INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-4, Issue-2, April-June-2014

ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 20th Feb-2014 *Revised: 30th March-2014* *Accepted: 6th April-2014*

Research article

GENETIC DIVERGENCE ANALYSIS AMONG TEN POPULATIONS OF *CONVOLVULUS ARVENSIS* L. BY RAPD-PCR

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ABSTRACT: Genetic diversity evaluation among ten *Convolvulus arvensis* genotypes, of which collected from different regions of Iran, was determined using RAPD technique.The extraction of total genomic DNA was carried out using MBST kit.A total of 6 RAPD primers product bands 49 bands, 33 (67.34%) of wich were poly morphic and 16 (23.65%) monomorphic. Molecular analysis of ten populations based on the UPGMA method assigned the genotypes in three main groups. In this grouping, C_1 genotype in Nyasar of Kashan and C_8 genotype in Alamut of ghazvin showed the most difference to other groups. And genotype in Ardebil and genotype in hamedan have not shown any difference. However, RAPD analysis was found to be a valuable diagnostic tool to evaluate genetic diversity of *Convolvulus arvensisgenotypes* in Iran.

Key words: Genetic diversity, of *Convolvulus arvensis* L., RAPD

INTRODUCTION

Convolvulus arvensis L. (field bindweed) is a species of bindweed in the morning glory family (Convolvulaceae), native to Europe and Asia [12]. It is a climbing or creeping herbaceous perennial plant growing to 0.5 – 2 m high [3]. The leaves are spirally arranged, linear to arrowhead- shaped, 2-5 cm long and alternate, with a 1-3 cm petiole [4]. The flowers are trumpet-shaped, 1-2.5 cm diameter, white or pale pink, with five slightly darker pink radial stripes [5]. Flowering occurs in the mid-summer, when white to pale pink, funnel- shaped flowers develop [5]. Flowers are approximately 0.75-1 in. (1.9-2.5 cm) across and are subtended by small bracts. Fruit are light brown, rounded and 1/8 in. (0.3cm) wide. Each fruit contains 2seeds that are eaten by birds and can remain viable in the soil for decades. [7, 8]Molecular approaches collectively represent a potential tool that can be applied for effective characterization of germplasme. It addresses the limitations associated with morphological and biochemical processes [9]. A common approach for assessing levels of genetic diversity is the use of molecular markers such as randomly amplified polymorphic DNA (RAPD) [10, 11]. RAPD technique is simple, reliable, efficient, and an economical means of cultivar identification and diversity analysis [1]. RAPDs have previously been used successfully to assess levels of genetic diversity genotype identification in several plants including Lilium species [13], Agave tequilana var. Azul [10], Heliconia [9] and Gladiolus species [5].

At present based on our knowledge, there is no report on the diversity analysis in convolvulus arvensis using DNA markers in Iran, therefore our study was undertaken to assess the genetic diversity among Convolvulus arvensis L. population of Iran using RAPD-PCR.

MATERIALS AND METHODS

Plant material ten genotypes of *Convolvulus arvensis L*. were collected from different parts of Iran in this study. The genotypes included are shown in table 1.

Table 1. Origins of ten populations of *Convolvulus arvensis* L. Used in the study

Population	City or town	Elevation	Locality/Origin
$C.a_1$	**1. Nyassar**	1645meters	N 33° 97' E 51° 14'
$C.a_2$	**2. Zahedan**	1385meters	29° 25' N E 60° 30'
$C.a_3$	**3. Hamedan**	1741meters	N 34°80' E 48° 52'
$C.a_4$	**4. Karaj**	1300 m	N 35° 82' E 50° 97'
$C.a_5$	**5. Damavand**	1903meters	N 35° 57' E 52°63'
$C.a_6$	**6. Dezful**	148meters	N 32° 38' E 48° 39'
$C.a_7$	**7. Ahvaz**	12meters	N 31° 20' E 48° 40'
$C.a_8$	**8. Ghazvin (Alamut)**	2163meters	N 36° 47' E 50° 58'
$C.a_9$	**9. Ardebil**	1,500meters	N 38° 30' E 48° 51'
$C.a_{10}$	**10. Abul**	480meters	N 29° 30' E 60° 51'

DNA Extraction

The total DNA was extracted from young leaves by method proposed by Parviz,Sh et al. using MBST kit (2007)[5]. The MBST kit contains sufficient reagents for 50 µl DNA preparations and components: Lysis buffer, Binding buffer, Proteinase K, Wash buffer, Elution buffer (Elution buffer is consist of 10 mM Tris-HCL pH 7.4 and 1 mM EDTA PH 8.0), and MBST- column. For extraction, briefly, the leaves were first lysed in 300 µl lysis buffer and the proteins were degraded with 20 µl proteinase K for 45 min at 60 oc. After addition of 580 µl Binding buffer and incubation for 15 min at 70o c, 440 µl ethanol (%100) was added to the solution and after vortexing, the complete volume was transferred to the MBST-column. The MBST-column was first centrifuged and washed twice with 500 µl Wash buffer. Finally, DNA was eluted from the carrier with Elution buffer. The quality of DNA was determined using agarose gel (1%) electrophoresis.

