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Analysis of genetic diversity in bindweed (*Convolvulus arvensis* L.) populations using random amplified polymorphic DNA (RAPD) markers

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Abstract

Bindweed (*Convolvulus arvensis* L.) is one of the most harmful weed species in Turkey. Using random amplified polymorphic DNA (RAPD) markers, It investigated the genetic diversity within and among five local populations of bindweed. Fourteen (14) RAPD primers produced a total of 429 bands, of which 142 (33.1%) were polymorphic. The mean Nei's gene diversity value for all five populations was 0.2053. Shannon's information index varied with a population (0.2278–0.3082), averaging 0.3047. Analysis of molecular variance (AMOVA) presented 53.8% variation within populations, and 32.9% among populations, showing a high variation within populations. Additionally, the variation between groups was 13.3%. The genetic differentiation among populations (GST) was 0.293, indicating that most genetic diversity occurs within populations. Gene flow (Nm) was low, at only 0.6032.

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Introduction

Convolvulus arvensis L. is an herbaceous perennial plant belonging to the genus *Convolvulus*, which is the second largest genus within the Convolvulacee family (Cronquist, 1981). Although the origin of *C. arvensis* is Europa and the species is predominantly in the temperate zones of Europe, Western Asia, and North America mainly, it is particularly damaging to cultivated fields of Europe (Holm *et al.,* 1977). It was reported that, *C.arvensis* is one of the 15 important harmful grasses of the world and the species is a serious problem for 32 different crops in more than 44 countries (Holm *et al.,* 1977; Schroeder, 1993). This species is commonly known as field bindweed or small-flowered morning glory. It is a perennial herbaceous broadleaf weed, which spread primarily by means of lateral roots and root buds (Holm *et al.,* 1977). The leaves are spirally arranged, linear to arrowhead-sheaped, 2-5 cm long and alternate, with a 1-3 cm alternate. The creping or twining thin-branched stem can form a dense, tanglet mat or can attain a height of up to 120 cm. The insect pollinated flowers of *C. arvensis* are regular with a funnel-shaped, with white or pale pink coloured corolla. After pollination, 2-compartimentalized capsules are formed, with one to four seeds produced per fruit; *C. arvensis* is polymorphic. Many biotypes and ecotypes were reported (Garcia-Baudin and Darmency, 1979). The chromosome number of this species has been reported as 2n=48 most commonly (Garcia-Baudin and Darmency, 1979). Bindweed is predominantly distributed in the temperature zones of Europe, Western Asia and North America (Austin, 2000). It is a serious weed in Australia, USA, France, New Zealand and South Africa. In Turkey, it is distributed along the whole country and mainly preferred light, warm and dry places (Coruh and Zengin, 2007). In addition to this, bindweed is found in a wide range of habitats such as roadsides, cropland, lakeshores, vineyards and orchards (Austin, 2000). It has an extensive underground root system; therefore, it is very droght resistant and a very competitive during drought spells (Vogelgsang, 1998). Due to its climbing ability, it is able to infest various levels of plant community. Bindweed competes with other species (i.e: alfalafa, asparagus) for sunlight, nutrients and moisture and it can reduce crop yield and increase irrigation costs. It was reported that bindweed is one of the most harmful weed species in the farmland (Austin, 2000). Bindweed typically develops large patches and is difficult to control. It is troublesome in numerous crops, but is especially problematic in cereals, beans and potatoes. On the other hand, this species can be controlled by using competitive crops (*Sorghum* or *Secale*) or herbicides such as 2,4-dichlorophenoxiacetic acid (2,4-D), dicamba, glyphosate that are applied either alone or in combination (Vogelgsang, 1998).

