African Journal of Biotechnology Vol. 11(43), pp. 10056-10062, 29 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2517
ISSN 1684–5315 © 2012 Academic Journals

*Full Length Research Paper*

# Investigation of genetic diversity in flixweed (*Descurainia sophia*) germplasm from Kerman province using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) molecular markers

**Kian Aghaabasi[1] and Amin Baghizadeh[2]***

[1]Department of Biotechnology, Kerman Graduate University of Technology, Kerman, Iran.
[2]International Center for Science, High Technology and Environmental Sciences, Kerman, Iran.

Accepted 20 April, 2012

**Flixweed plant bearing the scientific title of *'Descurainia sophia'* is recognized across the planet as well as in Iran, as an herbal medicine. The present study investigates the genetic diversity of germplasm of flixweed in Kerman Province using and inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. Flixweed seeds were initially collected from 15 fields in different regions of Kerman, and the DNA were extracted from the seeds utilizing cetyltrimethylammonium bromide (CTAB) method with slight alterations and elongation of DNA washing stages. The quantity and quality of the extracted DNA was measured using electrophoresis and Scandrop devices. 20 RAPD primers were utilized for higher band and relatively lower costs; 16 primers which had produced more lucid bands in polymerase chain reaction (PCR) as well as four ISSR primers in which band differentiation was thoroughly distinguishable were used for the analysis. The number of 146 polymorphism bands was obtained from the mentioned initial numbers and the scoring process was carried out. The obtained 0 to 1 matrix, employing NTSYS-pc, was turned into similarity matrix using Dice similarity coefficient and subsequently the dandrogram was drawn using un-weighted pair group method with arithmetic mean (UPGMA). With regard to the results, the 15 selected regions were categorized into four separate groups. Principle component analysis (PCA) was performed of which two and three dimensions graphs using 20 primers were drawn. Finally, the results show that RAPD and ISSR markers maintained desirable distinguishing power in determining the genetic diversity and family relations of the mass under discussion.**

**Key words:** Kerman, flixweed, random amplified polymorphic DNA**,** inter-simple sequence repeat markers, genetic diversity.

## INTRODUCTION

Flixweed is a wild plant by the scientific name of '*Descurainia sophia* (L.) Webb' or the similar name of '*Sisymbrium Sophia* L.' from Brassicaceae family, the height of which may even reach 1 m (Zargai, 1997). The silique fruit is of 15 to 35 mm length and 1 mm width which are vertically stationed inside the silique cavity of the seeds.

Flixweed seed is very small and dark yellow or brown bearing an uneven surface in a stretched oval form, one end of which is cut and maintains a transparent yellowish ring (Amin, 2001; Daryaei, 2007). Flixweed is native to southern Europe, Asia, South Africa, South America and New Zealand (Daryaei, 2007). With regard to its propagation, flixweed wildly grows in the west, north

*Corresponding author. E-mail: amin_4156@yahoo.com.

**Abbreviations: ISSR,** Inter-simple sequence repeat; **RAPD,** random amplified polymorphic DNA; **CTAB,** cetyltrimethylammonium bromide; **UPGMA,** un-weighted pair group method with arithmetic mean; **PCA,** principle component analysis.

(Amol), center (Tehran, Karaj, Yazd), and south of Iran (Kerman, Fars) in non-agronomical and fairly humid regions (Afshar, 1991; Dublin et al., 2000).

Studies indicate that consumption of flixweed in women during pregnancy leads to more successful deliveries and less instances of elongated insemination, delivery and pregnancy (Haji Sharifi, 2003). Flixweed seeds are used in China as laxative and antipyretic, and for the alleviation of skin inflammations such as hives. Flowers and leaves of flixweed are utilized as astringent as well as obviator of vitamin C deficiency. Flixweed seeds are also used for the excretion of ascarid and renal calculus. According to the Iranian traditional medicine, flixweed is of a warm and moist nature and bears properties such as stomach strengthening, alleviating of hoarseness, lightening complexion, as well as appetizer and as a treatment for measles and scarlatina (Mirheydar, 1997; Daryaei, 2007). Boiled flixweed is also used as treatment for chronic bronchitis, excretion of chest mucous and treatment of diarrhea (Amini, 1995; Dublin et al., 2000).

Linoleic acid, palmitic acid and oleic acid are among important fatty acids extant in flixweed seed. Compositions such as bansil, isothiocyanate, aldisulphates, etc. are found in the oily essence obtained from flixweed seed through distillation with vapor (Shokouhinejad, 1994). The obtained essences of fixweed in different parts of the world and even Iran are disparate regarding environmental and genetic conditions. The most of medicinal plants are still harvested from natural fields in Iran and no agronomical breed of them has yet been introduced. This may eventually lead to the gradual deterioration of desirable germplasms and our deprivation of breed items with higher quality as well as quantity. Molecular biological methods have nowadays proven most helpful in the recognition of plant. Analysis of the genetic diversity is a prerequisite to the recognition of plants through molecular methods (Farsi and Zolala, 2003). Extensive studies have been carried out aiming at investigating genetic diversity of plants using molecular markers especially polymerase chain reaction (PCR)-based DNA markers.

