traits but molecular markers. In particular, DNA-based markers provide the best assessment of genetic variation because they are plentiful and are not dependent on environmental effects. This made this evaluation more efficient and reliable (Dongre et al., 2007). After the development of polymerase chain reaction (PCR) technology (Saiki et al., 1985; Mullis et al., 1986), several PCR-based markers were developed and applied to assess the genetic variation among populations and genetic resources. These marker systems are different in technical principle, type of inheritance, reproducibility, amount of polymorphism and in their costs (Powell et al., 1995; Schulman, 2007). The amplified fragment length polymorphism (AFLP) technique (Vos et al., 1995) is one of the best marker systems that can be used to detect high levels of DNA polymorphism and is extremely promising for genetic diversity studies (Powell et al., 1996). Retrotransposon AFLP is a modification of the AFLP methodology. In this fingerprinting technique, amplification is achieved using one AFLP primer in combination with one sequence-specific retrotransposon primer. Sequence-specific amplified polymorphism (S-SAP) is one of the several retrotransposon-based marker systems that has been developed to assess the polymorphisms at the DNA level (Waugh et al., 1997). This marker system has shown higher levels of polymorphism than the standard AFLP (Labra et al., 2004; Sensi et al., 1996). The S-SAP method has robust reproducibility and is straightforward in the detection of polymorphism (Wegscheider et al., 2009).

The objective of this study was to estimate the genetic diversity among the accessions of *Hyoscyamus* germplasm using AFLP) and retrotransposon AFLP techniques, and assess the relative informativeness of retrotransposon AFLP and standard AFLP markers.

MATERIALS AND METHODS

Plant material and DNA extraction

The seeds of 45 accessions of Hyoscyamus *sp.* from different wild habitat areas of Iran were obtained from Research Institute of Forest and Rangelands (RIFR), Karaj, Iran (Table 1). Voucher numbers of the accessions studied were deposited in the Herbarium of RIFR. Gibberellic acid and cold treatments were used to break dormancy, and the germinated seeds were transferred into pots containing top soil and placed under greenhouse conditions in the Institute of Medicinal Plant, Karaj, Iran. After two weeks, fresh leaves from each pot were picked for genomic DNA extraction. The fresh Leaves of each sample were ground to a fine powder in liquid nitrogen and total genomic DNA was extracted using Dellaporta method (Dellaporta et al., 1983) with some modifications. The quality and quantity of the extracted DNA were tested on 1% agarose gel electrophoresis.

AFLP assays

The AFLP procedure was performed with appropriate modifications of the method described by Vos et al. (1995). Genomic DNA was double-digested with two restriction enzymes (*Eco*RI and *Mse*I). 10 µl of genomic DNA (250 ng) of each sample was mixed with 4 µl Tango buffer(10x), 5 µl sterile distilled water and 2.5 unit of each restriction enzyme and incubated for 3 h at 37°C and 1 h at 65°C, respectively. The digested fragments were ligated to double stranded adaptors appropriate with the *EcoRI* and *MseI* restriction sequences (Table 2) with 1.5 unit T4 DNA ligase and incubated for 3 h at 37°C. The ligated DNA fragments were diluted three times with sterile distilled water and stored at -20°C. This was done to prepare template DNA for first step of amplification and this is called preamplification.

Preamplification was carried out using non-selective primers (E_{000} and M_{000}) in a 25 µl reaction volume containing 3.75 µl of (1:3) diluted ligation product, 1 unit of *Taq* polymerase, 1X Taq polymerase buffer, 0.4 µM of each of the two primers, 150 µM of each of dATP, dCTP, dGTP and dTTP, and 2 mM $MgCl_2$. This amplification was performed in a thermocycler programmed for 25 cycles, each consisting of 1 min at 94°C, 1 min at 60°C and 72°C for 2 min. The final extension was done at 72°C for 7 min. The pre-amplification product was diluted 1:9 in sterile double distilled water to prepare template DNA for selective amplification. Selective amplification was performed in a 25 µl reaction mixture volume containing 3.75 µl of diluted pre-amplification product, 1x Taq polymerase buffer, 2 mM MgCl2, 1 Unit of *Taq* polymerase, 150 µM of dNTPs, and 0.4 µM of each of the two primers with two or three additional nucleotides at the 3´end. For the selective amplification step, the following cycle profile was used: 5 min at 94°C for pre-denaturing, 35 consecutive cycles each consisting of 1 min at 94°C for denaturing, 1 min at 65°C for annealing and 2 min at 72°C for extension. After these 35 cycles, a final extension step was done at 72°C for 7 min. 20 primer combinations were used for diversity assessment. All adaptors and primers are listed in Table 2. The PCR reactions with retrotransposon primers were performed in the earlier mentioned procedure by replacement of a retrotransposon primer with one selective primer. The PCR products were separated on denaturing 6% (w/v) polyacrylamide gel electrophoresis. For amplified fragment detection, silver staining method was used as described by Bassam et al. (1991).

