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Inter- and intra-specific genetic diversity of Iranian yarrow species *Achillea santolina* and *Achillea tenuifolia* based on ISSR and RAPD markers

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ABSTRACT. Cultivation and preservation of yarrow has recently attracted wide attention due to its beneficial properties; however, genetic variation of *Achillea* species is still relatively unknown. We used RAPD and ISSR markers to assess genetic diversity in 16 accessions of yarrow belonging to two species native to Iran. Seven ISSR and nine RAPD primers generated 187 amplified fragments, of which 159 were polymorphic. The similarity coefficient among *Achillea tenuifolia* accessions ranged from 61 to 86%, and from 40 to 84% among *A. santolina* accessions. A low similarity was observed between these two species (mean similarity = 0.36%). This low similarity is consistent with their geographical distribution. According to the results of cluster and PCA analyses, the two species completely separated from each other. These markers will aid in

the identification of elite genotypes for domestication and breeding programs.

Key words: Genetic diversity; *Achillea* species; ISSR; RAPD; Marker

INTRODUCTION

Yarrow (*Achillea* sp) is one of the youngest evolutionary genera of the Asteraceae family, which is present throughout the world (Farajpour et al., 2012). More than 100 species have been recognized in this genus (Goli et al., 2008). Yarrow is a perennial herb and well-known medicinal plant, widely used in folk medicine for gastrointestinal disorders (Bimbiraitė et al., 2008). These plants are native to Europe and western Asia but are also found in Australia, New Zealand, and North America (Rechinger, 1963).

During the 21st century, most studies have focused on the cultivation and preservation of medicinal and aromatic plants and the evaluation of their quality (Bimbiraitė et al., 2008). Molecular markers have proven to be valuable tools for the characterization and evaluation of genetic diversity among and within species and populations. It has been shown that different markers might reveal different kinds of variation (Powell et al., 1996). RAPD markers (Williams et al., 1990) and ISSR markers (Zietkiewicz et al., 1994) have been used with success to identify and determine relationships at the species, population, and cultivar levels in many plant species, including several aromatic and medicinal plants (Mattioni et al., 2002; Nan et al., 2003; Fracaro et al., 2005; Manica-Cattani et al., 2009; de Lima et al., 2011). However, there are limited reports on the genetic relationships of *Achillea* species. For example, Wallner et al. (1996) assessed the stability of oligonucleotide fingerprints and RAPD markers for discrimination between 2 *Achillea* species during micropropagation whereas Morsy (2007) reported the molecular variation of 5 populations of *Achillea fragrantissima* (Forssk) in Egypt using RAPD and isozyme markers.

Only a limited number of studies examine the genetic variation of *Achillea* species in Iran. Rahimmalek et al. (2009) have studied inter- and intra-genetic diversity of *Achillea* species using amplified fragment length polymorphism markers. Gharibi et al. (2011) applied ISSR markers to the study of genetic variation in two sub-species of *Achillea millefolium* in Iran.

No reports describe the application of RAPD and ISSR markers for the determination of genetic diversity in *A. santolina* and *A. tenuifolia*, but such studies can provide new insights into the evolutionary relationships of these species.

The objectives of this study were to identify genetic variation between 2 *Achillea* species using ISSR and RAPD markers and to evaluate their evolutionary connection in relation to their geographical locations. The results of the study have implications for accurate identification of elite genotypes for domestication and breeding programs.

MATERIAL AND METHODS

Samples were obtained from the gene bank of the Research Institute of Forests and Rangelands in Tehran. The seeds were collected from the north, northwestern, western, and central regions of Iran (Figure 1). Young leaves were transported to the laboratory and stored at -80°C for subsequent analysis. DNA from young leaves was extracted using a modified cetyl-

trimethylammonium bromide (CTAB) procedure described by Murray and Thompson (1980). DNA concentration and quality was estimated electrophoretically and spectrophotometrically. The amplification conditions for RAPD and ISSR were an initial step of 5 min at 94°C followed by 35 cycles for 1 min at 94°C for denaturation and 45 s at 30° to 33°C (RAPD) or 50° to 53°C (ISSR), extension for 2 min at 72°C, and a final extension of 5 min at 72°C (Table 1). Amplification products were resolved with electrophoresis on 1.2% (w/v) agarose gels in Tris-borate-ethylenediaminetetraacetic acid buffer. DNA fragments were stained with ethidium bromide and digitized under ultraviolet light for further analysis. RAPD and ISSR amplicons were scored for presence or absence in each accession and the data were entered into a binary matrix as discrete variables. Genetic similarities in RAPD and ISSR data were calculated using the Jaccard similarity index according to the method of Sneath and Sokal (1973). Dendrograms showing the genetic relationships of the 16 genotypes were constructed using the unweighted pair group method with arithmetic mean. The Mantel test (Mantel, 1967) was used to detect the correlation between the two dendrograms. The cophenetic correlation coefficient was measured using a cophenetic algorithm to evaluate the degree of fit of the clusters in the dendrogram to the data in the similarity coefficient matrix. Cluster analysis and principal component analysis (PCA) were conducted using NTSYSpc version 2.02 (Rohlf, 1998).

