E3 Journal of Biotechnology and Pharmaceutical Research Vol. 3(4), pp. 69-73, June 2012
Available online at http://www.e3journals.org/JBPR
ISSN 2141-7474 © 2012 E3 Journals

Full Length Research Paper

Genetic relationships among yarrow based on Random Amplified Polymorphic DNA markers

Mohsen Ebrahimi[*1], Mostafa Farajpour[1], Mina Beigmohamadi[2] and Mehdi Ebrahimi[1]

[1]Department of Agronomy and Plant Breeding, Collage of Abouraihan, University of Tehran, Tehran Iran.
[2]Institute of Biotechnology of Urmia University, Urmia, Iran.

Accepted 20 April, 2012

The genetic relationship among 37 Achillea millefolium accessions was studied using Random Amplified Polymorphic DNA (RAPD) markers. Nine RAPD primers were used for the estimation of genetic diversity. All of the 9 primers were polymorphic. Nine RAPD primers generated 118 amplified fragments, most of which were polymorphic. The number of polymorphic bands produced in the 37 yarrow accessions range from 8 to 19. Primer g11 Produced 19 polymorphic bands, while primers e3 produced 8 polymorphic bands. The jaccards similarity indices (J), based on RAPD profiles, were subjected to complete linkage analysis. The highest genetic similarity was observed between Am4 and Am22 (81%), whereas the lowest value was found between Am33 and Am12 (18%). The dendrogram generated revealed five groups. The principle component analysis (PCA) indicated that first three principal component account for more than 62% of the total variation. This study confirms the efficacy of RAPD markers for the identification of plant genotypes. This information should be helpful for breeding and genome mapping programs.

Key word: Genetic diversity, *Achillea milefolium*, RAPD, PCA

INTRODUCTION

Yarrow (*Achillea milefolium*) is an important medicinal plant with different pharmaceutical uses (Gharibi et al., 2011). It is one of the youngest evolutionary genera of the Asteracea family, which is present throughout the world (Farajpour et al., 2011). More than 100 species have been recognized in this genus (Goli et al., 2008). These plants are native to Europe and western Asia but are also found in Australia, Newzeland and North America (Rechinger, 1963). Two major subspecies A.M. millefolium and A.M. elbursensis are recognized in Iran (Rechinger, 1963). RAPD markers (Williams et al., 1990) 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 (Manica-Cattani et al., 2009; Nan et al. ,2003; Fracaro et al., 2005; Mattioni et al., 2002). However, there are limited reports on the genetic relationships of *Achillea millefolium*. Wallner et al. (1996) assessed the stability of two *Achillea* species during micropropagation using RAPD markers. Rahimmalek et al. (2009) studied inter and intra genetic diversity of *Achillea* species using AFLP markers. Gharibi et al (2011) studied genetic diversity between two *Achillea milefolium* subsp (*millefolium* and *elbursensis*) using ISSR markers. Furthermore, there are no reports on the application of RAPD markers on genetic studies of yarrow. Objective of present study was identification of genetic diversity among *Achillea millefolium genotypes* using RAPD marker.

*Corresponding author. Email: Mebrahimi@ut.ac.ir. Tel: +982923040615. Fax: +982923040615

Abbreviation: Random Amplified Polymorphic DNA (**RAPD**), Principle Component Analysis (**PCA**), Amplified Fragment Length Polymorphism (**AFLP**)

Table 1. Numbers of polymorphic bands of RAPD primers used for 37 A. millefolium accessions.

Primer	Sequence	Annealing Temp	N° of bands	N° Polymorphic bands	Polymorphic (%)
g11	TGCCCGTCGT	32	20	19	95
m2	ACAACGCCTC	32	11	11	100
m3	GGGGGATGAG	32	11	9	81
a2	TGCCGAGCTG	33	15	14	93
a10	GTGATCGCAG	30	12	9	75
e3	CCAGATGCAC	32	9	8	89
f19	CCTCTAGACC	32	13	12	92
b10	CTGCTGGGAC	33	14	11	78
b11	GTAGACCCGT	31	13	10	77
Total			118	103	
average			13.11	11.44	87

