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Full Length Research Paper

Assessment of genetic diversity in *Achillea millefolium* subsp*. millefolium* and *Achillea millefolium* subsp*. elbursensis* using morphological and ISSR markers

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Yarrow (*Achillea millefolium* L.) is an important medicinal plant with different pharmaceutical uses. In this research, ISSR and morphological markers were used to assess genetic diversity in several accessions of yarrow from various geographical regions of Iran. Fourteen primers were used to amplify 228 bands out of which 199 (87.28%) were polymorphic. Cluster and Principle coordinate analyses (PCoA) showed that most genotypes were grouped based on their geographical regions. Analysis of Molecular variance (AMOVA) revealed that the differences among groups only accounted for 32.55% of the total variation, whereas differences among populations and within populations were 40.94 and 26.51%, respectively. Genetic distances among eight Iranian populations showed that the minimum gene diversity over loci was observed in North-Western (NW) population (0.021 ± 0.015), while the highest gene diversity was found in North (N) (0.129 ± 0.08) population. The results of morphological analysis in most cases corresponded to those obtained through molecular analyses.

Key words: *Achillea millefolium*, ISSR, genetic diversity, morphology.

INTRODUCTION

Yarrow (*Achillea* L.) belongs to Asteraceae family and more than 100 species have been recognized in this genus (Rechinger, 1963). To date, different medicinal applications of yarrow, such as spasmolytic, choleretic, treatment of wounds and anti-inflammatory activities, has been recognized (Benedek et al., 2007). Nineteen species of *Achillea* have been recognized in Iran distributed in different geographical and ecological regions (Rechinger, 1963). Among these species *A. millefolium* has extensive application in pharmaceuticals (Rahimmalek et al., 2009a). Populations of *A. millefolium* species in Iran are dispersed in North (N), West (W), North-West (NW), and West-Central (CW) regions based on Flora Iranica reports (Rechinger, 1963). Two major subspecies, *A. millefolium* subsp*. millefolium* and *A. millefolium* subsp*. elbursensis* are recognized in the country (Rechinger, 1963) with the latter being endemic to Alborz Mountain in Northern regions of Iran.

The ongoing processes of habitat destruction and degradation have restricted the distribution of many plant species and increased the distance between the remaining populations (Peterson et al., 2008). Habitat fragmentation, the reduction of a continuous habitat into several smaller spatially isolated remnants, reduces the size and increases the spatial isolation of plant populations. Small population size and isolation may have a number of negative effects on populations, such as genetic erosion and increasing genetic divergence (Kery et al., 2000; Peterson et al., 2008). Furthermore, the medicinal characteristics of plants, such as essential oil yield and morphological properties can be affected by

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their genetics, as well as environmental factors. For traditional medicinal plants, the major constraint in achieving higher essential oil yield is lack of genetic variability (Rahimmalek et al., 2009b). Therefore, information on the levels of genetic variation within and among natural populations provides fundamental insights on the genetic diversity and for improving the effective selection of the populations for pharmaceutical purposes (Domyati et al., 2011; Zhang et al., 2011).

In the recent years, the use of molecular markers has become an important tool to study genetic diversity of plants. The use of molecular markers with morphological data can improve the classification and genetic diversity studies in many plant species. Inter-simple sequence repeat (ISSR) markers (Zietkiewicz et al., 1994; Wang et al., 2010) are widely used in genetic diversity of different plants because they need no prior DNA sequence information, development costs are low, and laboratory procedures can easily be transferred to any plant species.

Rahimmalek et al. (2009c) used AFLP markers to assess the genetic variation among and within five *Achillea* species collected from different geographical regions of Iran. Furthermore, there are no reports on the application of ISSR markers on genetic studies of yarrow and such studies could provide valuable information about evolutionary process of yarrow in the country along with their genetic structure to improve conservation strategies.

The objectives of the present study were: (1) to determine the level of genetic diversity and differentiation among populations of *A. millefolium* in different geographical regions of Iran using ISSR markers; (2) to assess the genetic relationships of Iranian populations with a focus on their genetic structure and 3) to compare the results of molecular and morphological classifications.

MATERIALS AND METHODS

Plant materials

Forty accessions from eight populations of *A. millefolium* were collected from different geographical regions of Iran based on Flora Iranica (Rechinger, 1963) (Table 1). Sampling strategy was designed to cover the most distribution range of the species as widely as possible (Figure 1). Young leaves were collected and transported to the laboratory and stored in a -80°C freezer until use.

Morphological data analysis

The major morphological characters of all accessions were measured in three replicate and the mean were used for analysis. The dendrogram for morphological characters was constructed using SPSS ver17.

Extraction of genomic DNA

DNA from young leaves was extracted using the modified CTAB procedure as described by Murry and Thompson (1980). The quality and quantity of DNA was estimated spectrophotometrically and electrophoretically. The DNA was diluted to a working concentration of 10 ng/µl.