RAPD amplification

Amplification of RAPD fragments was performed according to Jayoti et al [2], A total of 6 different random 10-mer primers were used for RAPD analysis [12]. The sequences of the selected primers are presented in Table 2. Polymerase chain reaction (PCR) was conducted with 50µl reactions containing 6µl genomic DNA, 10 µ primer.9 µl H2O and 25 µl Ampliqon Master Mix (including Tris-HCL pH 8.5, (NH4)2 SO4 , 2mM MgCL2 , 0.2% Tween 20, 0.4mM dNTPs, 0.2 units/µl Ampliqon Tag DNA polymerase). The PCR reactions was carried out in a thermo cycler (Perkin Elmer, Massachusetts, USA) with following conditions, i.e. denaturation at 97oC for 2 min, followed by 40 cycles of denaturation at 94oC for 1 min, primer annealing at 35oC for 1min and primer extension at 72oC for 2 min. A final extension step was carried at 72oC for 4 min.

Detection of PCR products

The amplified products were detected using agarose gel electrophoresis (1% gel in TAE buffer), stained with ethidium bromide and visualized with UV transilluminator and photographed using gel documentation system.

Data analysis

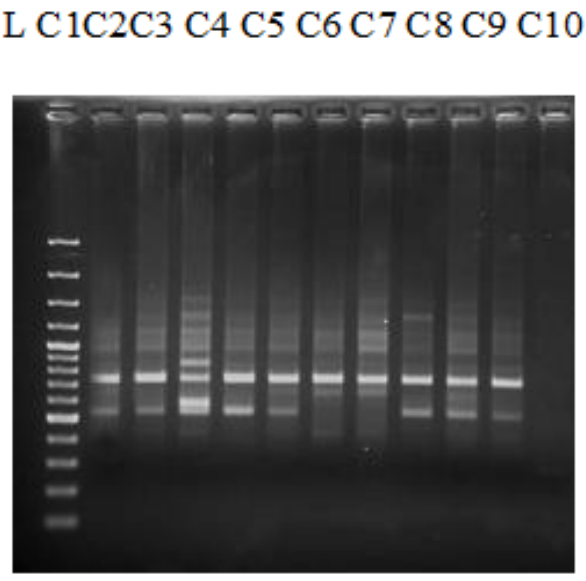
The RAPD bands were scored as ‘’1’’ for presence and ‘’0’’for absence across all Convolvulus arvensis accession for each primer. The data was analyzed for genetic diversity with the help software NTSYS 2.2 version and genetic distances were calculated on the basis of Jaccard’s Pair wise Similarity Coefficients .Clustering was performed using on weighted Pair Group Method of Arithmetic Means (UPGMA) and dendrogram was constructed using graphic function in the program.

Table 2: List of random dacamer primers used for RAPD analysis.

Primer number	Sequence
OPC-01	5'-TTCGAGCCAG -3'
OPN-06	5'-TGAGACGCACA -3'
OPN-04	5'-GGACCGACCCA -3'
OPC-17	5'-TTCCCCCCAG -3'
OPC-20	5'-ACTTCGCCAC -3'
OPE-01	5'-CCCAAGGTCC -3'