MATERIALS AND METHODS

Samples obtained from gene bank of Research institute of Forests and Rangelands in Tehran. These seeds were collected from north, northwestern, western, southern and central region of Iran. Young leaves were transported to the laboratory and stored in a -80◦C freezer for subsequent analysis. DNA from young leaves was extracted using the modified CTAB Procedure as described by Murray and Thompson (1980). DNA concentration and quality was estimated electrophoretically and spectrophotometrically. The amplification conditions for RAPD were an initial step of 5 min at 94° C followed by 39 cycles of 1 min at 94° C for denaturing 45 s at 30 to 33° (Table 1) for annealing, and 2 min at 72° C for extension and a final extension of 5 min at 72° C. Amplification products were resolved by electrophoresis in 1.2% ($w.v^{-1}$) agarose gels in TBE buffer. DNA fragments were stained with ethidium bromide and digitalized under UV light for further analysis. RAPD amplicon was scored for presence or absence in each accession, and the data were entered into a binary matrix as discrete variables (1 presence and 0 absences). Genetic similarities for RAPD data was calculated by using the jaccard similarity index, according to Sneath and Sokal (1973). Dendrograms showing genetic relationships of the 37 genotypes were constructed using the complete linkage. Mantel test (Mantel, 1967) was used to detect the correlation between the two dendrograms. The cophenetic correlation coefficient was generated by means of a COPH algorithm to check the goodness of fit between the cluster in the dendrogram and the similarity coefficient matrix. The cluster and PCA analyses were conducted using the software NTSYSpc version 2.02 (Rohlf, 1998).

RESULTS AND DISCUSSION

The RAPD analysis, a total of 118 bands were detected, among which 103 bands (87%). Were polymorphic with the mean of 11.4 per primer (Table 1). For each primer, the number of bands ranged from 9 to 20, with an average of 13.1. The number of polymorphic bands produced in the 37 yarrow accessions range from 8 to 19. Primer g11 Produced 19 polymorphic bands, while primers e3 produced 8 polymorphic bands. Mantel analysis between the Jaccard similarity coefficients calculated based on the presence or absence of RAPD marker showed that they were low correlated (r=0.53), indicating a low fitness between the dendrogram clusters and the similarity matrices. The highest genetic similarity was observed between Am4 and Am22 (81%), whereas the lowest value was found between Am33 and Am12. According to the dendrogram, five main groups were produced (Figure 1). Group1 was composed of the *Achillea millefolium* subsps *elbursensis*, this subsps is endemic of north of Iran. The mean of essential oil obtained from dried plants of this subsps was more than the other genotypes (0.31%cc) (data are not shown). This cluster was divided into two subgroups. The first subgroup was genotypes (Am36, Am33, Am35 and Am37). The second subgroup accessions (Am32 and Am34) belonged to the Golestan province (Table 2). Group 2 consists of twelve genotypes which were gathered from north and western part of the country. This cluster was divided into three subgroups. The first subgroup consists of Am18, Am21 and Am14 which were gathered from the north of Iran. Am21 was gathered from Golestan province while the others were collected from Gilan province. Second subgroup consists of Am2, Am13, Am12 which were gathered from north and west of the Country. Subgroup3 consists of Am26 to Am31. The average of essential oil yield of dried plants of these genotypes was 0.22% cc (data are not shown). Group3 was two prototypes (Am11 and Am8). Am11 was collected from the center of the Country (Arak). Genotypes of Group4 were gathered from the west and center of the Country. This cluster was divided into two subgroups. The subgroup I consisted of genotypes (Am1, Am25 and Am15) which were collected from Isfahan and Markazi provinces. The second subgroup consisted of Am6, Am9 and Am19; all of these genotypes were collected from Kurdistan province. The second subgroup consisted of genotypes (Am4, Am7, Am10, Am22 and

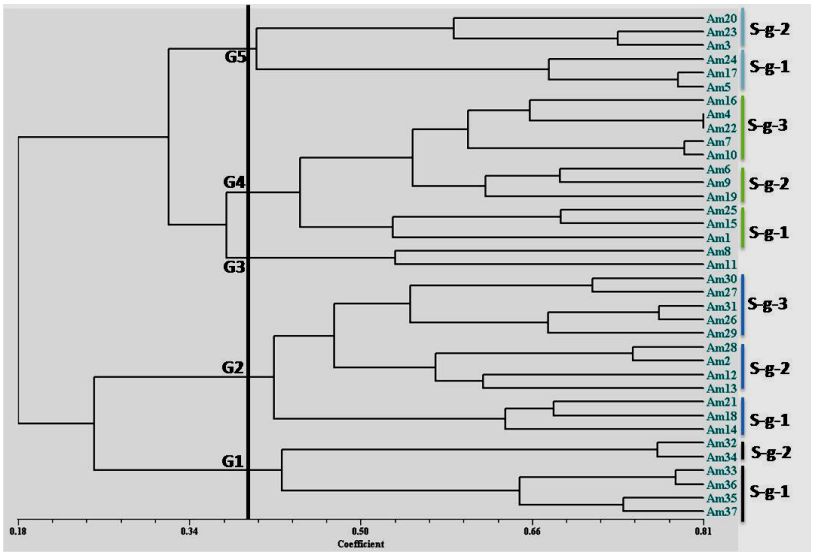

Figure 1. Dendrogram of 37 *Achillea millefolium* accessions (Am1-Am37) based on RAPD data using complete linkage method.

