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Genetic diversity in *Aloe vera* accessions from Iran based on agro-morphological, phytochemical and random amplified polymorphic DNA (RAPD) markers

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Aloe vera **is highly valued for anthraquinones compounds found in their leaves. The main objectives of the present study were to (1) evaluate phenotypic and molecular diversity among Iranian Aloe accessions; (2) compare Aloin content among accessions collected from different ecological areas; (3) determine correlations between Aloin content and morphological traits; and (4) estimate the broad-sense heritability (h^2_b) for the traits evaluated. In this study a combined analysis of variance was used to evaluate 10 Iranian accessions of *Aloe* over a 2-year period. Traits investigated include leaf weight, root weight and Aloin content. RAPD markers were also used to evaluate molecular diversity. Statistical analysis showed that accessions had significant effect on all evaluated traits. Simple correlation analysis showed that leaf weight had positively significant correlation with root weight and also with Aloin content (r = 0.534 and 0.493, respectively). The highest estimate of broad-sense heritability was that of leaf weight (h^2_b = 67.2%). Cluster analysis based on agro-morphological/phytochemical traits or RAPD markers did not reveal a clear relationship between diversity pattern and geographical origin. In conclusion, genetic diversity of Iranian accessions of Aloe as shown in this study should play a critical role in future selection and breeding of Aloe.**

Key words: Broad-sense heritability, breeding, anthraquinones, aloin.

INTRODUCTION

Family Liliaceae, with over 300 species, is naturally present in various parts of the world including Africa, Madagascar and Arabia (Adams et al., 2000). Plants belonging to this family are known by their substantial amount of anthraquinone especially in their leaves (Kuzuya et al., 2001). Anthraquinones from Aloe plants have been reported to exhibit a wide range of biological characteristics such as antimicrobial, antifungal, anti-inflammatory, immunostimulant, antiseptic, wound and burn healing, antiulcer, antitumor, and antidiabetic (Loots et al., 2007). Common Aloe (*Aloe vera* L.) is one of the most important species belonging to this family (Nayanakantha et al., 2010), which grows naturally in South Africa and in Asia (Rajasekaran et al., 2006). *A. vera* is a succulent perennial xerophytic plant with smooth leaves surfaces but with spiny teeth on the margins. Aloe may also be propagated means of by seeds, which are produced by the hermaphrodite flowers usually found in dense racemes. The flowers (not used medicinally) are yellow (Nayanakantha et al., 2010). The aloe plant has long (up to 20 inches long and 5 inches wide), fleshy leaves that have spikes along the edges.

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The fresh parenchymaous gel found in the leaf is clear; this part is sometimes dried to form *A. vera* concentrate or diluted with water to create aloe juice products. The sticky latex liquid is derived from the yellowish green pericyclic tubules that line the leaf (rind); this is the part that yields laxative anthraquinones (Hu et al., 2003).

So far, anthraquinones have been detected in Aloe, the most important being aloin (10-β-glucopyranosyl-1, 8-dihydroxy-3-hydroxymethyl-anthracen-9-one) followed by barbaloin, the C-glycoside of aloe-emodin anthrone which yields the yellow dye (Yang et al., 2004; Rajesh et al., 2010).

Aloe leaf is known to be used in traditional herbal medicine such as stomach ailments, gastrointestinal problems, skin diseases, constipation radiation injury (Cardenas et al., 2006; Arunkumar and Muthuselvam, 2009; Pandey and Mishra, 2009) and for wound and burns healing (Rodriguez et al., 2005) since 1500 BC. Aloe is cultivated in some countries such as Iran (Mozafarian, 1998; Rezaie et al., 2004). Cultivation of Aloe in Iran has been increased, because the prevailing climate and fertile lands favorable for growing of various medicinal plants include *A. vera* (Rezaie et al., 2004). However, in recent years, extensive research has been conducted in identification, determination and improvement of anthraquinones compounds in Aloe (Kuzuya et al., 2001; Yang et al., 2004).