ISSR analysis

Of the 20 ISSR primers screened, 14 that produced a higher number of reproducible bands were selected for the ISSR analysis of yarrow accessions (Table 2). PCR reactions were carried out in a volume of 15 µl containing 10 ng total DNA, 10× PCR buffer, 2% formamide, 0.25 mM each dNTP, 10 pM of each primer, 4 mM $MgCl_2$ and 1 U *Taq* DNA polymerase. The optimum annealing temperature was determined for each primer (Table 2). PCR cycling conditions for all accessions were 2 min initial denaturation (94°C); followed by 40 cycles of 1 min at 94°C, 1 min at the specific annealing temperature, and 2 min at 72°C; ending with a final extension step of 10 min at 72°C. Amplified DNA fragments were separated in a 2% agarose gel at 100 W for 3 h in 1× TBE buffer (100 mM Tris–Borate, pH 8.0, 2 mM EDTA) and stained with ethidium bromide.

Data analysis

The polymorphic ISSR bands in each gel were scored as present (1) or absent (0). The cluster analysis and principal coordinate analysis (PCoA) were conducted by the software NTSYSpc Version 2.02 (Rohlf 1998). Polymorphic information content (PIC) was calculated by applying the simplified formula (Anderson et al., 1993): $PICi = 2fi\ (1 - fi)$, where fi is the percentage of the ith amplified band present. Genetic similarity among all accessions was calculated according to Simple Matching (SM) similarity index, using the similarity of qualitative data (Simqual) routine. The dendrogram was constructed using the unweighted pair group method average (UPGMA) clustering procedure. A Mantel test (Mantel, 1967) was used to detect the correlation between two dendrograms. The cophenetic correlation coefficient was generated by means of the COPH routine in order to check the goodness of fit between the clusters in the dendrogram and the similarity coefficient matrix. A bootstrap analysis of 100 replicates was employed using Winboot software (http://www.irri.org/science/software/winboot.asp). Gene diversity and analysis of molecular variance (AMOVA) were calculated among Iranian populations using Arlequin version 3 software.

RESULTS

Primer Selection and amplification

Fourteen primers were selected for the assessment of genetic relationships among yarrow accessions based on the number of amplification products, the quality of the profiles, the level of polymorphism, and the reproducibility of bands (Table 2). The selected primers generated 228 amplified fragments, with an average of 16.28 fragments per primer in the whole genotypes (Table 3). The size range of the amplified products was 200 to 2000 bp, and the number of products per primer varied from 6 in P22 [$(TCC)_5YR$)] to 23 in P25 [$(CA)_8VT$)]. The average percentage of polymorphic bands was 87.28%, which is

Table 1. List of yarrow accessions examined for polymorphism in present study.

Accession codes	Place of collection	Subspecies	Latitude	Longitude	Altitude (m)
Cwa1	Ganjname, Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2100
Cwa2	Ganjname, Hamadan , Iran	*millefolium*	34° 47'N	48° 30'E	2100
Cwa4	Ganjname, Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2100
Cwa5	Ganjname, Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2100
Cwa6	Ganjname, Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2100
Cwb1	Ganjname-b, Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2150
Cwb2	Ganjname-b , Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2150
Cwb3	Ganjname-b , Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2150
Cwb4	Ganjname-b , Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2150
Cwb5	Ganjname-b , Hamadan, Iran	*millefolium*	34° 47'N	48° 30'E	2150
Nk1	Kandovan, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	3013
Nk3	Kandovan, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	3013
Nk4	Kandovan, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	3013
Nk5	Kandovan, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	3013
Nk6	Kandovan, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	3013
Npz1	Pole-zangoole, Mazandaran, Iran	*elbursensis*	36° 7'N	51° 17'E	2820
Npz3	Pole-zangoole, Mazandaran, Iran	*elbursensis*	36° 7'N	51° 17'E	2820
Npz7	Pole-zangoole, Mazandaran, Iran	*elbursensis*	36° 7'N	51° 17'E	2820
Nsia1	Siahbishe, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	2950
Nsia2	Siahbishe, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	2950
Nsia12	Siahbishe, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	2950
Nsia13	Siahbishe, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	2950
Nsia23	Siahbishe, Mazandaran, Iran	*millefolium*	36° 7'N	51° 17'E	2950
Wsan1	Sanandaj, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
Wsan1	Sanandaj, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
Wsan2	Gardane khan, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
Wsan3	Gardane khan, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
Wsan4	Gardane khan, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
Wsan5	Gardane khan, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
Wsan6	Gardane khan, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
Wsan7	Gardane khan, Kordestan, Iran	*millefolium*	35° 18'N	47° 2'E	1750
NWor1	Orumieh, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1190
NWor3	Orumieh, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1190
NWor4	Orumieh, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1190
NWor5	Orumieh, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1190
NWor6	Orumieh, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1190
NWu2	Ghasemloo, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1650
NWu3	Ghasemloo, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1650
NWu4	Ghasemloo, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1650
NWu5	Ghasemloo, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1650
NWu6	Ghasemloo, Azarbaijan gharbi, Iran	*millefolium*	37°32'N	45°05'E	1650