Random amplified polymorphic DNA (RAPD) is among the said markers; RAPD maintains advantages such as lower need for genomic DNA in each analysis, no need for prior knowledge of sequences, facility of analysis and measurement, and generalizability (Zamani et al., 2006). Furthermore its application does not entail costly equipments and radioactive materials for marker detection (Justus et al., 2004). Kroth et al. (2005) utilized RAPD markers for the evaluation of six instances of hulless barley and seven instances of malt barley (Kroth et al., 2005). Doldi et al. (1997) analyzed the genetic diversity of 18 genotypes of soya using simple sequence repeat (SSR) and RAPD markers. Munir et al. (2011) also studied the germplasm of *Brassica juncea* based on molecular markers in Pakistan in 2011. By analyzing the genetic diversity of germplasm of *Brassica napus* using 20 samples from China, 25 samples from Slovakia, two from Germany and France, and one sample from Britain, Shengwu et al. (2003) concluded that there is a significant genetic diversity between the Chinese and European samples.

Inter SSR (ISSR) fingerprinting was developed such that no sequence knowledge was required. ISSR markers are used for the purpose of genotype recognition as well as the study of population structure of plant species such as corn (Kantety et al., 1995), potato (Prevost and Wikinson, 1999), orange (Fang et al., 1997), wheat (Nagaoaka and Ogihara, 1997), grass (Tabatabaei et al., 2008), melon (Fabriki et al., 2008), etc (Godwin et al., 1997; Bornet and Branchard, 2001). A study carried out by Shi et al. (2010) on 22 types of *Cornus* sp. using 19 ISSR markers also showed that ISSR molecular markers can be considered appropriate instruments for DNA fingerprinting.

Since flixweed is considered to be an extremely important plant in Iran, India, China, etc. and an important weed in the US, China and Europe, numerous studies have been carried out concerning its chemical compositions, physiology, and methods for controlling its propagation. However, no studies are known to have investigated the genetic diversity of flixweed across the world. This present paper therefore analyzes the genetic diversity of flixweed using RAPD and ISSR markers for the first time, aiming at a more extensive knowledge of flixweed and protection of its germplasm.

## MATERIALS AND METHODS

Flixweed seed from 15 fields in Kerman province (Figure 1) were collected using the assistance of the Head of Herbarium of the Research Center of Ministry of Agriculture in spring and summer 2010.

### DNA extraction

Flixweed seed sustain a dormancy period and requires different treatments such as vernalization for germination. For reasons of economy in time, flixweed seeds were utilized for DNA extraction instead of the leaves. Several protocols were used for extraction, due to essence, secondary metabolites and high protein content of flixweed seed.

The genomic DNA was extracted by cetyltrimethylammonium bromide (CTAB) method in Doyle and Doyle model (1987) with minor modifications in the DNA washing stages and amount of the materials. Considering the purpose of the present study as to the investigation of differences between flixweed ecotypes, seeds pertaining to each region were mixed and finally 0.3 to 0.5 g of seeds was prepared for extraction.

### DNA quality and quantity analysis

DNA quantity and quality were both estimated using a Scandrop device (Analytic Jena Co., Germany) by measuring absorbance at A260 and A280 nm and on 1% agarose gel electrophoresis by comparing band intensity with DNA ladder of known concentrations. As a result, samples with OD 260/280 = 1.8 to 2 and desirable