Band scoring and statistical analysis

Polymorphic bands obtained from both marker systems were constructed by scoring 0 and 1 for absence and presence of bands, respectively. The generated data matrixes were subjected to statistical analysis using the NTSYS-pc analytical software (Rohlf, 1998). Genetic similarity estimates, were calculated using Dice coefficient of similarity (Nei and Li, 1979). The accessions were grouped by cluster analysis using the unweighted pair-group method (UPGMA).

RESULTS AND DISCUSSION

Molecular markers, in particular DNA based markers provide reliable genetic information because of the independence of the confounding effects of environmental factors (Powell et al., 1995). In this study, two DNA based marker systems were used and compared for assessing the genetic relationships among 45 accessions of *Hyoscyamus sp.* Both marker systems however, were similar in principle but they showed some differences in amount of polymorphism detected.

Analysis of banding patterns of 18 AFLP primer combinations revealed 264 polymorphic bands with an

Table 1. Codes, species, localities and voucher numbers of accessions.

Accession code	Species	Locality	Voucher number
1	*Senecionis*	Yazd, Taft	20984
2	*Pucillus*	Yazd, Mehriz	22106
3	*Senecionis*	Yazd, Mehriz	21062
4	*Niger*	Balouchestan	19226
5	*Pucillus*	Yazd, Ardakan	21120
6	*Ppucillus*	Yazd, Taft	10323
7	*Niger*	Birjand	24944
8	*Niger*	Kordestan, Saghez	9442
9	*Niger*	AzarbayejanGharbi, Mahabad	19673
10	*Niger*	Zanjan	19251
11	*Niger*	Markazi,Shazand	12746
12	*Niger*	Golestan, Maraveh Tappeh	16436
13	*Niger*	Lorestan, Azna	7490
14	*Niger*	Lorestan, Aligoodarz	8982
15	*Niger*	Mazandaran, Alasht	16545
16	*Niger*	Golestan, Kalaleh	16340
17	*Niger*	Golestan, Azadshahr	16638
18	*Niger*	Kordestan, Sanandaj	12008
19	*Niger*	Hamadan, Asadabad	21931
20	*Niger*	Markazi, Khomeyn	15619
21	*Niger*	Esfahan, Khansar	9927
22	*Niger*	Chaharmahal, Farsan	12688
23	*Niger*	Ardabil	11891
24	*Niger*	Ardabil, Khalkhal	11869
25	*Niger*	Sanandaj, Baneh	19881
26	*Niger*	Hamadan, Avaj	11927
27	*Reticulatus*	khorasan	3609
28	*Reticulatus*	Semnan	21683
29	*Reticulatus*	Khorasan, Mashhad	1418
30	*Niger*	Sanandaj, Marivan	11964
31	*Muticus*	Esfahan	23894
32	*Reticulatus*	Yazd, Taft	4516
33	*Niger*	Semnan, Ahovan	17269
34	*Niger*	Semnan, Shahmirzad	21570
35	*Niger*	Qum	17617
36	*Niger*	Esfahan	18171
37	*Niger*	Yazd, Taft	3103
38	*Niger*	Markazi, Arak	15038
39	*Niger*	Fars, Shiraz	12296
40	*Niger*	Gilan, Siahkal	21151
41	*Niger*	Karaj, Ghachsar	16893
42	*Senecionis*	Yazd, Bafgh	10283
43	*Muticus*	Bushehr	21217
44	*Muticus*	Bushehr, Dashtestan	12877
45	*Muticus*	Bushehr, Tangestan	12888

average of 14.7 fragments per primer combination. The highest and the lowest number of polymorphic bands per assay were 23 and 5 bands, respectively (Table 3). Moreover, the five retro/AFLP primer combinations generated 42 clearly scorable polymorphic bands across 45 genotypes. The number of polymorphic fragments for each primer pair, varied from 5 to 12 with an average of 8.4 polymorphic fragments per primer combination (Table

Table 2. Adaptors and primers used for pre-amplication and selective amplication of 45 accessions of *Hyoscyamus* species.