Aloe grows naturally in various parts of Iran such as in the south and north (Ghahreman, 1999). In Iran, Aloe cultivation has a historical background due to its importance in wound healing (Rezaie et al., 2004). Nowadays, Aloe, is cultivated mostly in the Mazandaran province North, Iran. Recent rise in demand for Aloe as an important species has led to over harvesting of this naturally grown species. An essential factor for promotion of natural aloin as a commercial commodity is that it should be produced with high quality in plentiful amounts and it should be affordable (Rezaie et al., 2004). Efforts have been made in recent years to increase the quality and quantity of the antheraquinones content by genetic manipulation (Rezaie et al., 2004) but considering the cost, the field production still remains the best way.

It seems that selection of Aloe cultivar based on improved agro-morphological characteristics such as leaf density, leaf thickness, high growing rate and high content of anthraquinone metabolites, is the best solution to overcome this problem (Nayanakantha et al., 2010; Nejatzadeh-Barandozi et al., 2011).

The improvement in traits can be achieved through understanding the nature and amount of variability present in the genotypes of Aloe used in breeding programs. Furthermore, this evaluation will provide information about uniqueness and distinctness of genotypes, which is of vital importance in optimal and effective conservation of genotypic variability (Bisrat et al., 2000).

In recent years, attempts have already been made to study the genetic diversity in *A. vera* (Viljoen, 1999; Rajasekaran et al., 2006; Nayanakantha et al., 2010; Nejatzadeh-Barandozi et al., 2012).

Genetic diversity is best estimated if agro-morphological, biochemical and marker studies are used together (Lattoo et al., 2008). Nowadays, molecular marker, have become an important tool to evaluate genetic diversity in plants more efficiently. Among various molecular markers, the random amplified polymorphism DNA (RAPD) is most widely used. It does not require prior genomic information and are simpler and more economical than other DNA marker techniques (Lattoo et al., 2008; Khan et al., 2009). The main objectives of the present study were to (1) evaluate phenotypic and molecular diversity among Iranian Aloe accessions; (2) compare Aloin content among accessions collected from different ecological areas; (3) determine correlations between Aloin content and morphological traits; and (4) estimate the broad-sense heritability (h^2_b) for the traits evaluated.

MATERIALS AND METHODS

Plant

Aloe genotype accessions were collected from natural growing areas all over Iran during October to November, 2007. Sampling was done in a total of 10 areas, according to the information obtained from local agricultural extension offices and producers all over Iran. Geographical origins of the samples are listed in Table 1. Suckers of each accessions genotypes were grown directly in experimental sites.

Site description

In order to study the morphological and agronomical divergence among collected genotypes, filed experiments were conducted for two years using complete randomized block design with three replications at the Experimental Station of National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran, during two growing seasons of 2007 to 2008. The geographical location of the station was 35°47' N and 50°56' E with altitude of 126 m to the sea level and an average annual precipitation of 380 mm.

The soil texture was silt-loam with pH 7.9. The field in which the Aloe Suckers were planted had been abandoned for more than 3 years. Rendered manure was applied at a rate of 10 tons/ha; 150 kg/ha phosphor were added as phosphate ammonium in addition to 210 kg/ha ammonium nitrate was added at three stages including once at sowing date, once at middle of growing season and once immediately after flowering. For the second year 140 kg/ha ammonium nitrate was added at two stages including middle of growing season and immediately after flowering.

Collected suckers were sown by hand using complete randomized block design with three replications. Each experimental plot size of 4 m × 4 m and suckers of each sample were grown in 6 rows with a 60 cm distance between lines and 30 cm within lines and the four middle rows were considered for 2-year agro-morphological and phytochemical evaluation. In this way, a total of 78 plants were produced in each plot, out of which 52 plants in the middle rows were evaluated. The field was kept weed free by hoeing and plants were irrigated when necessary.