comparable to some results in similar studies in Asteraceae family using ISSR markers (Petros et al. 2008; Sabzalian et al. 2009). The number of polymorphic bands per primer ranged from 4 to 21, with an average of 14.21 (Table 3). The average PIC value for the amplification products was 0.316 (Table 3). Primers P1 [$(CT)_8G$)] and P11 [$(CA)_8RT$)] showed the highest and lowest PIC values, respectively (Table 3). The banding pattern produced by primers anchored at the 3` end was more clear than those anchored at the 5` end. The higher clarity of banding pattern for 3` anchored primers is also reported in various studies (Pradeep Reddy et al., 2002; Rahimmalek et al., 2009b). In this research, the primers that had AC and AG motifs produced bands with higher

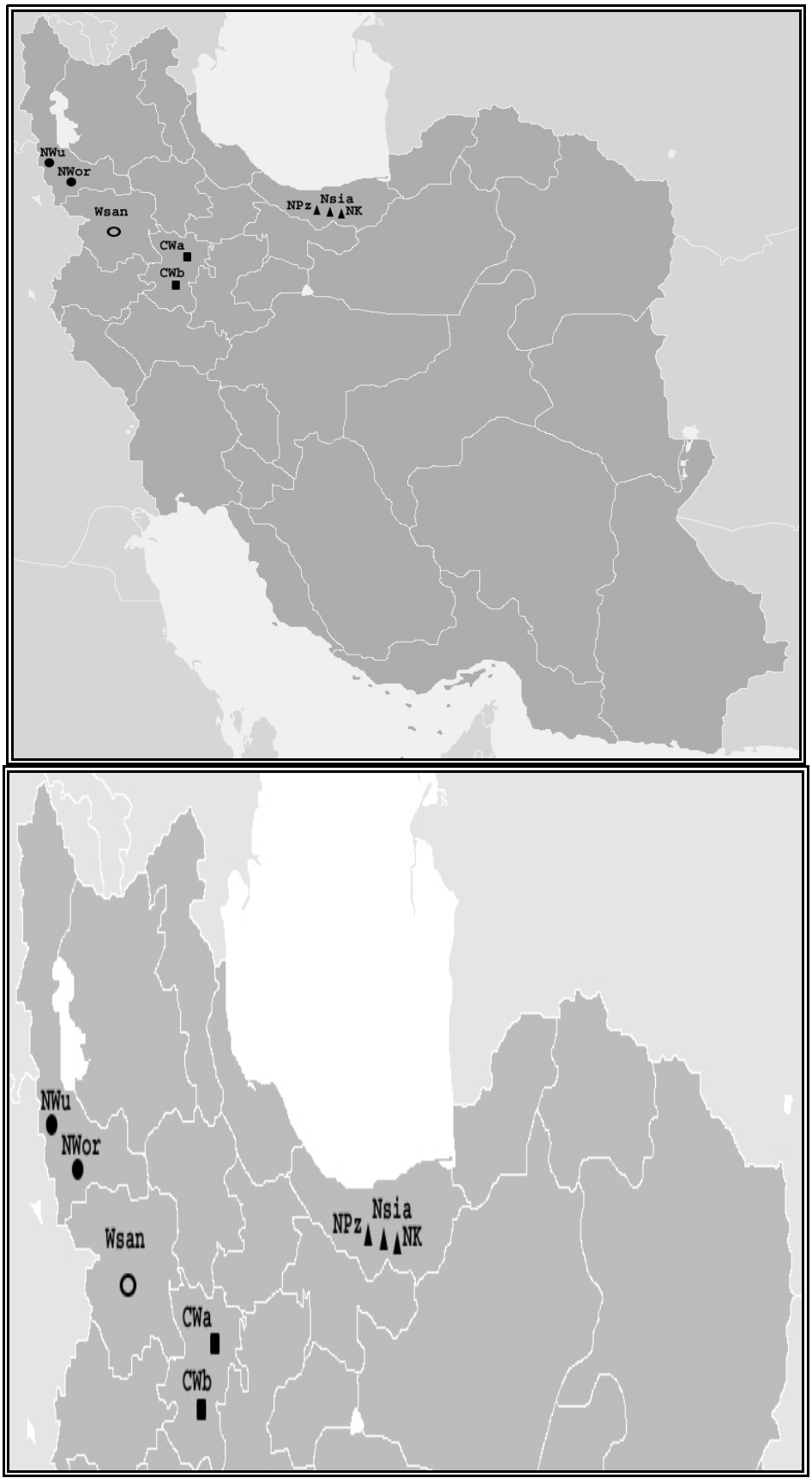

Figure 1. Geographical locations of *A. millefolium* population in Iran.