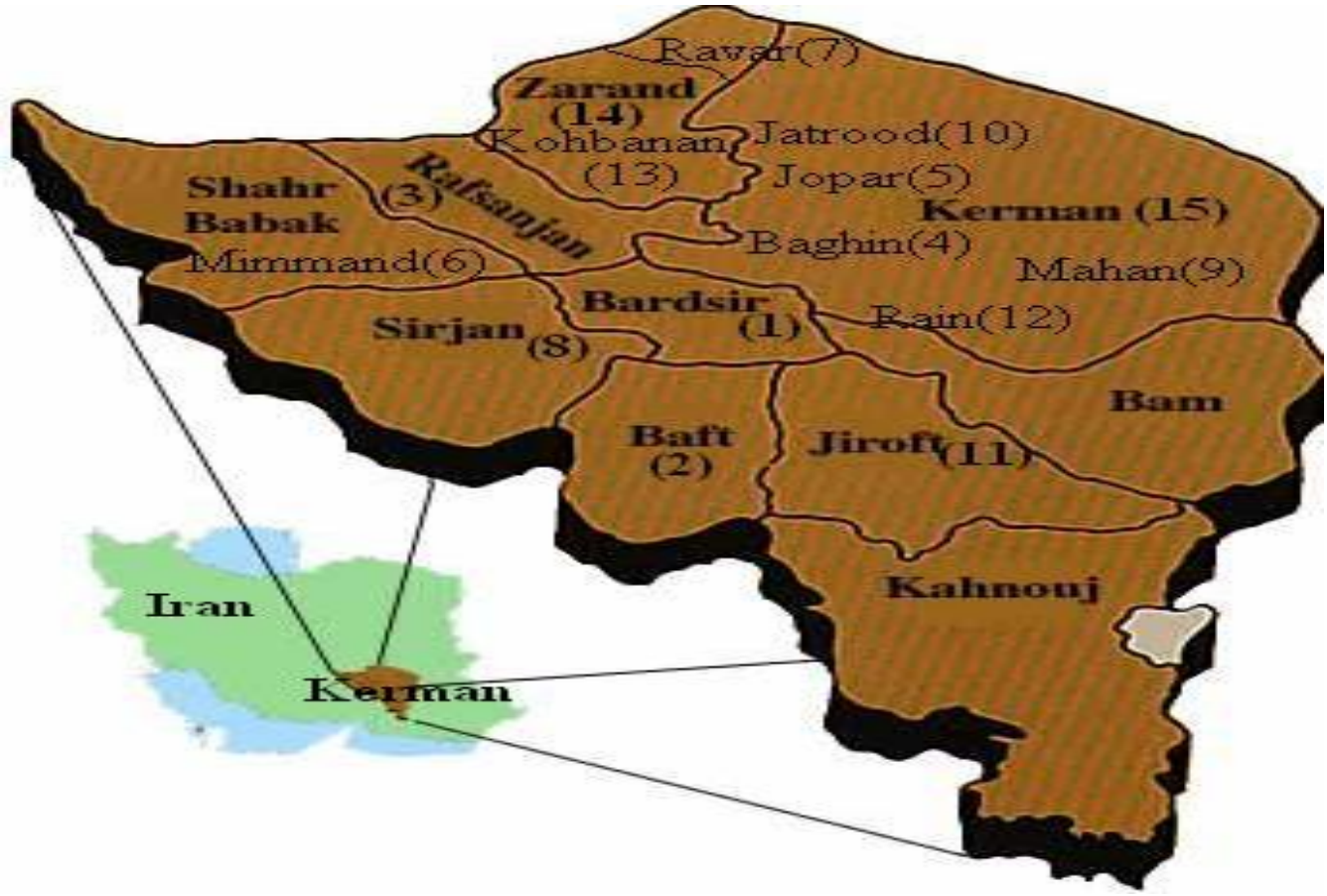


**Figure 1.** The geographical regions of the collected flixweed masses across Kerman province.

purity were selected. Both of the ways showed that yield and quality of DNA extracted by this method was sufficient and desirable for RAPD and ISSR analysis etc.

**PCR amplification**

PCR cycle according to Wanntorp's method (Wanntorp et al., 2006) was used with slight alterations for RAPD and ISSR Primers. 20 random RAPD primers, and four ISSR markers manufactured by Sinagen Co, were employed in this study. Taq DNA polymerase enzyme, dNTPs mixture in addition to PCR buffer (10x), as well as $MgCl_2$ were prepared from Sinagen Co. A volume of 25 µL PCR mixture was prepared for each primer.The thermocyclers utilized in the present study were of Mastercycle models in simple and gradient types manufactured by Eppendorf Co. The optimum annealing temperature was calculated for each primer using gradient thermocycler (Table 1) subsequent to PCR which took more than 4 h, and were stored in the temperature of -20°C for a later experiment of electrophoresis and PCR.

**Electrophoresis**

PCR products were conducted for each RAPD and ISSR primer on agarose gel and tris-borate-EDTA (TBE) buffer. Horizontal electrophoresis E 132 manufactured by Consort Co. of Belgium was employed in this study. The gel was placed in gel documentation Syngene device to be examined under UV light and photographed in different formats using GeneSnap software (v 6.08.04). The PCR products were separated by electrophoresis on 1% agarose gel and subsequently stained with ethidium bromide. Six macro liters of 100 bp Ladder, manufactured by Fermentase Company was utilized for the purpose of estimation of the molecular mass of polymerase chain reaction products.

**Analysis of RAPD and ISSR data**

16 RAPD primers, maintaining more clear bands along with four ISSR primers were selected for analysis. For statistical data analysis, clear bands were determined in every gel subsequent to which the size of every clear band in each was determined considering 100 bp standard marker and according to standard length and molecular weight using GeneTools software, and finally, the sizes of observed bands were calculated. The presence and absence of each and every observed band for all 20 primers were scored as 1 and 0, respectively, which led to the design of a 0 to 1 matrix using Excel for the prepared primers. The data was later transferred to NTedit, the entrance section of NTSYS software. The similarity matrix was designed using DICE similarity coefficient and application of NTSYS-pc (v 2.02), followed by cluster analysis through un-weighted pair group method with arithmetic mean (UPGMA) and drawing of pertinent dendrogram (Figure 3). Principle component analysis (PCA) was performed. Finally, two and three dimensions graphs using 20 primers were drawn (Figures 4 and 5).