Table 1. Geographical locations of Iranian aloe populations.

Population	Origins	Climate[a]	Latitude, (N)	Longitude, (E)	Altitude (m)
1	Borazjan	Warm temperate-humid	50° 28 'N	27° 59 'E	5
2	Boushehr	Warm-arid	29° 15 'N	51° 12 'E	94
3	Sar korreh	Warm-arid	28° 53 'N	51° 17 'E	43
4	Hormozgan	Warm temperate-humid	27° 75 'N	56° 05 'E	9
5	Bashagerd	Warm-arid	50° 31 'N	29° 34 'E	10
6	Minab	Warm temperate-humid	25° 55 'N	48° 05 'E	8
7	Bandar pol	Warm temperate-humid	53° 21 'N	29° 30 'E	9
8	Bidkhon	Warm-arid	28° 15 'N	50° 12 'E	90
9	Dehno	Warm-arid	53° 15 'N	28° 50 'E	5
10	Asaloye	Warm-arid	38° 51 'N	50° 17 'E	43

[a]Yearly mean temperature in warm-arid and Warm temperate-humid are 30-50°C and 30-40°C, respectively. Yearly mean rainfalls are warm- arid and warm temperate-humid climates are 100-300 mm, 300-400 mm respectively.

Field investigated characters

In order to obtain the quantitative yield of the crop, dry leaf weights (kg/ha) and dry root weights (kg/ha) were estimated over two growing seasons during 2007 to 2008. At the end of first growing season (December 2007), of the four middle rows in each plot, leaves of 39 plants belonging to the first three rows were harvested from all plots and Aloe roots of 13 plants belonging to the fourth row were dug up to keep for further evaluation in the succeeding year. The harvested materials were dried at room temperature and were weighed on an analytical balance to estimate weight of dry leaf production and dry root production for each plot, and were calculated in kg per hectares.

The whole leaves of *A. vera* were cut into thin pieces, put onto glass plate, lyophilized in vacuo for 2 days, and then ground into a fine powder for further use. The collected leaf samples were ground in a blender and were stored in paper bags in a dry place before the aloin analysis. At the end of second growing season (December 2008), sampling of leaves and roots were repeated as explained earlier for 39 of 2-year-old plants belonging to middle 3 rows. The ground second year leaf samples were also prepared in the same way.

Phytochemical analysis

After drying, leaves were ground in a blender and the aloin contents were evaluated according to the simple method described by Siebenborn et al. (2002) with few changes. Fifty milligrams of ground leaf samples were mixed with 45 ml of distilled water using ultrasound mixer and were fermented for 12 h. Then, they were transferred to water bath at 70°C for 1 h, and thereafter 45 ml of ethanol was added to the samples. The samples were then adjusted to 100 ml by adding 50% ethanol after they were filtered through a 0.2 mm filter (Millipore). Ten milligrams of aloin ($C_{19}H_{22}O_{10}$, Fluka 05560) were dissolved in 200 ml of pure ethanol using an ultrasonic bath (standard I). To prepare the final standard solution (2 mg/100 ml), a 20 ml sample of standard was mixed with 30 ml of methanol and was adjusted to 100 ml by adding distilled water.

The absorbance values of all ethanol extracts and standard solution were measured at 250 nm using a spectrophotometer (Perkin Elmer UV/VIS Lambda 20).

Total aloin content based on aloin standards was calculated according to following expression:

$$\textit{Total aloenin content}\ (\%) = \frac{100}{50} \times \frac{\textit{Standard concentration} \times \textit{Sample absorbance value}}{\textit{Standard absorbance value}}$$