Table 2. Sequence and appropriate annealing temperature of ISSR primers used in this experiment.

Primer name	Motif	Sequence	Annealing temperature (°C)
P1	5′-(CT)8 G-3′	5′-CTCTCTCTCTCTCTCT G-3′	51
P2	5′-(CA)8 G-3′	5′- CACACACACACACACA G-3′	51
P3	5′-(TC)8 C-3′	5′- TCTCTCTCTCTCTCTCC-3′	51
P5	5′-(AC)8 G-3′	5′ -ACACACACACACACAC G-3′	52.5
P8	5′-(AC)8 YG-3′[a]	5′ -ACACACACACACACAC YG-3′	51
P9	5′-(AG)8 T-3′	5′ -AGAGAGAGAGAGAGAG T-3′	53
P11	5′-(CA)8 RT-3′[a]	5′- CACACACACACACACA RT-3′	48
P12	5′-(GA)8 T-3′	5′- GAGAGAGAGAGAGAGA T-3′	48
P15	5′-BDB (TCC)7-3′ [a]	5′-BDB TCCTCCTCCTCCTCCTCCTCC-3′	55
P16	5′-HVH (TCC)7-3′ [a]	5′- HVH TCCTCCTCCTCCTCCTCCTCC-3′	55
P22	5′-(TCC)5 YR-3′[a]	5′- TCCTCCTCCTCCTCC YR-3′	54
P24	5′-(GA)8 YG-3′	5′- GAGAGAGAGAGAGAGAYG-3′	52
P25	5′-(CA)8 VT-3′	5′- CACACACACACACACA VT-3	53
P26	5′-CCA (CT)8 -3′	5′-CCACTCTCTCTCTCTCTCT-3′	53

[a] Type of degenerate nucleotide: R = A/T, Y = G/C, B = T/G/C; D = A/T/G, H = A/T/C, V = 3A/G/C.

Table 3. Summary of ISSR primers characteristics in different *A. millefolium* accessions.

Primer name	Number of scorable bands	Number of polymorphic bands	Polymorphisms (%)	PIC/primer
P1	21	20	95.23	0.200
P2	20	19	95	0.360
P3	17	15	88.23	0.218
P5	17	15	88.23	0.308
P8	12	10	83.33	0.307
P9	19	15	78.94	0.327
P11	14	13	92.8	0.405
P12	14	13	92.8	0.375
P15	23	21	91.3	0.208
P16	11	8	72.8	0.331
P22	6	4	66.66	0.396
P24	13	10	76.92	0.339
P25	23	22	95.65	0.309
P26	17	14	82.35	0.353
Total	199	228	-	
Average	14.2	16.2	87.28	0.316

resolution than those with other motifs. This was similar to findings of other researchers (Blair et al., 1999; McGregor et al., 2000).

Genetic diversity analysis

A dendrogram generated by the SM similarity matrix and UPGMA method revealed genetic relationships among yarrow genotypes (Figure 2). A high cophenetic correlation coefficient of 0.91 between the Jaccard similarity data matrix and the cophenetic matrix was obtained, indicating a good fit between the dendrogram clusters and the similarity matrices. The dendrogram revealed three groups. Group 1 included the Central-west populations (CWa and CWb). Group 2 consisted of three populations ((Nsia, Nk and Npz)) from Northern regions of the country. Of the three populations in this group one (Npz) belonged to *A. millefolium* subspecies*. elbursensis* and the other two (Nsia and Nk) belonged to *A millefolium* subsp*. millefolium*. Group 3 comprised of two populations (Nwor and Nwu) from NW and one population