Adaptor	Sequence (5' – 3')
E adaptor1	CTCGTAGACTGCGTACC
E adaptor2	AATTGGTACGCAGTCTAC
M adaptor1	GACGATGAGTCCTGAG
M adaptor2	TACTCAGGACTCAT
Primers	Sequence (5' – 3')
M000	GATGAGTCCTGAGTAA
M35	GATGAGTCCTGAGTAAGAG
M22	GATGAGTCCTGAGTAACCC
M20	GATGAGTCCTGAGTAACAT
M17	GATGAGTCCTGAGTAACAA
M-GC	GATGAGTCCTGAGTAAGC
M-TA	GATGAGTCCTGAGTAATA
E000	GACTGCGTACCAATTC
E-46	GACTGCGTACCAATTCGTC
E-11	GACTGCGTACCAATTCAGG
E-8	GACTGCGTACCAATTCACT
E-2	GACTGCGTACCAATTCAAC
E-TG	GACTGCGTACCAATTCTG
E-TAT	GACTGCGTACCAATTCTAT
E-TA	GACTGCGTACCAATTCTA
E-CG	GACTGCGTACCAATTCCG
Tagermina	AGAGGAGGATATCCCAACAT
Bar1	CTAGGGCATAATTCCAACA

Table 3. Effectiveness of AFLP and retro transposon AFLP markers in detecting of polymorphism of *Hyoscyamus sp.* accessions.

Parameter	AFLP	Retro/AFLP
Number of assay units	18	5
Polymorphic fragments scored	264	42
Minimum polymorphism scored per assay	5	5
Maximum polymorphism scored per assay	23	12
Average polymorphism scored per assay	14.7	8.4

3). The average polymorphism scored per assay was 14.7 with AFLP compared to 8.4 with retro/AFLP, whereas higher levels of polymorphism for retro/AFLP system compared to standard AFLP were reported in earlier studies (Sensi et al., 1996; Labra et al., 2004). This higher level of polymorphism for AFLP compared to retro/AFLP may be due to the use of unsuitable retro/AFLP primers. In addition, the number and the kinds of primers used (18 primer combinations in AFLP compare to 5 primer combinations in retro/AFLP) may also affect the levels of polymorphism among different markers. Considering earlier reports, the number of primer combinations may also affect the correlations among different marker systems (Belaj et al., 2003).

Estimates of genetic similarity

A summary of the genetic distance estimated between pairs of genotypes, calculated for each marker technique is reported in Table 4. Retro/AFLP data showed the highest range of genetic distance among 45 accessions (from 0.08 up to 1) with an average of 0.65. Minimum, maximum and average values of dissimilarities based on the 264 AFLP markers were found to be 0.16, 0.69 and 0.44, respectively. These results revealed that retro/AFLP data gave higher average genetic distance than AFLPs. Estimates of genetic distance of both marker systems based on the 306 polymorphic bands between 45 accessions ranged from 0.15 to 0.7 with an average of

Table 4. Average, maximal and minimal values of genetic distance using 3 combinations of marker system AFLP, retro/ AFLP and AFLP + retro/ AFLP) among 45 *Hyoscyamus sp.* accessions.

Parameter	Marker system		
	AFLP	Retro/ AFLP	AFLP + Retro/AFLP
Average	0.44	0.65	0.46
Maximum	0.69	1	0.7
Minimum	0.16	0.08	0.15