RAPD analysis

Young leaves from 20 plants selected randomly from each population were harvested three weeks after emergence. Genomic DNA was extracted according to CTAB method (Saghai-Maroof et al., 1984) with minor modifications. DNA was quantified via spectro-photometric measurement of UV absorption at 260 nm (Shimadzu UV-260). For RAPD analysis of genomic DNA, 17-decamer arbitrary primers (Operon DNA Technologies, Alameda, CA, USA) were used (Table 2). Polymerase chain reaction (PCR) was performed in a volume of 25 µl containing 50 ng of genomic DNA, 2.5 polymerase buffer, 1.0 mM of each dNTP, 1.0 mM $MgCl_2$, 1.1 µM of primer, and 5 unit of *Taq* DNA polymerase. Amplification was performed in a Techne Progene thermal cycler (Techne Cambridge Ltd.), cycling conditions were: initial denaturation at 94°C for 4 min followed by 35 cycles at 95°C for 1 min, at 37°C for 1 min, at 72°C for 2 min, and a final extension step of 10 min at 72°C. After amplification, 5 µl of loading buffer was added in each reaction tube and the amplification products were separated on 1.6% agarose gels in TBE buffer. The gels were stained with ethidium bromide (1 mg/L H_2O) for 10 to 15 min and were washed in distilled water for 10 to 15 min. The RAPD bands were then visualized through UV-Transluminator and were photographed.

Statistical analysis

Combined analysis of variance was performed for agronomical and phytochemical evaluated characters (that is, eight different population and 2 years) using SAS software, version 6.08 (SAS 2002). The extent of genetic variation among accessions was estimated as broad-sense heritability, which is defined as the ratio of the genetic variance ($\partial^2 g$) arising between accessions to the total phenotypic variance ($\partial^2 p = \partial^2 g + \partial^2 e$) according to the following formula:

Table 2. Primers with 5'- 3' sequence used to assess genetic diversity of Iranian aloe populations.

Primer number	Primer code	Sequence 5'- 3'
1	D-03	GTCGCCGTCA
2	D-05	TGAGCGGACA
3	D-07	TTGGCACGGG
4	D-09	CTCTGGAGAC
5	D-11	AGCGCCATTG
6	D-13	GGGGTGACGA
7	D-15	CATCCGTGCT
8	D-17	TTTCCCACGG
9	D-19	CTGGGGACTT
10	D-20	ACCCGGTCAC
11	LC-76	GTGACGTAGG
12	LC-77	GGGTAACGCC
13	LC-80	CAGCACCCAC
14	LC-81	TCTGTGCTGG
15	LC-82	TTCCGAACCC
16	LC-83	AGCCAGCGAA
17	LC-87	AGGTGACCGT

Table 3. Summary of descriptive statistics for evaluated traits in the 2-year period.

Trait	First year			Second year		
	Min	Max	Mean±SD[a]	Min	Max	Mean±SD[a]
Aloin content (%)	2	5	3±0.11	0.4	4.7	3±0.019
Leaf weight (kg/ha)	20	49	30±11	560	650	608±29
Root weight (Kg/ha)	19.85	61.36	41.4±0.02	62.7	1000	534±40.77

[a]Standard deviation.

$$h_b^2 = \left[\frac{\partial_g^2}{\partial_g^2 + \partial_e^2}\right] \times 100$$

In this experiment, the estimates of $\partial^2 g$ and ∂_e^2 are (Table 3):

$$\partial_g^2 = \frac{MS_g - MS_{gy}}{ry}$$

$$\partial_e^2 = MS_e$$

where MS_g, MS_{gy}, r, y and MS_e are population mean square, mean square for population × year, number of replications, number of years, and error mean square, respectively.

In addition, simple statistics (that is, mean, maximum, minimum and coefficient of variation) were used in order to compare genetic variation between accessions and the means of results were compared by Duncan's multiple range tests. Simple correlations (r) between values of the accessions for different botanical and phytochemical traits were evaluated across the 2-year period. A cluster analysis of the data was performed based on phenotypic traits and phytochemical contents by using Unweighted Pair Group Method Using Arithmetic Averages (UPGMA). Correlation and UPGMA analyses were performed using SPSS 9.0 program package (SPSS 1999).