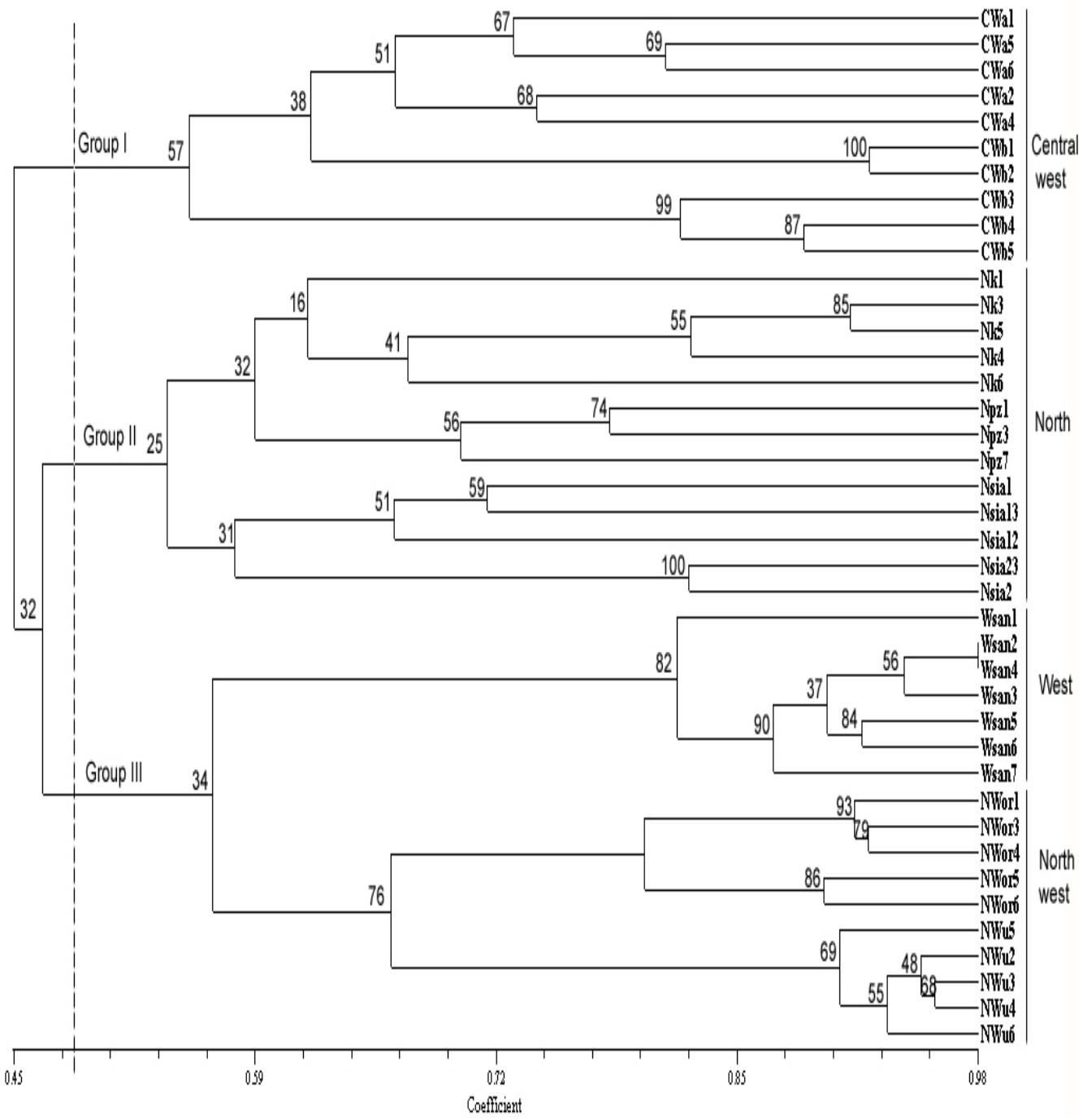

Figure 2. UPGMA dendrogram based on Jaccard similarity coefficient among *A. millefolium* accessions collected from different geographical regions of Iran. Bootstrap analysis was conducted using Winboot software with 100 bootstrap sub-samples of the data matrix.

(Wsan) from W which was grouped together (Figure 2).

Principle Coordinate Analysis (PCoA) was performed to visualize the association among accessions in more details (Figure 3). The results showed that the first three principal coordinates explained 66.8% of the total variation. The results of PCoA analysis in most cases corresponded to those obtained through cluster analysis but classified genotypes in two groups. Group 1 contained genotypes from Northern (N) and West-Central (CW) ones together, while the NW and W populations were classified with each other. The similarity matrix for cluster analysis confirmed the more similarity of CW