The genetic structure of plant populations reflects the interactions of various processes, including the long-term evolutionary history of the species, mutation, genetic drift, mating system, gene flow, and selection (Schaal *et al.,* 1998). Recent developments in molecular techniques provide the weed scientist with a larger array of genetic tools for studying genetic diversity of populations than was previously available (O'Hanlon *et al.,* 2000). In the past few decades, random amplified polymorphic DNA (RAPD) markers have been used to study the genetic diversity of weed populations. This technology *via* the polymerase chain reaction (PCR) has fast become a means of investigating genetic diversity within and between populations, and the technique has been widely used to survey population genetic structure, because its application does not require any prior information about the target sequence in the genome (Li *et al.,* 2006). The genetic diversity of several weed has been successfully assayed using RAPD markers, including several annual plant species (Ward and Jasieniuk, 2009) and *Thymus* sp. (Sunar *et al.,* 2009). In experimental studies of the invasive plant species, both our country homeland and outside the homeland (Argentina and United States), *Convolvulus arvensis* was observed to possess higher infestation success in America and Argentina. One of the most important questions in invasion biology is, why are the invasive species more successful outside homeland

themselves?

In the present study, RAPD markers were used to investigate the genetic diversity of five populations of bindweed located in the Eastern Anatolia region of Turkey which were sampled and analyzed. Genotype origins, genetic structure across an invasion and native centers of diversity are important clues for determining locations of potential biological control agents. The aims of the study were to the world's most harmful weed, *C. arvensis* species classified and causing serious yield losses in our country, and explore the amount and distribution of genetic variation.

Materials and methods

Sample Collection

During July 2010, five populations (Askale, Erzurum, Ispir, Oltu and Tortum) of bindweed were sampled from Eastern Anatolia (Fig. 1). Populations were separated geographically by at least 50 km. 15 individuals were collected from each population (for a total of 75 samples), by collecting roughly 50 g of fresh leaves per sample. To avoid duplicate sampling of the same genome, each individual sample from a population was collected randomly from locations at least 50 m from each other. The samples were placed in ziplock bags with silica gel and stored in a freezer at -80° C until further tests.

Fig. 1. Geographic distribution of five sampled populations of bindweed.

Genomic DNA isolation and RAPD Procedures

Genomic DNA was extracted from powdered plant materials using a method described by Li and Quiros (2001). The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed using 0.8% agarose gel electrophoresis against known concentrations of unrestricted lambda DNA.

Samples were screened for RAPD variation using standard 10-base primers supplied by operon (Operon Technologies Inc., Alameda, CA, USA). 30 µl of reaction cocktail was prepared as follows: 10x Buffer 3.0 µl, dNTPs (10mM) 1.2 µl, magnesium chloride (25mM) 1.2 µl, primer (5µM) 2.0 µl, taq polymerase (5unit) 0.4 µl, water 19.2 µl sample DNA 3.0 µl (100 ng/ µl). Forty-five (45) oligonucleotide primers were screened, and among them, 14 primers were selected and used for further studies (Table 1). The thermal cycle was: 2 min. at 95°C; 2cycles of 30 sec. at 95°C, 1 min. at 37°C, 2 min. at 72°C; 2 cycles of 30 sec. at 95°C, 1 min. at 35°C, 2 min. at 72°C; 41 cycles of 30 sec. at 94°C, 1 min. at 35°C, 2 min. at 72°C; followed by a final 5 min. extension at 72°C then brought down to 4°C.

Electrophoresis

The PCR products (27 µl) were mixed with 6x gel loading buffer (3 µl) and loaded onto an agarose (1.5% w/v) gel electrophoresis in 0.5x Tris-Borate- EDTA (TBE) buffer at 70 V for 150 min. Amplification products separated by gel were stained using an ethidium bromide solution (2 µl Etbr/100ml 1x TBE buffer) for 40 min. The amplified DNA product was detected by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge Electronic Design Ltd, Cambridge, UK).

Data Analysis

PCR products were scored as presence (1) and absence (0) of the band for each population and analyzed. Based on the binary matrix obtained in the study, genetic diversity indexes, including the percentage of polymorphic loci (%*P*), the observed number of alleles (n_a), the expected number of alleles

(n_e), Neil's gene diversity (H), Shannon's information index of diversity (I), genetic differentiations among populations (G_{ST}), and gene flow (N_m) were calculated in the dominant diploid of the POPGENE v. 1.31 program (Yeh *et al.*, 1999). The additional measure for partitioning genetic variation was estimated based on Shannon's information index, or obtained with AMOVA (Arlequin version 3.11). A dendrogram was generated by neighbor joining trees using POPULATION 1.2.28 software based on Nei's unbiased genetic distance coefficient matrix calculated with POPGENE software.