Moreover, polymorphic RAPD fragments were scored as either present (1) or absent (0) across all accessions. Only the distinct, well-resolved fragments were scored. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. Binary matrix was used to estimate the genetic similarities between pairs, by employing Dice (Nei and Li, 1979) method. Phenogram was also constructed based on UPGMA using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC), version 2.02 (Applied Biostatistics) program (Rohlf, 2006).

RESULTS

Agro-morphological and phytochemical diversity

Analysis of variance (ANOVA) applied on agro-morphological and phytochemical evaluated characters showed that the differences among accessions were

Table 4. Duncan' s multiple range tests analysis for evaluated traits across the 2-year period.

Population	Leaf weight (kg/ha)	Root weight(kg/ha)	Aloin content (%)
1	225^{DEF}	211.8^{D}	3.1^{BC}
2	206^{EFG}	327.22^{C}	2.3^{E}
3	320^{C}	437.24^{AB}	3.2^{BC}
4	150^{GH}	209^{D}	2.2^{CH}
5	110^{H}	20.6^{F}	2.5^{DE}
6	190^{FG}	131.5^{E}	1.8^{F}
7	253^{DE}	244^{D}	3^{C}
8	260^{D}	480^{A}	3.56^{AB}
9	386^{B}	197.9^{DE}	3.9^{A}
10	490^{A}	389.3^{BC}	3^{CD}

Different letters indicate statistically significant differences.

Table 5. Pearson correlation coefficient of evaluated traits of Iranian aloe populations.

Trait	Aloin content (%)	Leaf weight (kg/ha)	Root weight(kg/ha)
Aloin content (%)	1	-	-
Leaf weight (kg/ha)	0.493*	1	-
Root weight(kg/ha)	0.528*	0.534 *	1

Table 6. Broad-sense heritability estimates for evaluated traits of Iranian aloe populations.

Trait	$*\partial^2 g$	$*\partial_e^2$	$*h_b^2$ (%)
Leaf weight (kg/ha)	0.31	0.15	67.32
Root weight (kg/ha)	1517.33	3511	30.17
Aloin content (%)	0.001	0.0013	43.47
$*\partial^2 g = \left((MS_g - MS_{gy})\right)/ry$	$*MSe = \partial_e{}^2$	$*h_b^2 = (\partial_g^2/\partial_g^2 + \partial_s^2) \times 100$	-
$MS_g = \partial_s^2 + r\partial_{gy}^2 + ry\partial_g^2$	$MS_{gy} = \partial_s^2 + r\partial_{gy}^2$	-	-

highly significant ($p < 0.01$). While, the interaction between replication × year effects showed no significant difference of these traits. The effect of year and year × population was significant for all morphological traits ($p < 0.01$). Interaction between year × population was significant for aloin content ($p < 0.01$) while year did not have any significant effect on aloin content.

Genetic variation

A summary of descriptive statistics of the characters measured at main trial is shown in Table 3. Mean aloin contents were almost the same across during the 2-year period, the leaf and root weights varied greatly. Duncan's multiple range tests showed that the accessions of Aloe were significantly different in most evaluated traits (Table 4).

Simple correlation coefficient

Simple correlation was observed between the evaluated traits (Table 5). Due to the small number of accessions ($n = 10$) a higher significance level was considered ($\alpha = 10\%$). Simple correlation between traits (Table 5) showed that leaf weight and root weight had positive correlation with aloin content ($p < 0.1$ and $r = 0.493$ and 0.528, respectively). Moreover, leaf weight showed significant positive correlation with root weight ($p < 0.1$ and $r = 0.534$).

Broad-sense heritability (h^2_b)

According to these results (Table 6), within *A. vera* spe-

cies leaf weight was most heritable trait (h^2_b = 67.62%). Root weight and aloin content showed lower heritability estimates (30.17 and 43.47%, respectively).