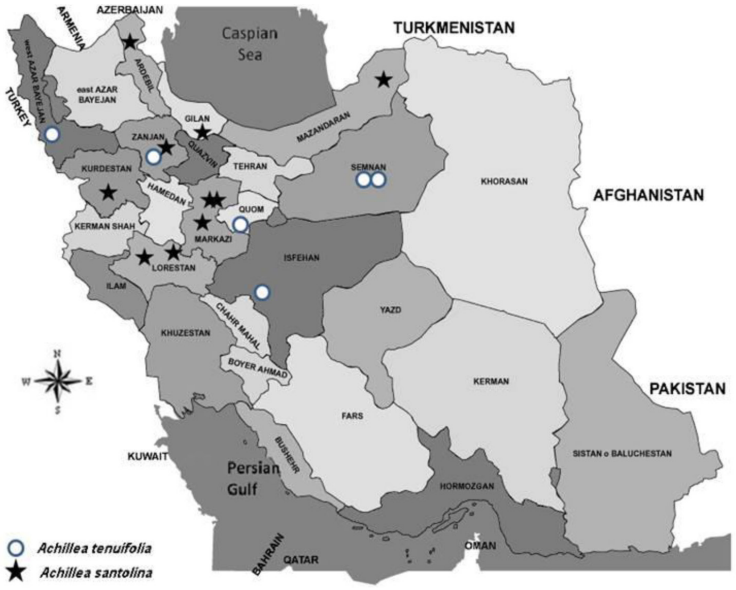

Figure 1. Geographical distribution of the Iranian *Achillea* accessions analyzed.

RESULTS AND DISCUSSION

The RAPD analyses, detected 116 bands, of which 101 bands (87%) were polymorphic and the mean was 11.22 per primer (see Table 1). For each primer, the number of bands for RAPD ranged from 9 to 20, with an average of 12.88. The ISSR analysis detected 71 bands, the number ranging from 7 to 15, with an average of 10.14 (see Table 1). Polymorphic

bands comprised 81.7% of the bands. Selected ISSR and RAPD primers amplified 187 bands, most of which were polymorphic (85%). Mantel analysis on the Jaccard similarity coefficients, calculated based on the presence or absence of RAPD and ISSR markers, showed that the similarity coefficients were highly correlated (0.90), indicating a high degree of association between the dendrogram clusters and the similarity matrices. Two main groups stand out in the dendrogram (Figure 2).

Table 1. PCR specifications, number and percent of polymorphic bands of ISSR and RAPD primers used for 16 *Achillea* accessions.

Primer	Sequence	Annealing temperature	No. of bands	No. polymorphic bands	Polymorphic bands (%)
ISSR1	$(CA)_8$-GT	51	15	13	87
ISSR2	$(GA)_8$-G	53	11	8	72
ISSR3	$(GT)_8$-C	51	9	8	89
ISSR4	$(AC)_8$-TG	51	8	6	75
ISSR5	$(AGTG)_4$	52	10	8	80
ISSR6	$(GATA)_5$	50	7	7	100
ISSR7	$(TCT)_6$	51	11	8	72
Total	-	-	71	58	-
Average	-	-	10.14	8.28	81.7
RAPD-g11	TGCCCGTCGT	32	20	18	90
RAPD-m2	ACAACGCCTC	32	11	11	100
RAPD-m3	GGGGGATGAG	32	10	9	90
RAPD-a2	TGCCGAGCTG	33	15	14	93
RAPD-a10	GTGATCGCAG	30	12	9	75
RAPD-e3	CCAGATGCAC	32	9	8	89
RAPD-f19	CCTCTAGACC	32	13	12	92
RAPD-b10	CTGCTGGGAC	33	13	11	84
RAPD-b11	GTAGACCCGT	31	13	9	69
Total	-	-	116	101	
Average	-	-	12.88	11.22	87
Total (RAPD+ISSR)	-	-	187	159	
Average (RAPD+ISS)	-	-	11.68	9.93	85