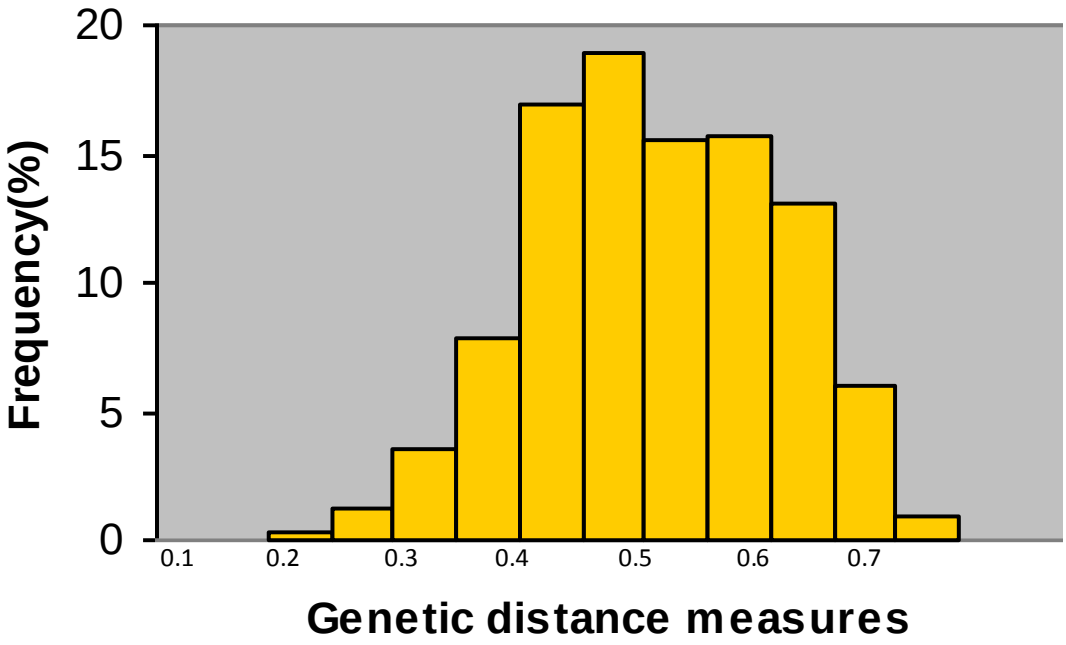

Figure 1. Frequency distribution of genetic distance between pairs of accessions based on both marker systems data. Genetic distance estimates were calculated based on all combinations (n = 990).

0.46. The genetic distance patterns between *Hyoscyamus sp.* accessions, expressed by the AFLP and retro/AFLP dendrograms were relatively different. For example in AFLP analysis, two accessions from the Golestan province (16 and 17), had the lowest genetic distance (0.16), while in retro/AFLP analysis, the lowest genetic distance (0.08) were detected between Mahabad accession (9) and Ardabil accession (23). The frequency distribution of genetic distance between pairs of accessions based on both marker systems indicates a normal distribution (Figure 1). The same results have been reported in *B. carinata* genotypes (Genet et al., 2005).

Cluster analysis

The dendrograms generated with hierarchical unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on data obtained by AFLP, retro/AFLP and AFLP+ retro/AFLP are shown in Figures 2,3 and 4. The cophenetic correlations between the similarity matrix and corresponding dendrogram were found to be 0.88, 0.87 and 0.9 for AFLP, retro/AFLP and AFLP+ retro/ AFLP, respectively (Mantel, 1967). The dendrograms derived from AFLP and AFLP+ retro/AFLP markers were more similar to each other when compared to the dendrogram generated based on retrotransposon AFLP data.

Cluster analysis of AFLP + retro/AFLP data classified all 45 accessions into ten different groups. According to the analysis, the first cluster contained 29 accessions of *H. niger* and four accessions of *H. reticulatus* distributed into several sub-clusters based on the species and the origin of accessions. Two other accessions of *H. niger* (4 and 30) were represented as individual branches in the cluster. All accessions of *H. muticus* (31, 43, 44 and 45) were clustered as four individual groups. Among the three studied accessions of *H. senecionis,* two accessions (1 and 3) from the Yazd province were placed