Cluster analysis

The 10 Aloe accessions were clustered based on phenoltypic and phytochemical traits, by UPGMA. Clustering procedure showed 3 main groups (Figure 1). Five of the 10 accessions (Po 3, 6, 9, 7, 10) were included in group A, while groups B contained four accessions (Po 1, 5, 2, 4) and 1 population (Po 8) was included in cluster group C (Table 2).

Genetic diversity

A total of 140 polymorphic bands were produced by 17 primers. The primers produced a maximum of 17 (primer 9) and a minimum of 5 (primer 13) polymorphic bands. Primers 9 and 13 produced the highest and lowest number of polymorphic bands, respectively. The reported similarities between the accessions, using Dice coefficient, ranged between 94% (accessions 4, 11) and 78% (accessions 4, 11) with an average of 44%. The cluster analysis based on RAPD marker data showed two main groups (Figure 2). In this regard, six of the 10 accessions (Po 1, 3, 8, 9 10, 6) were included in cluster group I and four accessions in cluster group II (Po 2, 7, 4, 5).

DISCUSSION

Agro-morphological and phytochemical diversity

The results showed that accessions of *A. vera* were significantly different in evaluated agro-morphological and biochemical traits, suggesting that selection for breeding programs could be possible.

This diversity could be seen in Duncan's multiple range tests (Table 4). Highly significant effect of year on leafweight and root weight indicates the differential response of accessions in the 2-year period of study. The means of leaf weight and root weight were multiplied by 20 and 13, respectively, in the second year, while the aloin content remained almost constant in the 2-year period (Table 3). Hasanuzzaman et al. (2008) also reported progressive increases in leaf and root weight.

There are various studies concerning agronomical (Hasanuzzaman et al., 2008), phytochemical (Kuzuya et al., 2001) and diversity aspects (Viljoen, 1999; Rajasekaran et al., 2006; Nayanakantha et al., 2010; Nejatzadeh-Barandozi et al., 2012) of Aloe but no concrete information explaining the heritability estimates for these traits is available. Plant breeders frequently use heritability estimates to distinguish proportion of total phenotypic variation as a result of genotype and environmental influences. This estimate is then used to design appropriate breeding methodologies (Thavarajah et al., 2009). Furthermore, broad-sense heritability of traits with significant different genetic variations would be the best descriptor for selection and breeding program (Figliuolo et al., 2001). According to this result, high broad heritability was found for leaf weight (Table 6). High broad heritability estimate indicates genetic variations with lesser influence of the environment and the potential of effectiveness of its selection (Marame et al., 2009). Leaf weight is quite easy to score and in addition is correlated with industrially important traits (root weight and aloin content) (Table 5). This allows for easy field screening and thus these three traits are known as efficient descriptors for selection and evaluation of aloe accessions.

In this study, estimates of broad-sense heritability (h^2_b) were moderate for aloin content (43.47%, Table 6). Also, Duncan's multiple range tests showed significant differences among accessions for aloin content as an industrially important trait. Furthermore, Duncan's multiple range tests showed highly potential for aloin production in the evaluated accessions, as 7 out of 10 accessions, were qualified as Duncan's groups A to C (Table 4). Potential of aloin production was also reasonable in comparison with other accessions from Japan (Kuzuya et al., 2001). These results implied that, it may be possible to select aloe clones with enhanced ability to produce essential antheraquinone components and improved qualitative traits. The fairly low heritability of root weight (h^2_b = 30.17%, Table 6) probably shows that environment has considerable impact on underground parts production. Correlation analysis showed clearly relationships within evaluated traits (Table 5). Obviously an increase in leaf weight can stimulate the photosynthesis and other metabolic pathways in plant. This fact probably describes the significant and positive correlation between leaf weight and the other traits.