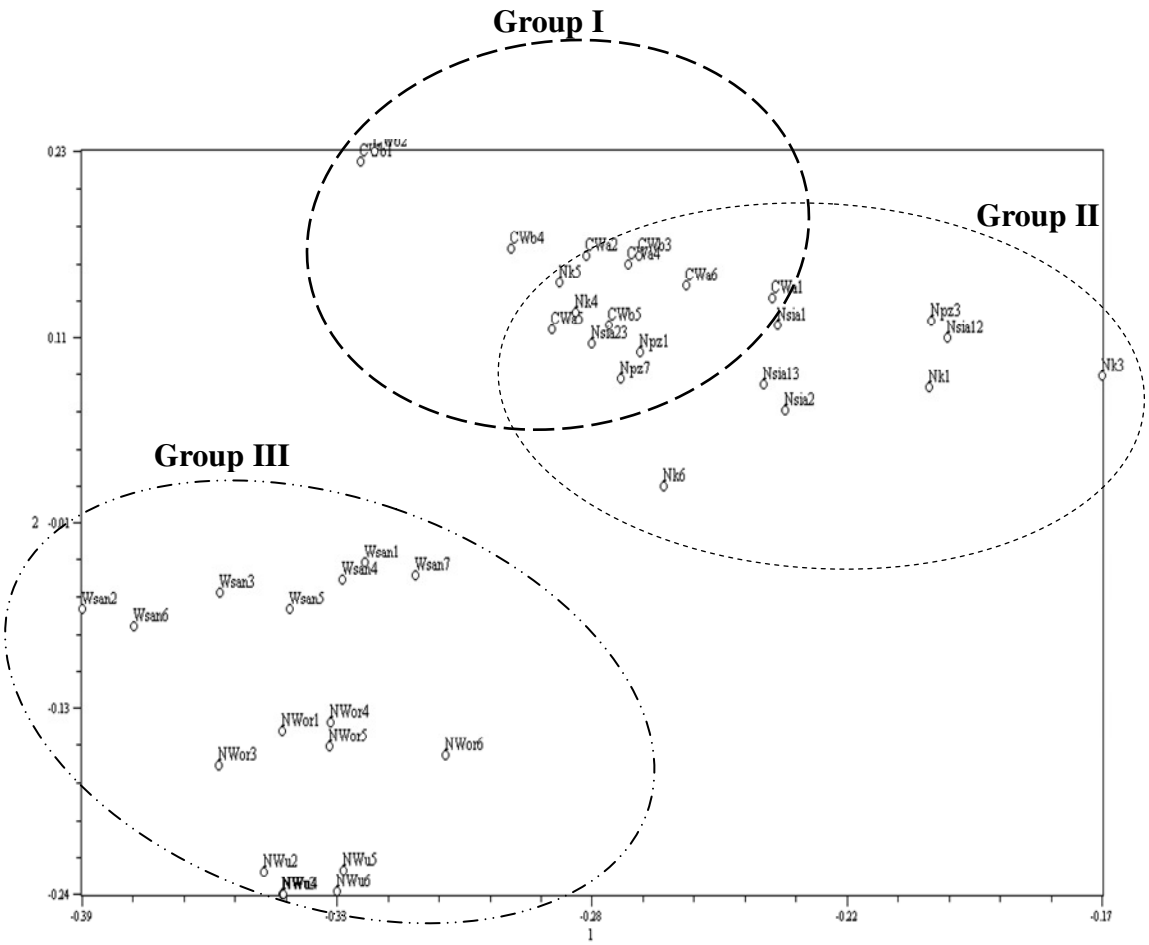

Figure 3. Patterns of relationships among *A. millefolium* accessions revealed by PCoA based on ISSR data.

populations with N populations as it has shown in PCoA analysis.

Genetic structure

Genetic distances among eight populations showed that the minimum gene diversity over loci was observed in NWu population (0.021± 0.015), while the highest gene diversity was found in Nk (0.129± 0.08) population (Table 4). In this study, genotypes belonging to W and NW (Wsan, NWor and NWu), N (Nsia, Nk, Npz) and CW (CWa, CWb) regions of Iran were assumed as three major groups for AMOVA analysis. Genetic differentiation based on pairwise Φ_{ST} comparisons of genotypes ranged from 0.294 (the least distance between Nk and Nsia genotypes) to 0.929 (the highest distance between Npz and NWu genotypes). All of the genetic distances in the matrix were significantly different ($P<0.05$). About 41% of total genetic variation was detected among the assumed groups, while 26.5% of total variation observed among populations within groups (Table 5). AMOVA analysis among the specified geographic regions showed that the majority of genetic variance was found among geographical groups and relatively low variance was detected within population (Table 5).

Morphological results

The mean, Maximum, Minimum and coefficient of phenotypic genetic variation for each trait is summarized in Table 6. A dendrogram generated using Ward's method revealed relationships among yarrow genotypes according to their morphologic characteristics. The morphologic analysis confirmed the molecular results in most cases. As a result, the samples were classified into two major groups (Figure 4). Group 1 consisted of two subgroups. Similar to molecular analyses populations from NW and W of Iran were grouped together in Group 1 (Figure 4). In Group 2, the N populations were classified with CW populations. The comparison of molecular and morphological analyses showed that Central-West populations (CWa and CWb) were more similar to

Table 4. Basic gene diversity information of eight yarrow population in Iran.

Statistics	CWa	CWb	Nk	Npz	Nsia	Wsan	NWor	NWur	Mean	sd
No. of gene copies	5	5	5	3	5	5	7	5	5	1.00
No. of loci	199	199	199	199	199	199	199	199	199	0.00
No. of usable loci	122	170	48	110	98	107	140	175	121.25	38.49
No. of polymorphic. loci	27	41	13	19	25	8	16	11	20	10.03
Expected heterozygosity	0.114±0.07	0.127±0.07	0.129± 0.08	0.115±0.08	0.122±0.07	0.029±0.02	0.053±0.03	0.0.26±0.01	0.089±0.04	-
Average gene diversity over loci	0.161	0.146	0.14	0.15	0.20	0.058	0.07	0.01	0.125	-

Table 5. Analysis of Molecular Variance among and within eight Populations of Iranian yarrow classified in three major groups.