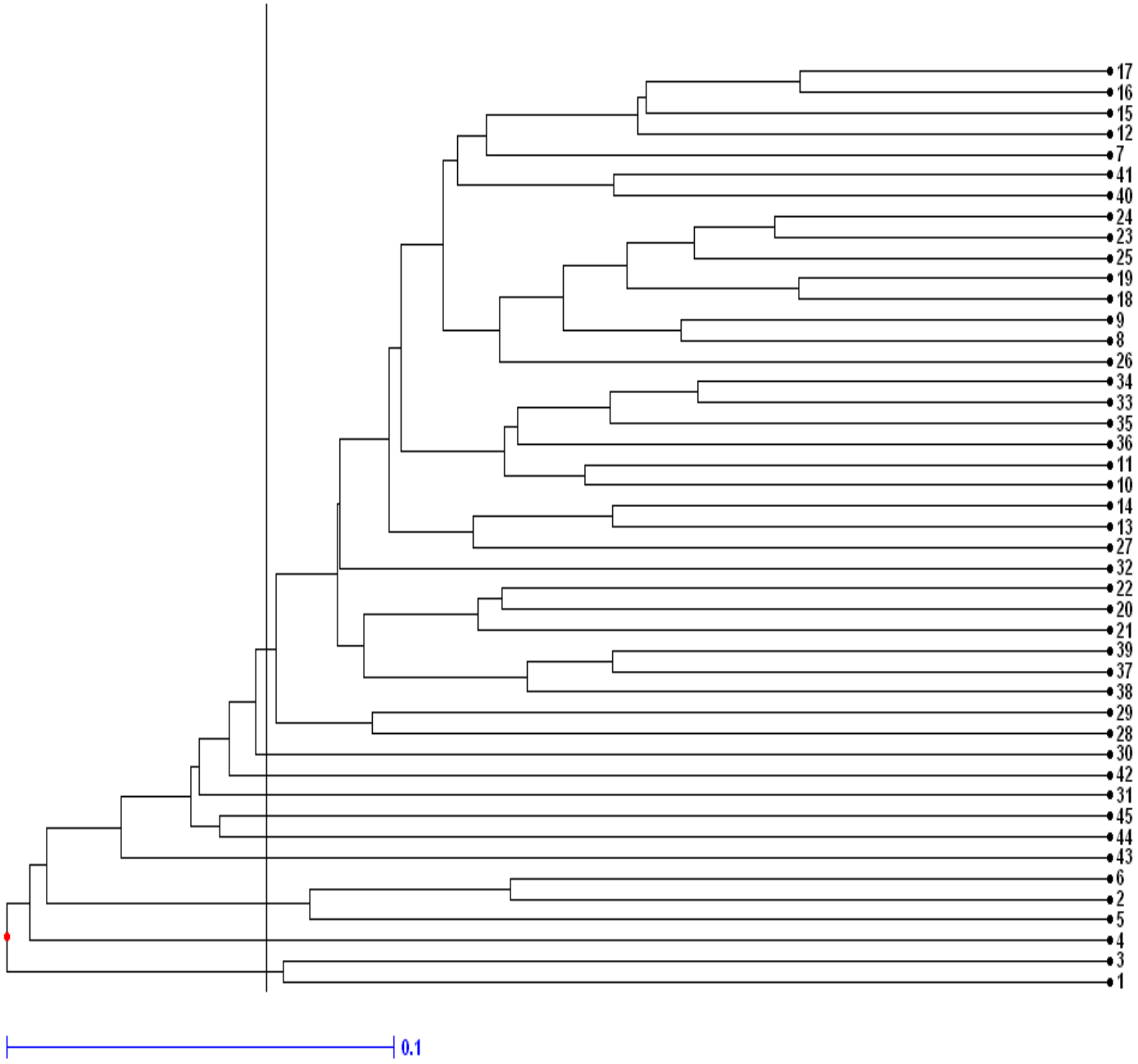

Figure 2. Dendrograms generated with hierarchical UPGMA cluster analysis based on AFLP data.

in the same group but another accession (42) was represented as individual branch in the cluster. All accessions of *H. puccillus* (2, 5 and 6) from the Yazd province were classified in the same group. Similar results were obtained from the dendrogram of AFLP. For instance, among the 31 accessions of *H. niger*, 29 accessions clustered together in the same group and all accessions of *H. puccillus* (2, 5 and 6) from the Yazd province were classified in the same group but some differences were found between the dendrograms of retro/AFLP and AFLP+ retro/AFLP, as well as in the retro/AFLP dendrogram accessions 43 and 44 grouped together, whereas these were in different subgroup in the AFLP+ retro/AFLP dendrogram.

The results of cluster analysis showed that the classification of accessions was almost according to their origins and species. For instance, three accessions (2, 5 and 6) of *H. pucillus* from the Yazd province were classified in the same group using all three marker systems. Separation of the species *H. pucillus* is confirmed by isozyme analysis (Sharifi et al., 2006) and seed storage protein analysis (Sheidai et al., 2000). On