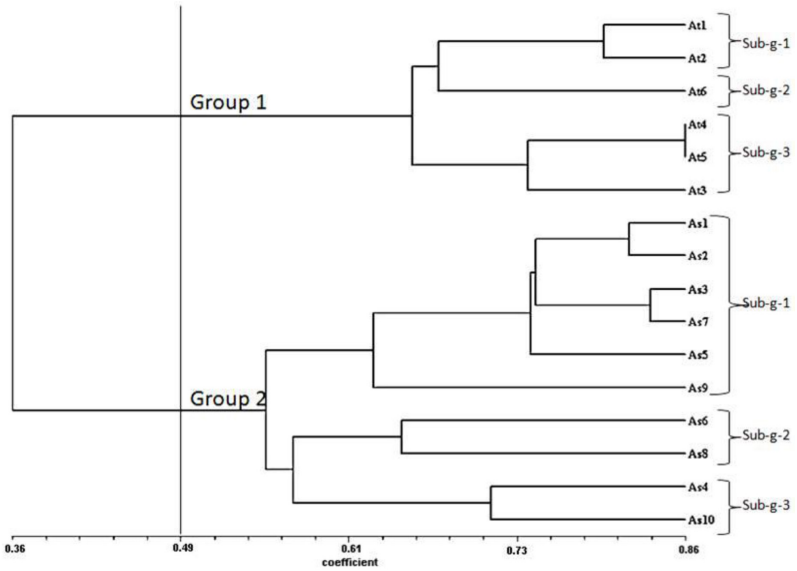

Figure 2. Dendrogram representation of cluster analysis for *Achillea* accessions constructed using UPGMA.

Group I is composed of the *A. tenuifolia* accessions, which are geographically scattered throughout central, northern and northwestern Iran. This species showed later flowering in comparison to that of *A. santolina* species. This cluster was further divided into 3 subgroups. One subgroup contained genotypes At1 and At2, which were gathered from northwestern part of the country. Another subgroup was a single prototype (At6) that was collected from the Ghom Province in the center of the country. The third subgroup consisted of At3, At4, and At5 genotypes that were gathered from the central regions of the country. The maximum similarity coefficient in the *A. tenuifolia* accessions was obtained for At4 and At5 (86%, Table 2), collected from the Semnan Province, whereas the minimum similarity coefficient was 54% between At2 and At5.

Table 2. Similarity coefficients of the 16 *Achillea* species accessions based on RAPD and ISSR data.

	At1	At6	At2	At4	At3	At5	As1	As5	As6	As3	As4	As8	As7	As10	As2	As9
At1	1															
At6	0.69	1														
At2	0.79	0.66	1													
At4	0.74	0.62	0.61	1												
At3	0.67	0.69	0.64	0.75	1											
At5	0.73	0.62	0.55	0.86	0.72	1										
As1	0.32	0.37	0.27	0.3	0.25	0.36	1									
As5	0.42	0.46	0.27	0.38	0.33	0.46	0.66	1								
As6	0.31	0.35	0.18	0.38	0.4	0.42	0.5	0.5	1							
As3	0.3	0.34	0.26	0.29	0.31	0.33	0.74	0.74	0.47	1						
As4	0.47	0.5	0.33	0.53	0.46	0.61	0.57	0.57	0.53	0.64	1					
As8	0.37	0.4	0.24	0.3	0.29	0.31	0.56	0.56	0.64	0.63	0.6	1				
As7	0.39	0.43	0.26	0.36	0.31	0.43	0.74	0.74	0.47	0.84	0.64	0.63	1			
As10	0.37	0.5	0.25	0.45	0.46	0.5	0.47	0.57	0.53	0.53	0.71	0.6	0.53	1		
As2	0.32	0.46	0.27	0.3	0.25	0.36	0.81	0.81	0.4	0.74	0.57	0.56	0.74	0.57	1	
As9	0.33	0.29	0.2	0.38	0.33	0.46	0.67	0.54	0.61	0.62	0.57	0.47	0.75	0.46	0.54	1

At = *Achillea tenuifolia*; As = *Achillea santolina*.