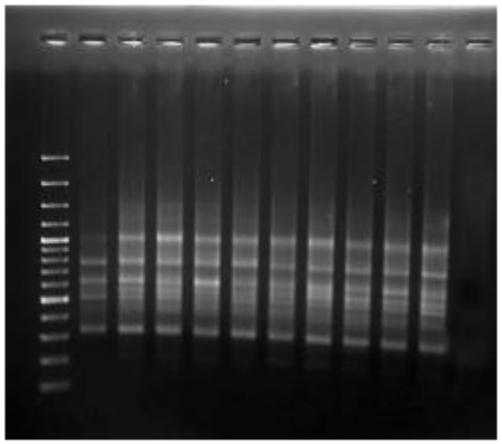
RESULT AND DISCUTION

A total of 6 random primers were used to establish RAPD-PCR seven cultivars belonging to *Convolvulus arvensis* L in Iran. All of 6 RAPD primers tested in this analysis produced a total of 49 bands and the number of bands generated by each primer ranged from 6 (OPE-01) to 11(OPC-20) with mean of 8/16 bands in per primer. Out of the total 102 bands, 33 (67/34%) were polymorphic and 16 (23/65%) monomorphic. The primer showing maximum number of polymorphic bands was OPC-20 (10 bands) followed by OPE-01(5 bands) (Figure1). The polymorphism shown by different primers ranged from 90/9% to 50% (Table3). The dendrogram based on genetic distances was constructed to investigate genetic diversity among ten genotypes and cluster diagram of this genotypes was presented in Figure 2 and revealed 3 main clad at 0/64 genetic distance. The clade A comprised of C.a_8 and clade B divided to two different sub clade, sub clade $B_1$and B2. Sub clade B1 contains C.a_3 and C.a_9 and C.a_3 showed the greatest similarity to C.a_9. Therefore, the most different population was C.a_1C.a8.



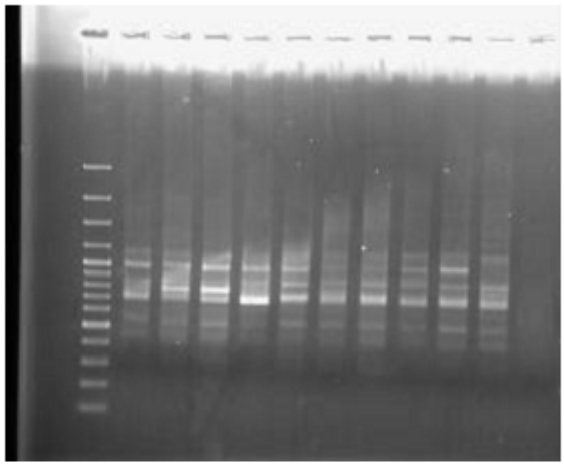
OPC-20

L C1C2 C3 C4 C5 C6 C7 C8 C9 C10 bP



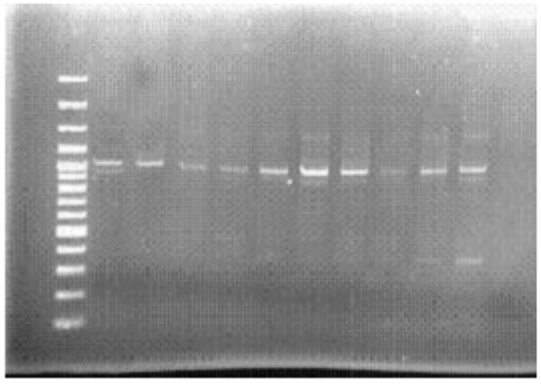
OPN-04

L C1C2 C3 C4 C5 C6 C7 C8 C9 C10 bP



OPC-01

L C1C2 C3 C4 C5 C6 C7 C8 C9 C10 bP



OPE-01

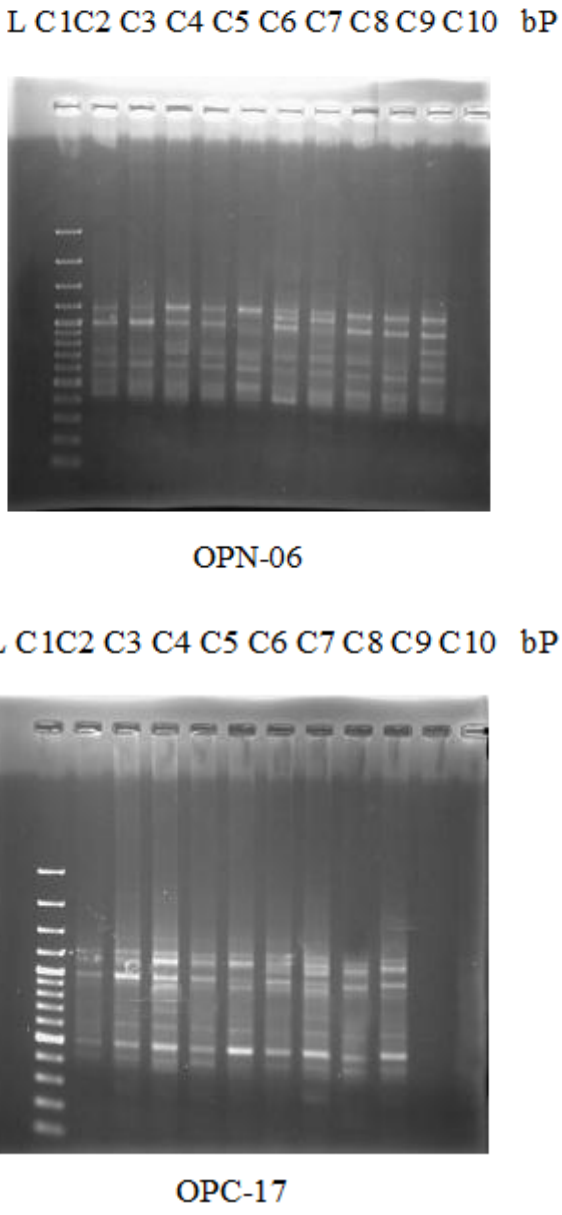


Figure1: Results of gel electrophoresis of RAPD products amplified with the random decamer primer OPC-20, OPN-04, OPC-17, OPN-06, OPE-01, OPC-01.