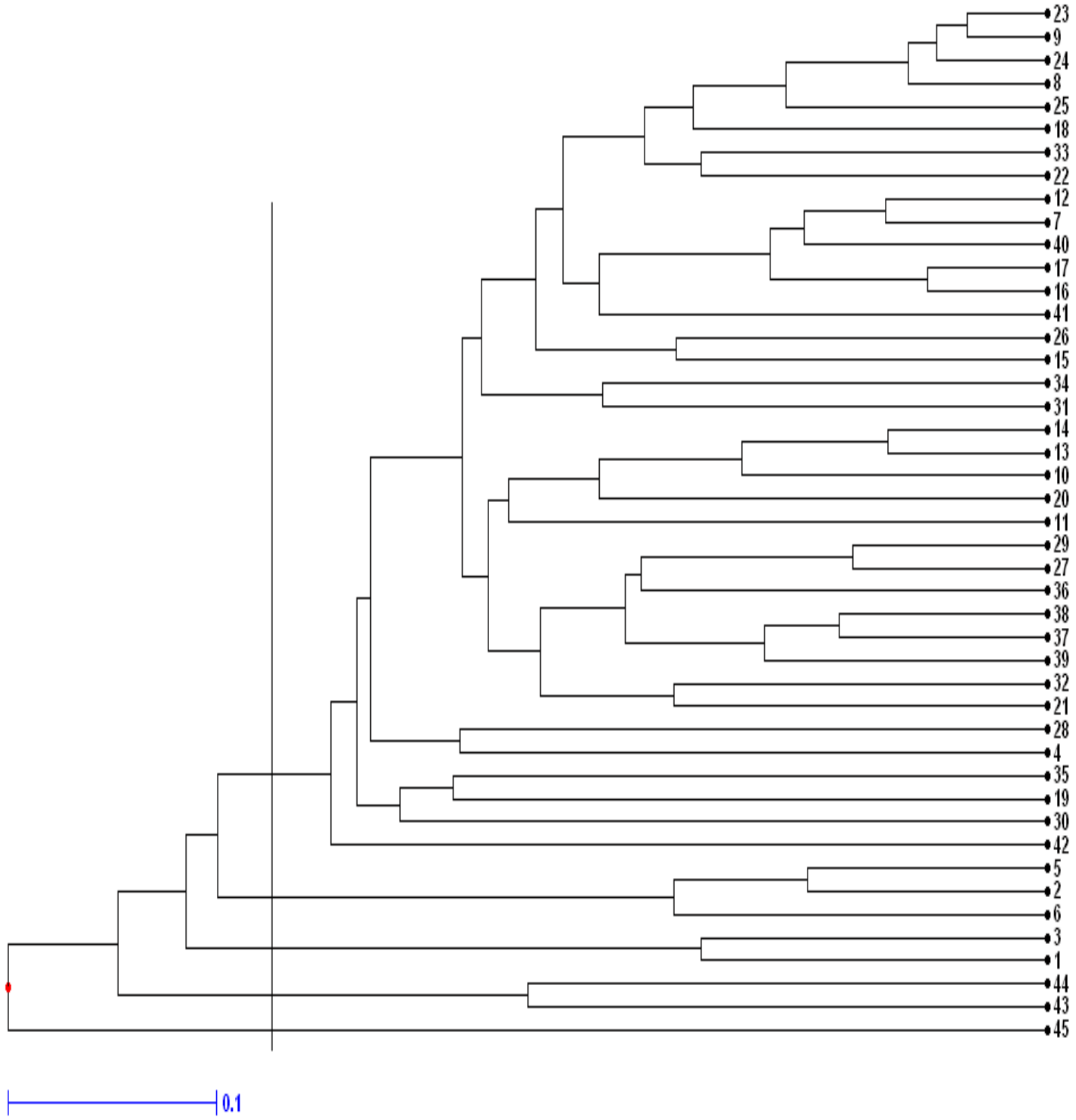

Figure 3. Dendrograms generated with hierarchical UPGMA cluster analysis based on retro/AFLP data.

the other hand, in some cases, the estimates of comparative genetic distances among accessions did not entirely correlate with geographical distances of their origins. For example, some accessions of *H. niger* originating from the Sanandaj province, such as 30 (Marivan accession) and 25 (Baneh accession) were placed in two separated sub cluster in contrast to their low geographical distance. This classification may be due to the unique genetic background of these accessions. This is in agreement with some earlier studies to assess