**Table 1.** Primers sequences and annealing temperature of 16 RAPD and four ISSR primers.

| Primer | Sequence | Annealing temperature (°C) | Primer | Sequence | Annealing temperature (°C) |
|---|---|---|---|---|---|
| 72 -RAPD | GAGCACGGGA | 58.6 | 56 -RAPD | CACTTACTAC | 48.2 |
| 69 -RAPD | GAGGGCAAGA | 52.8 | 58-RAPD | CACCTGGAAA | 59.1 |
| 396-RAPD | GAATGCGAGG | 51.8 | J-RAPD | CCTCACCTGT | 56.8 |
| 376 -RAPD | CAGGACATCG | 50.5 | A -RAPD | GGTCTCCTAG | 54.2 |
| 377-RAPD | GACGGAAGAG | 49.7 | D-RAPD | TGGGCTGGCT | 59.9 |
| 399-RAPD | TTGCTGGGCG | 60.7 | H -RAPD | GGTCAACCCT | 57.8 |
| 394 -RAPD | TCACGCAGTT | 58.1 | I -RAPD | GCGGGAGACC | 60.4 |
| 52-RAPD | TTCGGGAATA | 59.1 | B -RAPD | CGGAGAGCGA | 61.3 |
| P4-ISSR | ATGATGATGATGATGATG(18) | 46.9 | ISSR-8 | CTCTCTCTCTCTCTCTG (17) | 52.8 |
| P8-ISSR | ACACACACACACACACG(17) | 52.8 | ISSR-14 | GAGAGAGAGAGAGAGAC(17) | 52.8 |

## RESULTS

Subsequent to electrophoresis, a total number of 172 DNA bands were amplified from 20 selected markers. In total, 146 cases of the mentioned bands which is equal to 84.9%, exhibited poly-morphism (Figure 2). Primer A -RAPD dedicated to itself the lowest number of created bands whereas ISSR-8 maintained the highest number of amplified bands. Masses retrieved from Jiroft and Chatroud compared to those of Rafsanjan showed the lowest resemblance, while the highest resemblance was observed between masses of Jiroft and those of Chatroud. Results from the cluster (Figure 3) in the resemblance area (0.76) gave way to four categorizations: first group included a member, Rafsanjan; second group, Jopar and Kohbanan; third group only entailed Baghin; and the fourth group included masses from Bardsir, Baft, Chatrood, Jiroft, Ravar, Mahan, Zarand, Kerman, Mimand, Rain, and Sirjan. Group 4 included the most plants pertinent to 15 climates, the genetic diversity of most of which matched the geographical conditions. There were cases, however, for which no concordance was present; for instance the two masses of Jopar and Mahan, although geographically adjacent, were placed in different molecular groups. Thus, results indicate that adjacent masses geographically, may maintain significant diversities. Meanwhile, the obtained classification results of cluster analysis did not agree with the results of principle component analysis sufficiently. Six groups were obtained in 2D plot. The highest portion in creating diversity regarding the three components controlled 11.2, 9.3 and 7.71% of the total diversity, respectively. This study shows that the cophenetic correlation coefficient was 77.6% and UPGMA had high performance in grouping genotypes and with Similarity matrix has good consistent.

## DISCUSSION

The present study shows the presence of high genetic diversity in flixweed masses in Kerman province. The dendrogram of most of the masses were placed in group 4, the molecular diversities of which matched to a great extent with their geographical conditions. In 2011, a study done by Munir et al. (2011) based on molecular markers showed that molecular markers like RAPD have a good ability in assessing the *B. juncea*. Bearing in mind that *D. sophia* and *B. junce*a are in a same family, it can be said RAPD have an efficient role in determining the diversity of genetic and clustering this plant. By analyzing the genetic diversity of germplasm of *B. napus*, Shengwu et al. (2003) concluded that there was a significant genetic diversity between the Chinese and European samples.

Due to the diversity shown in *B. napus*, a study of *D. sophia* genetic diversity was conducted by using RAPD molecular markers in this research. A similar study carried out by Shi et al. (2010) on 22 types of *Cornus* sp. using ISSR markers showed that ISSR molecular markers can be considered appropriate instruments for DNA fingerprinting. The investigation on flixweed utilizing ISSR and RAPD markers showed that considering the desirable categorization and also indication of appropriate scope of diversity of the masses, the two mentioned markers proved efficient in the investigation of the genetic diversity of flixweed, and as a result they are capable of protecting flixweed germplasm. Since many medicinal properties were observed in flixweed, numerous