Table 3: Band statistics generated using RAPD primers in *Convolvulus arvensis* population.

NO.	Primers	Total no. of amplification product	Total no. of Polymorphic amplification product	Total no. of monomorphic amplification product	Polymorphism (%)
1	OPC-019	8	5	3	62.5
2	OPN-067	8	4	4	50
3	OPN-04	9	5	4	55.5
4	OPC-17	7	4	3	57.14
5	OPC-20	11	10	1	90.9
6	OPE-01	6	5	1	83.33

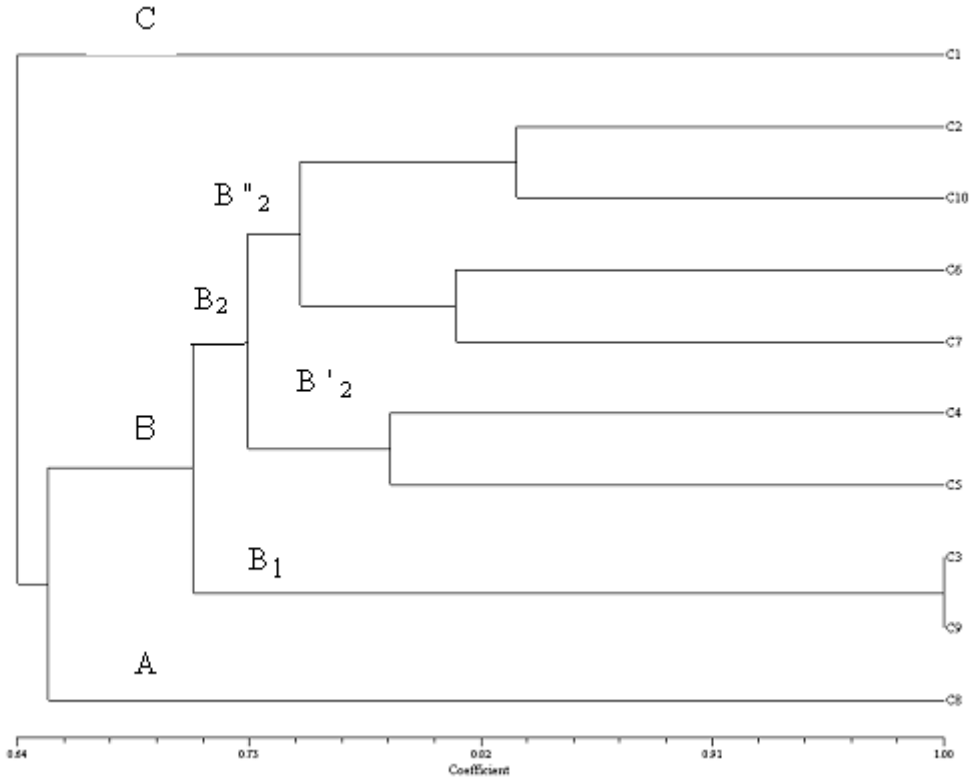


Figure 2: UPGMA dendrogram of ten *Convolvulus arvensis* population in regions of Iran.

To investigate the genetic diversity of Convolvulus arvensis (due to different weather climates) RAPD-PCR technique was used .To investigate the genetic diversity of plant Convolvulus arvensis, its leaves collected from 10 different Cities with different environmental conditions were used in Iran. Sampling was conducted in Kashan, Ardabil, Hamadan, Karaj, Damavand, Dezful, Ahvaz, Qazvin, Zahedan, and Zabol. DNA sample was used from the dried leaves of the plant and for data analysis of RAPD. 6 primers 10-mer were used applying random sequences. In total 49 Scorable bands were visible on agarose gel. Of total bands, 33 bands (67 /34%) were polymorphic and 16 bands (32/ 65%) were monomorphic. Using the software NTSYS VER. 2.2 and via Method Jaccard, similarity matrix was prepared and Dendrogram from 10 genotypes of Convolvulus arvensis was outlined from different areas .The results showed the maximum similarity between samples collected from Ardabil and Hamadan. The highest genetic diversity was from samples of Niyasar (Kashan) and Alamut (Qazvin).

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