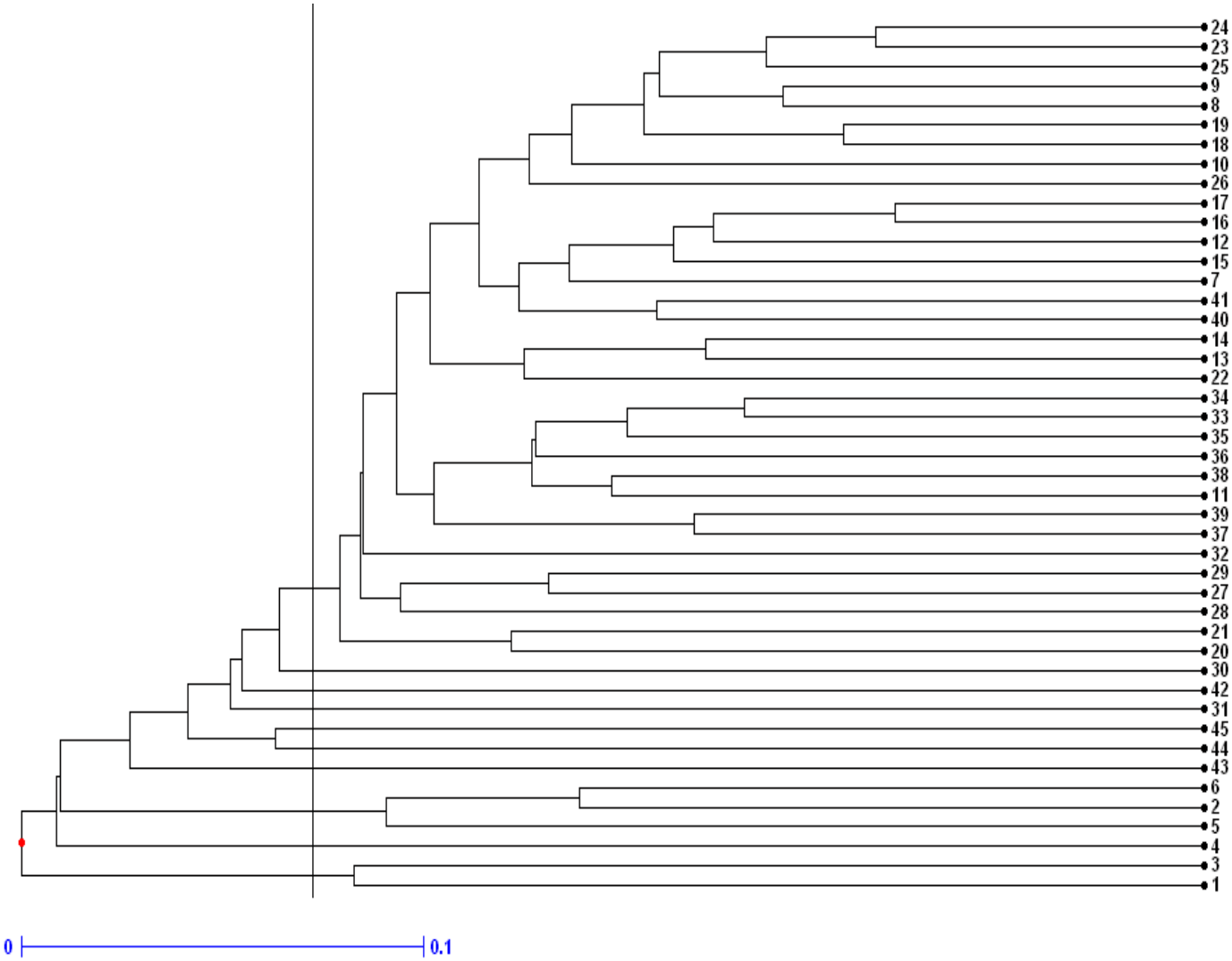

Figure 4. Dendrograms generated with hierarchical UPGMA cluster analysis based on AFLP and retro/AFLP data.

the genetic diversity using AFLP markers in *Daucus carota* (Bradeen et al., 2002), *Bunium persicum* (Pezhmanmehr et al., 2009), *Phaseolus vulgaris* (Martins et al., 2006) and *Matricaria chamomilla* (Solouki et al., 2008).

In conclusion, AFLP and retro/AFLP data sets showed a high level of polymorphism among Iranian accessions of *Hyoscyamus sp.*, reflecting their efficiency in the assessment of the genetic diversity. This can be used for germplasma management and breeding.

ACKNOWLEDGEMENTS

The financial support for this study by the Institute of Medicinal Plants (IMP), Tehran, Iran is kindly acknowledged. The authors thank the Research Institute of Forest and Rangelands (RIFR), Karaj, Iran for providing seeds of the accessions.

REFERENCES

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991). Fast and sensitive silver staining of DNA in acrylamide gels. Anal. Biochem. 196: 80-83.

Belaj A, Satovic Z, Cipriani G, Baldoni L, Testolin R, Rallo L, Trujillo I (2003). Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive. Theor. Appl. Genet. 107: 736-744.

Bradeen JM, Bach IC, Briard M, Le Clerc V, Grzebelus D, Senalik DA, Simon PW (2002). Molecular diversity analysis of cultivated carrot (*Daucus carota* L.) and wild Daucus populations reveals a genetically nonstructured composition. J. Am. Soc. Hort. Sci. 127: 383-391.

Dellaporta SL, Wood J, Hicks JB (1983). A plant DNA minipreparation: version II. Plant Mol. Biol. Rep. 1: p. 19.