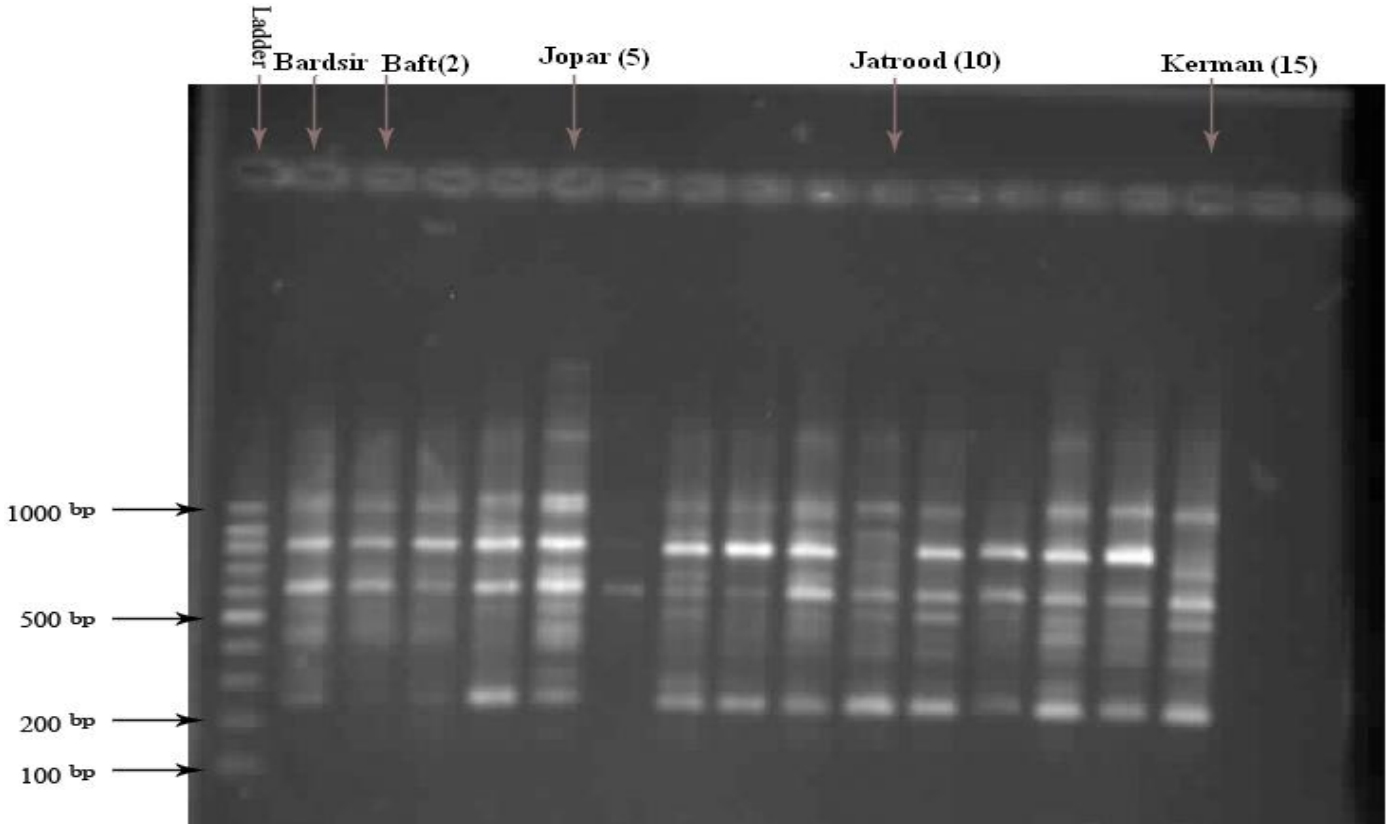


**Figure 2.** Polymorphism created bands with marker 376 -RAPD in flixweed masses in Kerman province subsequent to electrophoresis. Lane 1, Ladder 100 bp; lanes 2 to 16, sizes according to the provinces in Figure 1.