Results

After screening forty 10-base oligonucleotide primers (Operon Technologies, USA), 14 primers that displayed intense and reproducible bands were selected for further PCR amplification of 75 individuals (15 individuals per population) from five bindweed populations. These primers generated 429 amplified bands in total of which 142 bands were polymorphic (the percentage of polymorphism was 33.1%).

Table 1. Sequences of 14 primers used in this study.

primer	sequence 5'→3'
OPA04	AATCGGGCTG
OPH17	CACTCTCCTC
OPA02	TGCCGAGCTG
OPA01	CAGGCCCTTC
OPY07	AGAGCCGTCA
OPH19	CTGACCAGCC
OPW01	CTCAGTGTCC
OPW06	AGGCCCGATG
OPB08	GTCCACACGG
OPW08	GACTGCCTCT
OPBA07	GGGTCGCATC
OPBB11	TGCGGGTTCC
OPBB14	GTGGGACCTG
OPBC05	GAGGCGATTG

When each population was taken into account, percent polymorphic loci were 60.56% from Oltu and Tortum, and 42.96% from Erzurum, with a mean of 55.35%. The percentage of polymorphic loci (*%P*), Nei's gene diversity (H), and Shannon's index (I) was used to estimate the level of population genetic diversity (Table 2). The populations included in this study showed a comparatively high level of genetic diversity with H=0.2053 and I=0.3047, respectively. In the studied populations, Oltu population showed the highest level of genetic diversity (H=0.2381 and I=0.3488), while the Erzurum population had the lowest (H=0.1672 and I=0.2463). Using Stalkin and Barton's (1989) formula for estimated gene flow, $Nm=0.25*(1-G_{ST})/G_{ST}$ the gene flow between all populations was Nm=0.6032, lower than 1.0, a proposed gene flow within plant population. Significant ($P<0.001$) genetic differentiation was observed among the populations of bindweed: the genetic differentiation among populations (G_{ST}) was estimated as 0.2930. The observed number of alleles (n_a) per population varied from 1.5352 to 1.6056 (Table 2). The mean effective number of alleles for all 5 populations was n_e=1.3533, with a range from 1.3135 to 1.4042. The variance components of within and among populations detected with AMOVA were 53.8% and 32.9 % of the total variance, respectively. A Neighbor-joining tree (Fig. 2) based on Nei's genetic distance matrix showed that the five populations were clustered into two distinct groups, and two subgroups within group I.

In five populations of bindweed (Table 3), the genetic distance ranged from 0.0937 to 0.2091. The genetic distance between Oltu population and Ispir population was the biggest (0.2091), and the genetic identity between Oltu and Ispir populations was the lowest (0.8113). The genetic distance between Aşkale and Erzurum populations was the smallest (0.0937), and the genetic identity between Aşkale and Erzurum populations was the highest (0.9105). Oltu and Tortum also had lesser genetic distance (0.1011) and bigger genetic identity (0.9038). An example of amplified products generated with the RAPD maker OPW01 is shown in Fig. 3 and Fig. 4.

Table 2. Genetic diversity of five populations of bindweed detected by RAPD analysis *.

population	samples	Na	Ne	H	I	P	%P
aşkale	15	1.5915	1.3391	0.2019	0.3048	84	59.15
oltu	15	1.6056	1.4208	0.2381	0.3488	86	60.56
tortum	15	1.6056	1.4042	0.2330	0.3437	86	60.56
erzurum	15	1.4296	1.2891	0.1672	0.2463	61	42.96
ispir	15	1.5352	1.3135	0.1863	0.2801	76	53.52
mean	15	1.5535	1.3533	0.2053	0.3047	78.6	55.35

* n_a=observed number of alleles; n_e=expected number of alleles; *H*=Nei's gene diversity; *I*=shannon's index; *%P*=Percentage of polymorphic loci.