Source of variation	d.f	Sum of squares	Mean of Squares	Variance component	Percentage of variation	p-value
Among groups	2	65.828	32.91	1.62694	32.55	<0.001
Among populations within groups	5	57.047	11.54	2.04650	40.94	<0.001
Within populations	32	42.400	1.32	1.32500	26.51	<0.001
Total	39	165.27	42.37	4.99844		

Table 6. The mean, Maximum, Minimum and coefficient of phenotypic genetic variation among yarrow accessions.

Trait	Mean	Max/Genotype	Min/Genotype	Phenotypic genetic variation (%)
Plant height	30.81	41 (Nk6)	20 (NW0r3)	17.25
Number of lateral shoots	.857	2.6 (Nk5)	0 (NWu5)	95
Leaf length	9.65	15.5 (Nsia23)	2.4 (Nk1)	36
Leaf width	1.11	1.8 (CWb4)	0.5 (Npz7)	39
Inflorescence length	2.85	4.85 (CWa2)	1.2 (NWor5)	25
Flower diameter	3.22	4.3(CWa1)	1.6 (Npz3)	35
width of ligulae flowers	0.56	.73 (NK5)	0.3 (Wsan5)	21
Length of ligulae flowers	0.81	1.8 (NWu3)	0.55(Wsan6)	27
Day to flowering	62.75	93 (Nsia1)	37 (Nwor1)	33
Day to 50% flowering	84.15	105 (Nsia1)	67 (Nwu4)	16
Day to 100% flowering	101.42	120 (Nsia1)	76(Nwu4)	14
Number of florets in inflorescence	68.35	115 (CWa4)	30 (Nsia12)	39