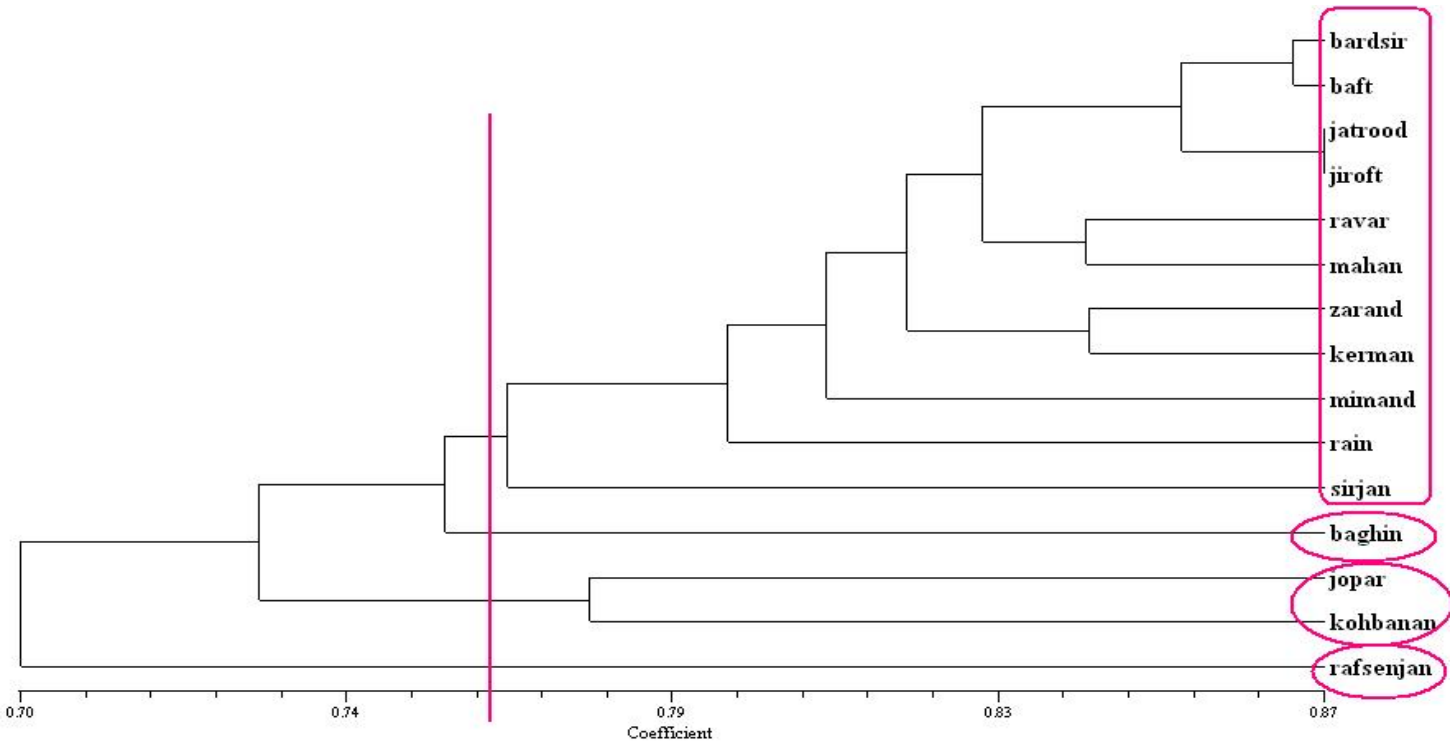


**Figure 3.** UPGMA dendrogram based on Dice similarity coefficient illustrating the relationships among 15 ecotypes.

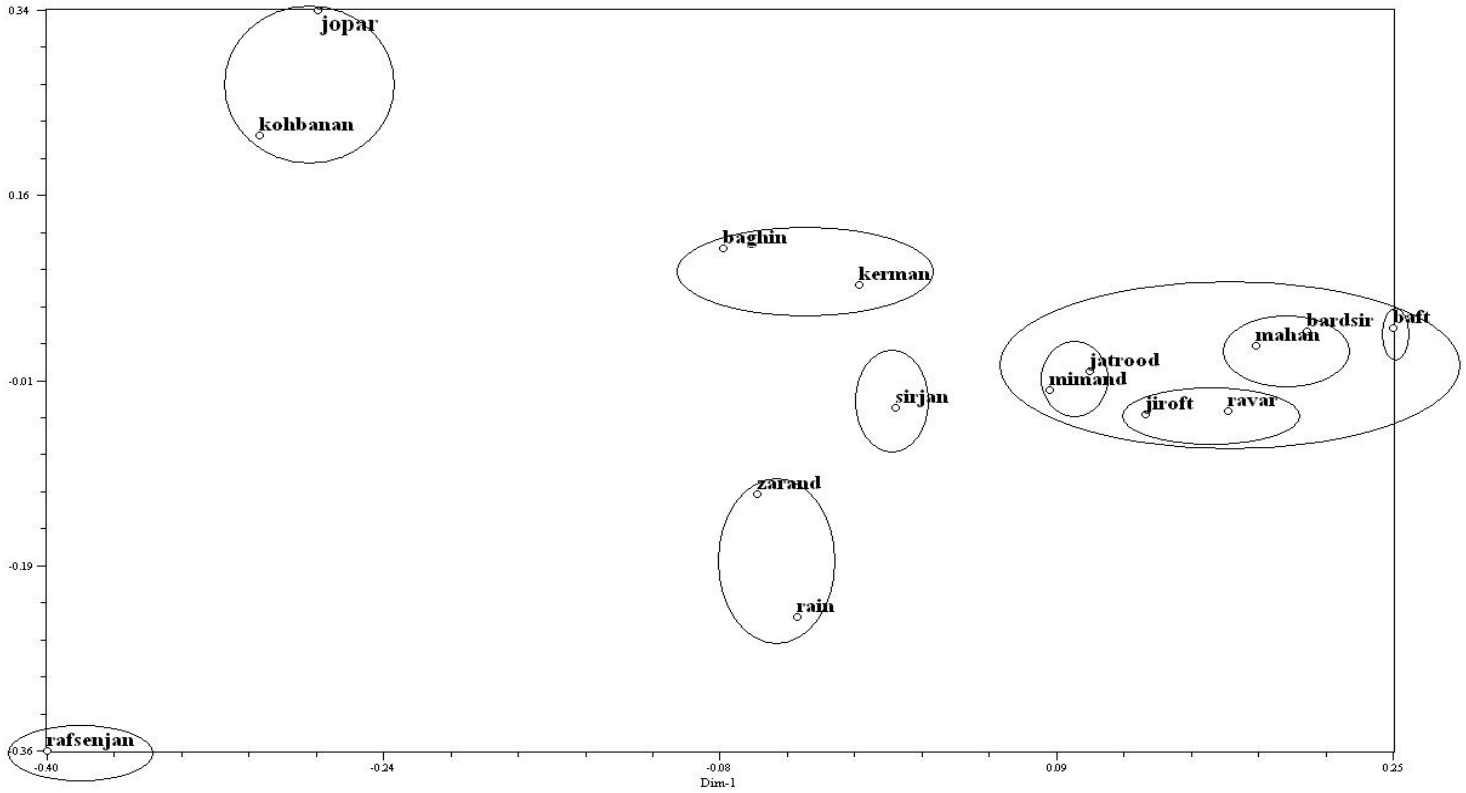


**Figure 4.** Two dimensional graph related to principle component analysis for 15 ecotypes using 20 primers.

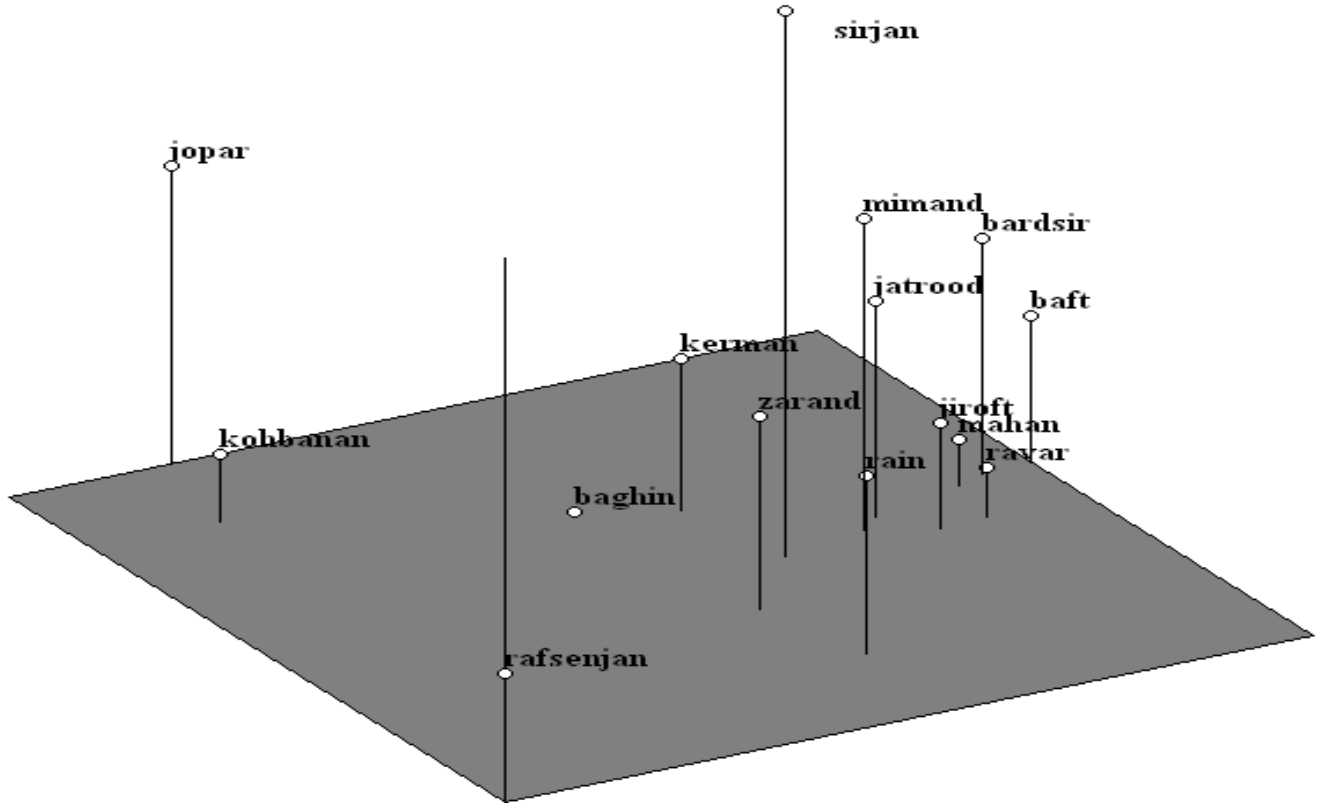


**Figure 5.** Three dimensional graph related to principle component analysis for 15 ecotypes using 20 primers.

studies have been carried out concerning its chemical compositions. In many countries such as the US and China, flixweed is considered as a weed resulting in studies on its physiology and methods for controlling its propagation. However, no studies are known to have investigated the genetic diversity of flixweed across the world. The present paper is thus considered the first study of genetic diversity of flixweed in Iran as well as across the world.