Table 2. List of the *Achillea millefolium* genotypes included in this study.

Genotype	Province	city	Genotype	Province	city
Am1	Markazi	Arak	Am20	Hamadan	Hamadan
Am2	Golestan	Gorgan	Am21	Golestan	Minodasht
Am3	Hamadan	Hamadan	Am22	Lorestan	Khoramabad
Am4	Lorestan	Azna	Am23	Isfahan	Chadegan
Am5	Fars	Estahban	Am24	Fars	Shiraz
Am6	Kordestan	Sanandaj	Am25	Isfahan	Kashan
Am7	Lorestan	Brojerd	Am26	Kordestan	Sanandaj
Am8	Isfahan	Samirom	Am27	Tehran	Taleghan
Am9	Kordestan	Marivan	Am28	Golestan	Ramyan
Am10	Kordestan	Kamyaran	Am29	Gilan	Siahkal
Am11	Markazi	ARAK	Am30	Ilam	Ilam
Am12	Lorestan	aligodarz	Am31	Golestan	Aliabad
Am13	Gilan	Rodsar	Am32	Golestan	Gorgan
Am14	Gilan	Siahkal	Am33	Gilan	Talesh
Am15	Markazi	Tafresh	Am34	Golestan	Ramyan
Am16	Kordestan	Bane	Am35	Golestan	Minodasht
Am17	Fars	Abadeh	Am36	Tehran	Damavand
Am18	Gilan	Talesh	Am37	Ghom	Jafarabad
Am19	Kordestan	Bijar	**-**	-	-

Am16) gathered from Lorestan province (except Am16). Group5 included six genotypes. This cluster was divided into two subgroups. The first subgroup consisted of three accessions (Am5, Am17 and Am24). These genotypes were collected from Fars province. The second subgroup consisted of Am20, Am3 and Am23 which is being planted in Hamadan in small scale and used for medical purposes (except Am23). The average of essential oil yield of dried plants of Am1-Am25 genotypes was 0.13% (data are not shown). The results of the clustering showed that *Achillea millefolium* subsps *elbursensis* is separated from other genotypes in the dendrogram

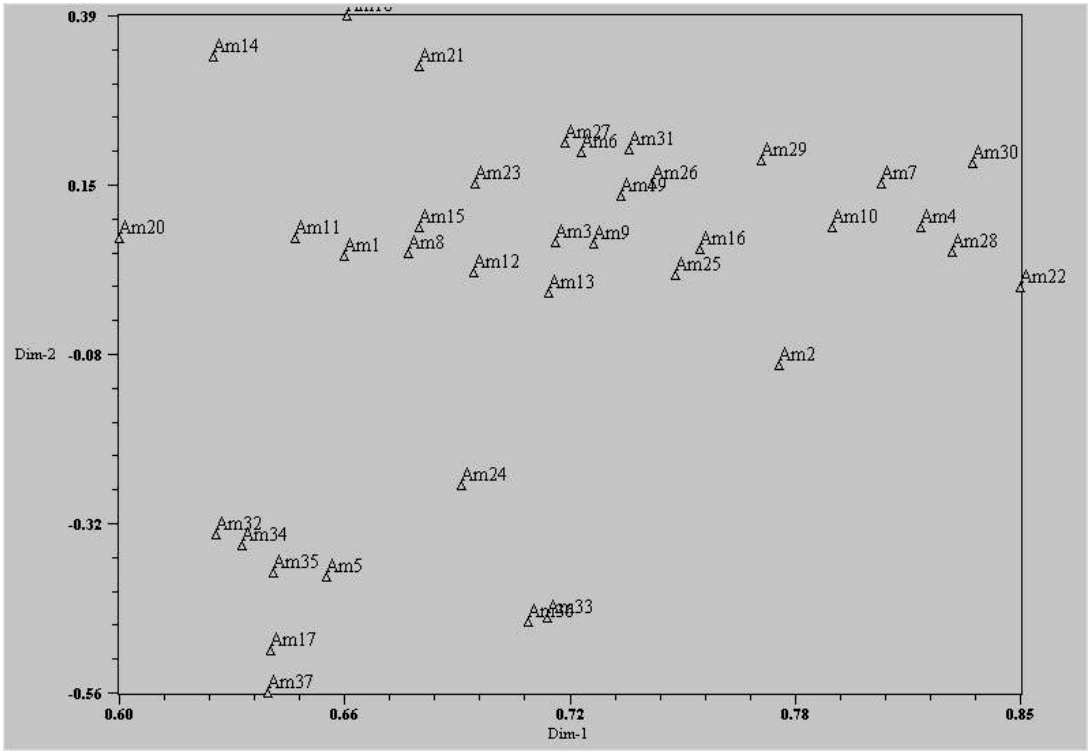

Figure 2. Patterns of relationships among 37*Achillea millefolium* accessions revealed by principal component analysis (PCA) based on RAPD data.