Table 3. Genetic identity and distance for five populations of bindweed from Eastern Anatolia*.

population	aşkale	oltu	tortum	erzurum	ispir
aşkale	****	0.8244	0.8386	0.9105	0.8759
oltu	0.1931	****	0.9038	0.8568	0.8113
tortum	0.1760	0.1011	****	0.9020	0.8786
erzurum	0.0937	0.1545	0.1031	****	0.8541
ispir	0.1326	0.2091	0.1294	0.1577	****

*Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Discussion

The RAPD amplification results and Popgene analysis indicated that 429 bands were marked for all individuals of the six populations, and of these, 142 were polymorphic. The percentage of polymorphic loci ranged from 42.96 and 60.56% within different populations; the average Nei's genetic diversity was 0.2053, and the average Shannon's information index was 0.3047. The polymorphism detected as a percentage of polymorphic loci was higher than the 43.7% reported by Fedorenko and Gritskikh (2008) using RAPD markers for analysis of molecular genetic polymorphism in *Arabidopsis thaliana*. It is widely accepted that some factors, such as the breeding system, life-form historical events, and geographic distribution, are decisive for determining the level of genetic variation and its partitioning within and among populations (Barrett, 1992). RAPD and Inter-simple sequence repeat (ISSR) analyses have shown that widespread outcrossing and woody species are generally characterized by low levels of among-population diversity and high differentiation within populations (Sun *et al.,* 2006; Qian *et al.,* 2008). Outcrossing plant species tend to present between 10 and 20% genetic variation among populations (Hamrick and Godt, 1989). Unlike outcrossing species, self-crossing plants such as *Senecio vulgaris* (38.9%) and *Antirrhinum subaeticum* (82.3%) have high levels of diversity among populations (Reisch *et al.,* 2005; Guo *et al.,* 2007). The major proportion (53.8%) of the total variation of bindweed was found within populations.

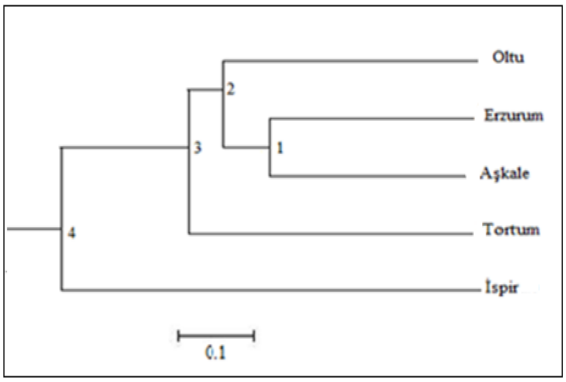

Fig. 2. Neighbor-joining tree of Nei's genetic distance among five populations of bindweed (0.1, standard genetic distance).

The remaining variation exists among groups (13.3%) and among populations (32.9%) respectively. This is in accordance with the findings of Hamza (2010), as he obtained higher variation (%67) within the three populations of invasive *Prosopis juliflora* and lower

variation among them (%33). When the proportion of genetic variation among populations is compared to mentioned information about out-crossing and self-crossing species, it is intermediate and these suggest that the breeding system of bindweed is mixed.

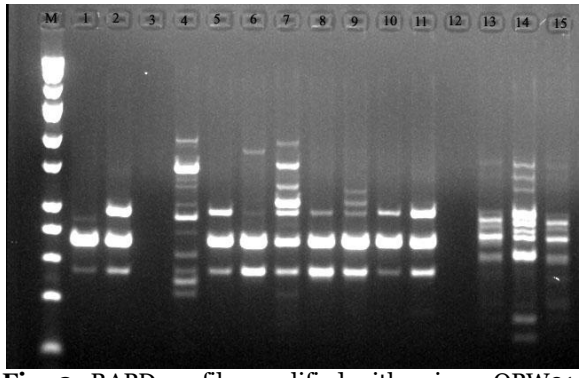

Fig. 3. RAPD profile amplified with primer OPW01 CTCAGTGTCC for 15 bindweed individuals from Tortum.