Relationship between cluster analysis and geographical origin

Cluster analysis undertaken based on agro-morphological and phytochemical traits (Figure 1) did not reveal a clear relationship between diversity pattern and geographical origin. For instance, accessions with the highest distance (Po 3 and 9) entered into the same cluster and also geographically close accessions (Po 5 and 6) entered in different clusters. For all evaluated characters, similarity among accessions was independent of their origin, and no trait in the present study, could clearly separate the accessions on the basis of geographical origin. Duncan's multiple range tests showed large variations within accessions, and most likely, the lack of clear clustering based on origins could

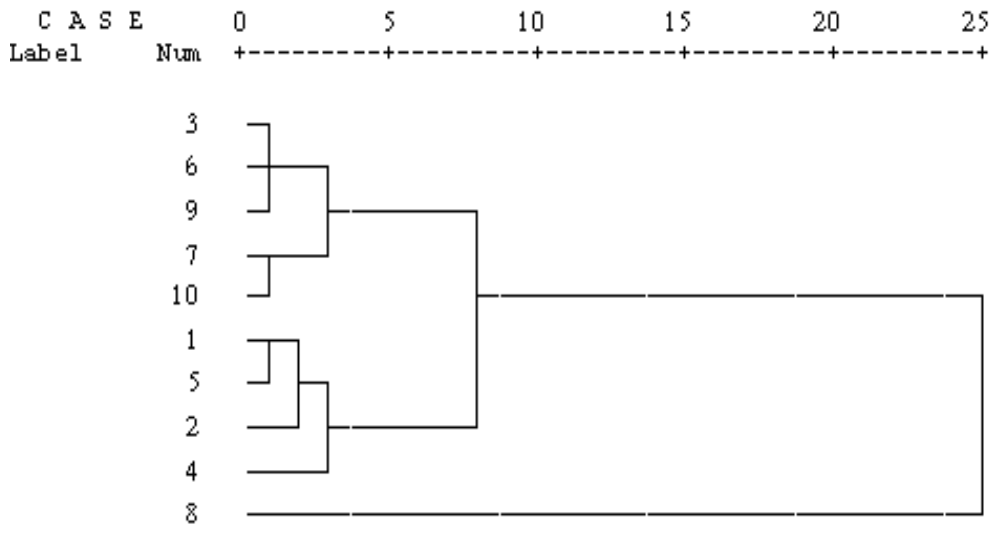

Figure 1. Dendrogram generated by cluster analysis of agro-morphological and phytochemical traits.