Group II included *A. santolina* accessions. This group is distributed over the central, northern, and northwestern parts of the country and is composed of three subgroups. One subgroup includes six genotypes that were gathered from the central and northwestern parts of the country. The maximum similarity coefficient among *A. santolina* accessions was observed for genotypes As3 and As7 (84%). Genotype As9 from the Zanjan Province was separated from the other accessions at this subgroup. Another subgroup consisted of As8 and As6 genotypes, which were collected from the western part of the country. The third subgroup was represented by 2 genotypes (As4 and As10) gathered from the northern regions of the country. The minimum similarity coefficient among *A. santolina* accessions was 46% between the As9 and As10 genotypes. According to the results of the cluster analysis, the two species adjoin with a 0.36% similarity coefficient. Rahimmalek et al. (2009) have reported a similar result on the range of the similarity coefficient of the adjoining two species.

In the systematic classification of Flora Iranica, *A. santolina* and *A. tenuifolia* have been grouped in the same section (Santolinolideae) (Rechinger, 1963). The results of the cluster analysis closely corresponded to the geographical origins of the genotypes. These findings are in line with the results of a previous study on the relationship between genetic variability and geographic distribution of *Achillea* species in Iran (Rahimmalek at al., 2009). The PCA indicated that the first 3 principal components accounted for more than 75% of the total ob-

served variation. The results of the PCA plot generated from RAPD and ISSR data suggested that the two major groups corresponded largely to those obtained through the cluster analysis (Figure 3). As6 is separated from the other *A. santolina* accessions in the PCA (see Figure 3).

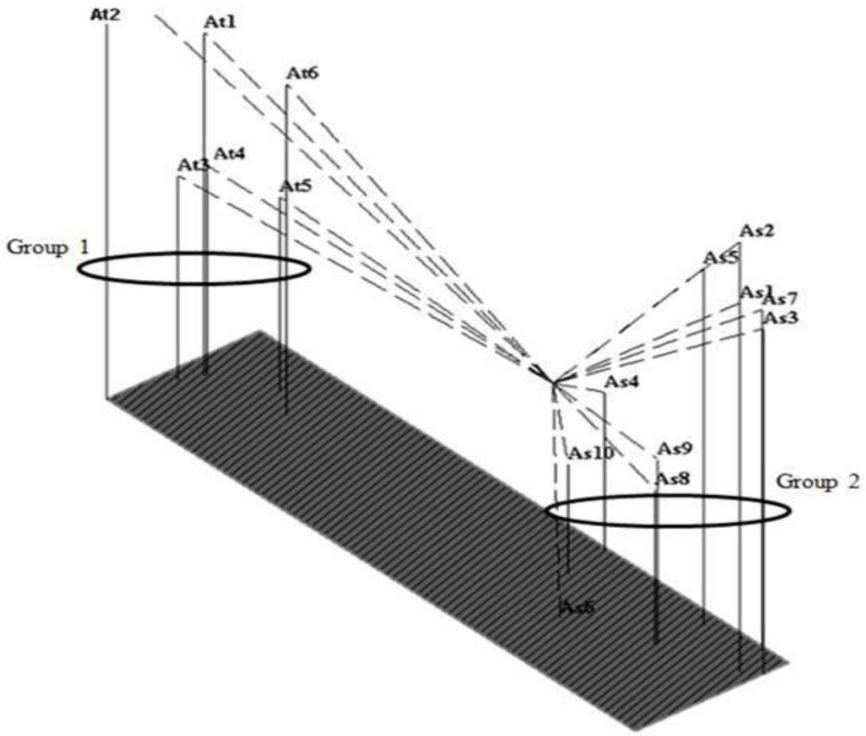

Figure 3. Three-dimensional plot derived from the principal component analysis of 16 Iranian *Achillea* accessions.

Our results indicated that the 2 molecular markers investigated in this study, namely ISSR and RAPD, efficiently identified and discriminated the *Achillea* species, allowing the characterization of all the accessions under study. The efficiency of these molecular markers for genotype identification in other aromatic and medicinal plants has been reported (Mattioni et al., 2002; Torres et al., 2003; Fracaro et al., 2005; Manica-Cattani et al., 2009).

The information obtained in this study will be useful in the management of wild *Achillea* species collection. The identity of *Achillea* species is difficult to establish based on morphological traits alone. We have demonstrated that RAPD and ISSR molecular markers can be effectively used to recognize certain accessions of *Achillea* species. Information about the genetic similarity of *Achillea* species can provide valuable insights into their systematic classification and can guide and improve the effectiveness of the breeding process.

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