## ACKNOWLEDGEMENTS

We appreciate the assistance of the officials of the International Center for Science, High technology and Environmental Sciences. Kerman, Iran, for placing at our disposal, the requirements for conducting the present study, and also Hekmat Parvan.

### REFERENCES

Afshar I (1991). Traditional Gift Of Iranian People. Homa Publication: 302.

Amin GH (2000). Traditional Herbal Drugs In Iran. Search and Education Assistance of Health Ministry Publication. Sari: pp. 1-9.

Amini A (1995). Pharmaceutical plant culture and Their Traditional Application in kordestan. Tagh bostan Pub., 176: 192, 213-214.

Bornet B, Branchard M (2001). Nonanchored inter-simple sequence repeat (ISSR) markers. reproducible and specific tools for genome fingerprinting. Plant Mol. Biol., 19: 209-215.

Daryaei M (2007). Treatment Miracles of plants in Iranian Medicine .Safirardehal Publication. First Printing, p. 218.

Doldi ML, Volmann J, Lelly T (1997). Genetic diversity in soybean as determined by RAPD and microsatellite analysis. Plant Breed., 116: 331-335.

Dublin S, Lydon-Rochelle M, Kaplan RC (2000). Maternal and neonatal outcomes after induction of labor without and identified indication. Am. J. Obstet. Gynecol., 183(4): 986-94.

Fabriki S, Shams M, Jalali M, Ahmadi J (2008). Analysis of Genetic Diversity of Iranian Melons (*Cucumis melo L.*) Using ISSR Markers. Iran. J. Biol., 22(2): p. 11.

Fang DQ, Roose ML, Fredric CT (1997). Fingerprinting trifoliate orange germplasm accessions with isozyme, RFLP and inter simple sequence repeat (ISSR) markers. Theor. Appl. Genet. 95: 211-219.

Farsi M, Zolala J (2003). Principles of plant biotechnology. Translate. mashhad university Publication, p. 495.

Godwin LA, Aitken EA, Smith LA (1997). Application of inter simple sequence repeat (ISSR) markers to plant genetics Electrophoresis, 18: 1524-1528.

Haji Sharifi A (2003). Secrets Of Herbal Drugs. Golshan publication. Tehran, p. 44.

Justus M, Ester W, Kahangi EM, Fusao M (2004). Genetic characterization of cultivated bananas and plantains in Kenya by RAPD markers. Sci. Hort., 99: 9-20.

Kantety RV, Zeng X, Bennetzen JL, Zehr BE (1995). Assessment of genetic diversity in Dent and Popcorn (*Zea mays* L.) inbred lines using Inter-Simple Sequence Repeat (ISSR) amplification. Mol. Breed., 1: 365-373.

Kroth MA, Ramella MS, Tagliari C, Francisco A, Arisi M (2005). Genetic similarity of Brazilian hulless and malting barley varieties evaluated by RAPD markers. Scientia Agricola, 62: 36-39.

Mirhidar H (1996). Herbal Education. Plants used in the prevention and treatment of diseases. Publishing Office Islamic Culture, 3: 173-175.

Munir I, Ali I, Arif M, Farhatullah I, Bakht J, Iqbal A, Ahmad D, Mian A (2011). Assessment of genetic diversity of *Brassica juncea* germplasm using randomly amplified polymorphic DNA (RAPD) markers. Afr. J. Biotechnol., 10(19): 3654-3658.

Nagaoka T, Ogihara Y (1997). Applicability of inter-simple sequence repeat polymorphism in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet., 94: 597-602.

Prevost A, Wikinson MJ (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet. 98: 107-112.

Rejhan M (1995). Treated by medical plant. Aba Publication, p. 54.

Shengwu H, Ovesna J, Vyvadilova M, Kucera V, Kucera L (2003). Evaluation of genetic diversity of *Brassica napus* germplasm from China and Europe assessed by RAPD markers. Plant Soil Environ., 49(3): 106-113.

Shi A, Kantartzi S, Mmbaga M, Chen P (2010). Development of ISSR PCR markers for diversity study in dogwood (*Cornus sp*.). Agric. Biol. J. North Am., 1(3): 189-194.

Shokouhinejad H (1994). Cure Garden.Boostan Publication. Tehran, pp. 78-95.

Tabatabaei B, Etemadi N, Farsani T (2008). Analysis of Genetic Diversity in Bermuda Grass (*Cynodon dactylon*) Accessions using Morphological Characteristics and ISSR Markers. J. Horticult. Sci. Technol., 9(2): 83-96.

Wanntorp LA, Kocyan R, van D, Renner S (2006). Toward a Monophyletic Hoya (Marsdeniease, Apocynaceae) Inferences from the *Chloroplasmtrn* L Region and the rbcL-atpB Spacer. Syst. Bot., 31: 586-589.

Zamani ZH, Sarkhosh, Fatahi M, Abadi H (2006). Evaluation of genetic diversity among some of Pomegranate to help RAPD Markers. Iran. J. Agric. Sci., 37(5): 865-873.

Zargai H (1997). Pharmaceutical plant. Tehran Univ. Pub., 1(7). pp. 213-214.