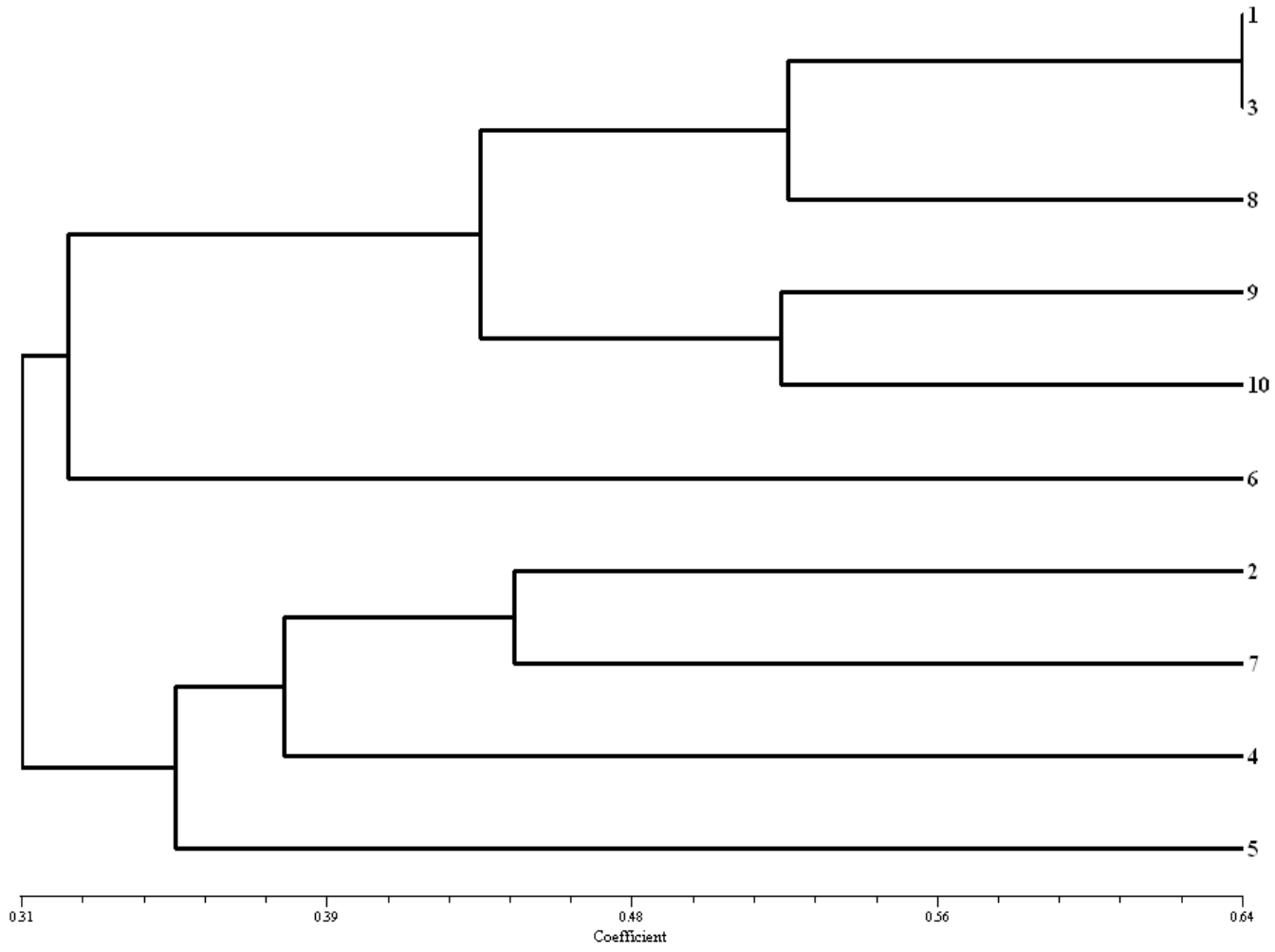

Figure 2. Dendrogram generated by cluster analysis base on RAPD marker data.

partly have been due to this reason.

Relationship between RAPDs diversity pattern and origins

Cluster analysis created based on RAPDs analysis using the same individuals (Figure 2) detected a high level of polymorphism between accessions, which led to a difficult interpretation of the results. However, these results did not reveal a clear relationship between diversity pattern and geographical origin. Since RAPD markers are known to be highly polymorphic, it would be interesting to undertake further studies with less polymorphic markers (e.g. isozymes) or markers that allow an easier analysis (e.g., microsatellites).

Relationship between agro-morphological/ phytochemical and molecular diversity

There was no association between agro-morphological/ phytochemical diversity (Figure 1) and molecular diversity (Figure 2). There could be many reasons for this dissimilarity. As an instance, RAPDs detect polymorphisms in coding as well as non-coding regions of the genome. Coding region is only a small portion of any genome (Khan et al., 2009). It is thus quite likely that the observed polymorphism was mainly from a non-coding region. In addition, there is more environmental influence accounting for the morphological and phytochemical diversity observed.

Therefore, in comparison with RAPD technique, morphological and phytochemical analyses are relatively less reliable for precise discrimination of closely related genotypes.

Conclusion

In this study, the analysis showed that accessions diversity for industrially important traits (leaf weight and aloin content) were highly significant, and therefore, could be successfully used in improving quantitative yield of this medicinal plant. Diversity encountered in Iranian aloe germplasm points out that there is a great potential for improvement of Aloe for both agronomic and quality traits. Our results provide a better knowledge of the genetic variation of vegetative and qualitative yield-related traits of these species.

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