(Figure 1). Some of the traits features of these genotypes, such as plant aroma, rhizome propagation and leaf shape differed from others. The principle component analysis (PCA) indicated that first three principal component account for more than 62% of the total variation. The results of PCA analysis plot indicated that subsps *elbursensis* genotypes were separated from the other *A. millefolium* accessions corresponded closely with the cluster analysis results (Figure 2). This study confirms the efficacy of RAPD markers for the identification of plant genotypes. Similar finding have proved the successful application of RAPD markers for estimation of genetic variability (Rand and Bhat, 2005; Khan et al., 2010; Hussein et al., 2007).This information should be helpful for breeding and genome mapping programs.

Conclusion

In the present study, RAPD markers provided a comprehensive insight into the genetic variation of the Iranian Achillea millefolium species. In addition, the results show that the RAPD markers efficiently identified Achillea millefolium genotypes, thus allowing the characterization of the accessions under study.

ACKNOWLEDGEMENTS

This study was supported by Forests, range and watershed management organization of Iran and university of Tehran.

REFERENCE

Farajpour M, Ebrahimi M, Amiri R, Noori SAS, Golzari R (2011). Investigation of variations of the essential oil content and morphological values in yarrow (Achillea santolina) from Iran. J. Med. Plants Res. 5:4393-4395.

Fracaro F, Zacaria J, Echeverrigaray S (2005). RAPD based genetic relationships between populations of three chemotypes of *Cunila galioides* Benth. Biochem. Sys. Ecol,. 33: 409-417.

Gharibi S, Rahimmalek M, Mirlohi A, Majidi MM, Tabatabaei BES (2011). Assessment of genetic diversity in *Achillea millefolium* subsp. *millefolium* and *Achillea millefolium* subsp. *elbursensis* using morphological and ISSR markers. J. Med. Plants Res., 5(11): 2413-2413.

Goli SAH, Rahimmalek M, Sayed TBE (2008). Characteristics and fatty acid profile of yarrow (*Achillea tenuifolia*) seed oil. Int. J. Agric. Biol., 10:355-357.

Khan AI, Khan IA, Awan FS, Sadaqat HA, Bahadur S (2010). Estimation of genetic distance based on RAPDs between 11 cotton accessions varying in heat tolerance.

Genet. Mol. Res., 10(1): 96-101.
Manica-Cattani MF, zacaria J, Pauletti G, Atti-Serafini L, Echeverrigaray S (2009). Genetic variation among South Brazilian accessions of *Lippia alba Mill*. (Verbenaceae) detected by ISSR and RAPD markers. Braz. J. Biol. 69, 375-380.
Mantel NA (1967). The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209–220.
Mattioni C, Casasoli M, Gonzalez M and IPINZA R (2002). Comparison of ISSR and RAPD markers to characterize three Chilean Nothofagus species. Theor. Appl. Genet., 104: 1064-1070.
Murray M, Thompson WF(1980). Rapid isolation of high molecular weight plant DNA. Nucl. Acid. Res., 8: 4321-4325.
Nan P, Peng S, Shi S, Ren H, Yang J, Zhong Y (2003). Interpopulation congruence in Chinese Primula ovalifolia revaled by chemical and molecular markers using essential oils and ISSRs. Zeitschrift für Naturforschung. J. Biosci., 58: 57-61.
Rahimmalek M, Sayed TBE, Arzani A, Etemadi N (2009). Assessment of genetic diversity among and within *Achillea* species using amplified fragment length polymorphism (AFLP), Biochem. Syst. Ecol. 37: 354-361.
Rand KR, Bhat KV (2005). RAPD markers for genetic diversity study among Indian cotton cultivars. Curr. Sci. 88: 1956-1961.
Rechinger KH (1963). Flora Iranica. No. 158. Akademische Druke-U. Verlagsanstalt, Wien, Austria. pp. 49-71.
Rohlf FJ (1998). NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, Version 2.00. Exeter Software, Setauket, New York.
Sneath PHA, Sokal RR (1973). Numerical taxonomy. San Francisco: Freeman.
Wallner E, Weising K, Rompf R, Kahl G, Kopp B (1996). Oligonucleotide fingerprinting and RAPD analysis of *Achillea* species: characterization and longterm monitoring of micropropagated clones. Plant. Cell. Rep. 15, 647-652.
Williams JGK, Kubelik AR, Livak KL, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acides Res., 18(22): 6531-6535.