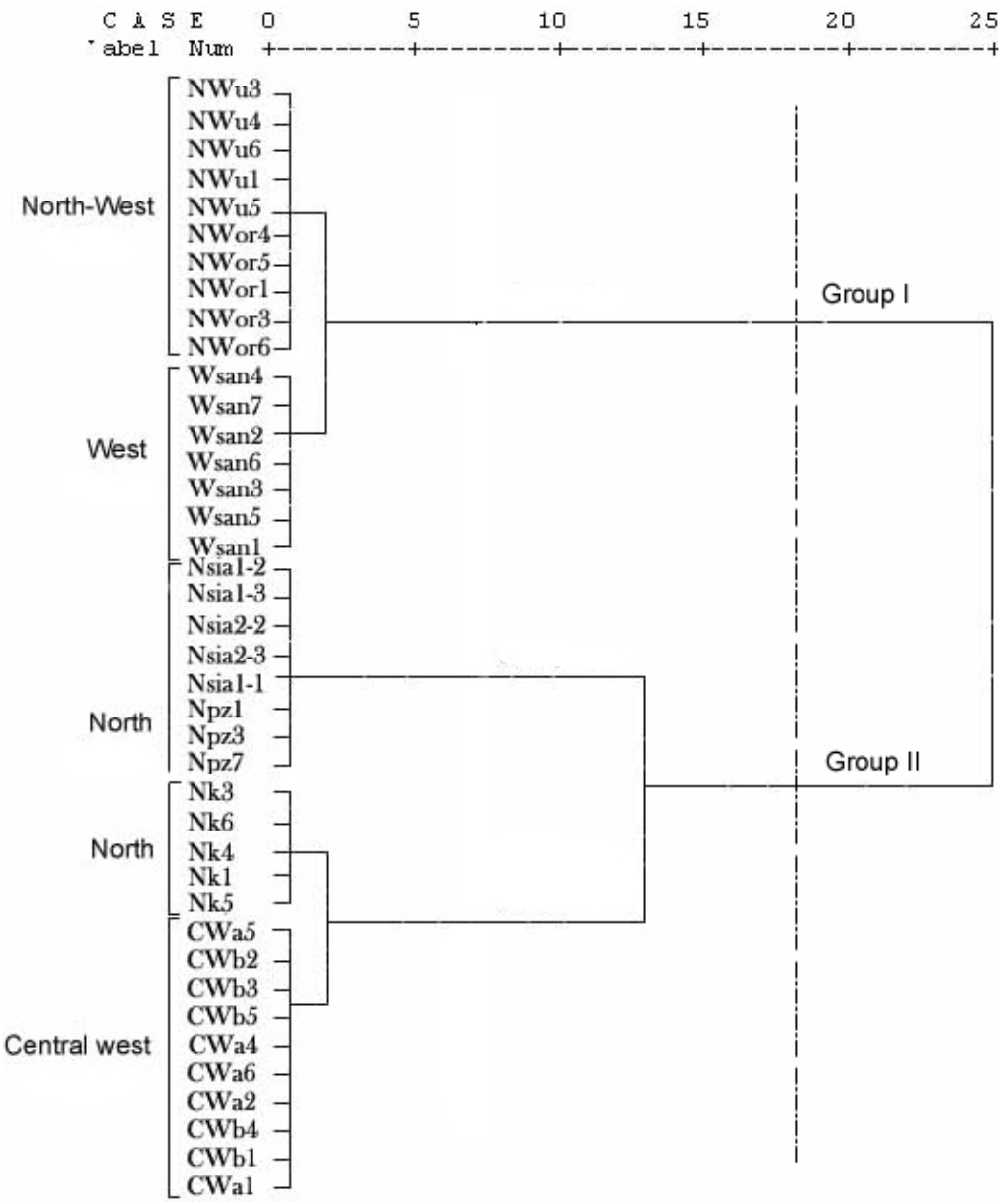

Figure 4. Morphological dendrogram based on Ward's method among *A. millefolium* accessions collected from different geographical regions of Iran.

Northern populations.

DISCUSSION

A relationship between genetic variability and geographic distribution has been observed in several species of aromatic plants of Anthemideae sub-family including *Artemisia annua* (Sangwan et al., 1999), *Tanacetum vulgare* (Keskitalo et al., 2001) and five *Achillea* species (Rahimmalek et al., 2009c). Similarly, in the present study, yarrow populations of Iran were grouped according to their geographical distribution. One probable reason for this might be the existence of Zagros (extended from NW to SW) and Alborz (extended in northern region) mountains in the country that has separated these populations during their course of evolution.

Several factors, such as population size, breeding system, pollinators and phylogenetic position, are important in explaining genetic diversity within species and the apportionment of the diversity among populations of species (Hamrick and Godt, 1996; Kim et al., 2005). In addition to determining the levels of diversity within species and populations, knowing how diversity is

apportioned within and among populations of a species is useful in formulating strategies for conserving diversity within taxa (Kim et al., 2005). This study indicated that the majority of genetic diversity of *A. millefolium* in Iran contributes to the diversity among populations within assumed groups. Among populations, NW (Nwor and NWu) and W (Wsan) had the lowest levels of genetic diversity (Table 4). The N (Nk, Nsia and Npz) and CW (CWa and CWb) populations had relatively higher genetic diversity in comparison with other populations.

The extensive genetic differentiation among yarrow populations that belonged to different geographical regions could be interpreted as a consequence of the fragmented geographical distribution (Figure 1). *A. millefolium* possesses no specialized mechanism for long-distance gene dispersal. Assuming insect pollination and seed dispersal by different factors, gene migration seems to be rare across such long distances (Lofgren, 2002).

The clustering (Figure 2) and the PCoA analyses (Figure 3) showed distinct grouping of populations from the same geographical regions together, supporting the idea of a limited gene flow due to fragmentation of the geographical range in this species. Other probable factors that may have played a role in high level of differentiation in some populations are breeding system and genetic drift (Ge et al., 2005). Reduced levels of genetic variation, especially in the smaller populations, will affect the species ability to adapt to changes in its habitat (Zong et al., 2008).

Considering the low genetic variation of small populations in the restricted regions, enhancing seed germination or vegetative propagation (using rhizome) can efficiently improve the spread of some populations. Lofgren (2002) reported that vegetative propagation in *A. millefolium* is relatively more common in comparison with reproductive propagation in the most habitats. So, it seems necessary to use conservation strategies to prevent such problems. For example, the limitation of grazing and plowing in special seasons might be effective for improving seed dispersal or vegetative growth of some populations.

In this study, geographical classification was more important than grouping according to subspecies. For instance, *A. millefolium* subsp. *elbursensis* and *A. millefolium* subsp. *millefolium* from the Northern regions of Iran were classified in the same group. It might be possible that some endemic subspecies of yarrow, such as *A. millefolium* subsp. *elbursensis* (NPz1, NPz3 and NPz7) have evolved along with another subspecies in a limited geographical region (eg. Alborz Mountains) and environmental barriers have limited their spared to other regions.

Morphological data in most cases confirmed the results of molecular analyses. Similar to molecular analysis, morphological data did not show the distinct separation of two subspecies in the cluster (Figure 4). On the other hand, in morphological analysis, the Central-west accessions had more similarity to Northern populations (Figure 4). It might be due to environmental variation in different geographical regions, which influence the morphological classification. Gurevitch (1992) reported that the environmental factors, such as temperature and altitude can affect some morphological characters, such as the size and compactness of leaves in yarrow. So, high phenotypic variation in some morphological traits could confirm the high genetic variation due to different geographical locations (Table 6).

In conclusion, the present study revealed the genetic variability among eight populations of Iranian yarrow. The results of this study may help to understand the genetic variation, genetic structure and evolutionary dynamics of yarrow with possible applications in breeding programs. The N and CW populations of Iran were found to be more diverse, and some locations in these regions may be good candidate sites for *in situ* conservation. Furthermore, selection of the diverse populations maybe beneficial to introduce new compounds for pharmaceuticals studies.

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