Dongre AB, Bhandarkar M, Banerjee S (2007). Genetic diversity in tetraploid and diploid cotton (*Gossypium* spp.) using ISSR and microsatellite DNA markers. Ind. J. Biotechnol. 6: 349-353.

Genet T, Viljoen CD, Labuschagne MT (2005). Genetic analysis of Ethiopian mustard genotypes using amplified fragment length polymorphism (AFLP) Markers. Afr. J. Biotechnol. 4(9): 891-897.

Kartle M, Kurucu S, Altun L (2003). Quantitative Analysis of 1-Hyoscyamine in *Hyoscyamus reticulates* L. by GC-MS. Turk. J.

Chem. 27: 565-569.
Labra M, Imazio F, Grassi F, Rossoni M, Sala F (2004). *Vine-1* retrotransposon-based sequence-specific amplified polymorphism for *Vitis vinifera* L. genotyping. Plant Breed. 123: 180-185.
Mantel NA (1967). The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209-220.
Martins SR, Vences FJ, Saenz De Miera LE, Barrosa MR, Carnide V (2006). RAPD analysis of genetic diversity among and within Portuguese landraces of common white bean (*Phaseolus vulgaris* L.). Sci. Hortic. 108: 133-142.
Mullis K, Faloona F, Schare S, Saiki R, Horn G, Erlich H (1986). Enzymatic amplification of DNA in vitro: the polymerase enzyme chain reaction. Cold Spring Harbor Symp. Quant. Biol. 51: 263-273.
Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269-5273.
Pezhmanmehr M, Hassani ME, Jahansooz F, Najafi AA, Sefidkon F, Mardi M, Pirseiedi M (2009). Assessment of genetic diversity in some Iranian populations of *Bunium persicum* using RAPD and AFLP markers. Iran. J. Biotechnol. 7(2): 93-100.
Powell W, Orozco-Castillo C, Chalmers KJ, Provan J, Waugh R (1995). Polymerase chain reaction-based assays for the characterisation of plant genetic resources. Electrophoresis, 16: 1726-1730.
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. Mol. Breed. 2: 225-238.
Rohlf FJ (1998). NTSYS-pc. Numerical taxonomy and multivariate analysis system, Appl. Biostatatistics. New York.
Saiki R, Scharaf S, Floona F, Mullis KB, Horn GT, Arnheim N (1985). Enzymatic amplification of β-Globin genomic sequences and restriction site analysis for diagnostic of sickle cell anemia. Science, 240: 204-207.
Sajeli B, Sahai M, Suessmuth R, Asai T, Hara N, Fujimoto Y (2006). Hyosgerin, a new optically active coumarinolignan, from the seeds of *Hyoscyamus niger*. Chem. Pharm. Bull. 54: 538-541.
Sensi E, Vignani R, Rohde W, Biricolti S (1996). Characterization of genetic biodiversity with *Vitis vinifera* L. Sangiovese and Colorino genotypes by AFLP and ISTR DNA marker technology. Vitis. 35: 183-188.
Sharifi G, Montasser S, Ebrahimzadeh H, Khatamsaz M (2006). Isozyme analysis of seedling samples in some species of *Hyoscyamus* from Iran. Pak. J. Biol. Sci. 9(9): 1685-1692.
Sheidai M, Khatamsaz M, Mosallanejad M (2000). Numerical taxonomy and seed protein analysis of *Hyoscyamus* species in Iran. J. Sci. I. R. Iran. 11(2): 83-91.
Schulman AH (2007). Molecular markers to assess genetic diversity. Euphytica, 158: 313–321.
Solouki M, Mehdikhani H, Zeinali H, Emamjomeh AA (2008). Study of genetic diversity in Chamomile (*Matricaria chamomilla*) based on morphological traits and molecular markers. Sci Hortic. 117: 281-287.
Suzuki K, Yun D, Chen X, Yamada Y, Hashimoto T (1991). An *Atropa belladonna* Hyoscyamine 6β- Hydroxylase Gene Is Differentially Expressed in the Root Pericycle and Anthers. Plant. Mol. Biol. 40: 141-152.
Vos P , Hogers R, Bleeker M, Rijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.
Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BBT, Powell W (1997). Genetic distribution of Bare-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). Mol. Gen. Genet. 253: 687-694.
Wegscheider E, Benjak A, Forneck A (2009). Clonal Variation in Pinot noir Revealed by S-SAP Involving Universal Retrotransposon-Based Sequences. Am. J. Enol. Vitic. 60: 104-109.