The genetic differentiation among populations of bindweed (GST = 0.2930) indicates that most genetic diversity occurs within populations. The genetic differance among 5 populations of bindweed in Erzurum in our study is far less than seen self pollinating species, where the reported G_{ST}=0.51 (Hamrick and Godt, 1989).

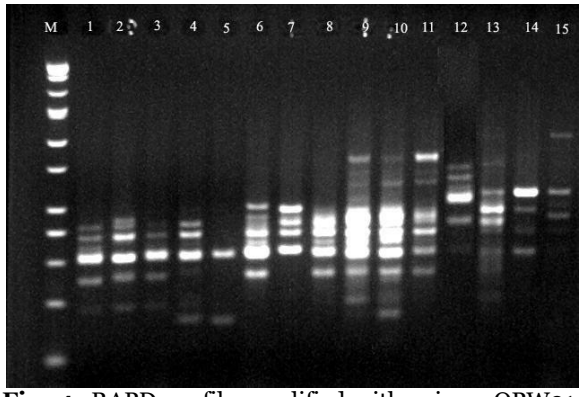

Fig. 4. RAPD profile amplified with primer OPW01 CTCAGTGTCC for 15 bindweed individuals from Ispir.

The value, however, is in close accord with G_{ST}=01 to 022 for mixed maturing and 0.10 to 0.20 fro outcrossing species (Hamrick and Godt, 1989). Gene flow was lower than 1 (Nm = 0.6032), a level of migration that will not prevent continued divergence among populations (Wright, 1951). Among populations, gene flow is generally limited by seed and pollen dispersal. The neighbor-joining tree, based on Nei's unbiased genetic distance, indicates that genetic differentiation is consistent with the geographic distance between bindweed populations. The majority of the populations from the same region clustered together. For example, Aşkale and Erzurum populations or Tortum and Oltu populations that are neighboring regions clustered together. In many plant species, there exist significant correlation between geographic and genetic distance among populations (Xia *et al.,* 2005; Nianxi *et al.,* 2006; Qian *et al.,* 2008), which can be explained by the isolation-by-distance hypothesis. Note that Oltu population is geographically closer to Ispir than it is to Aşkale, but genetic distance between Oltu and Ispir was higher than it was between Oltu and Aşkale. The observed differentiation between the populations of Oltu and Ispir could be explained by the existence of a barrier to gene flow. It suggests that this barrier is the Coruh River and chain of Mountains.

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References

Austin DF. 2000. Bindweed (*Convolvulus arvensis*, Convolvulaceae) in North America: from medicine to menace. Journal of the Torrey Botanical Society **127,** 172-177.

Barrett SCH. 1992. Genetics of weed invasions. In: Jain SK, Botsford LW (eds) Applied populationbiology. Kluwer, Dordrecht, 91–119 p.

Coruh I, Zengin H. 2007. Determination of economic threshold of field bindweed (*Convolvulus arvensis* L.) in spring wheat in Erzurum, Turkey. Journal of Ataturk University Agricultural Faculty **38,** 151-157.

Fedorenko OM, Gritskikh MV. 2008. Diversity of *Arabidobsis thaliana* (L.) Heynh. natural populations

at the northern limits of the species range: RAPD analysis. Russian Journal of Genetics **44(4),** 425–428.

Garcia-Baudin JM, Darmency H. 1979. Differences intraspecifiques chez *Convolvulus arvensis* L. Weed Research **19,** 219-224.

Guo HB, Lu BR, Wu QH, Chen JK, Zhou TS. 2007. Abundant genetic diversity in cultivated *Codonopsis pilosula* populations revealed by RAPD polymorphisms. Genetic Resources Crop Evoutionl **54,** 917-924.

Hamrick JL, Godt MJW. 1989. Allozyme diversity in plant species. In: Brown A.H.D., M.T. Clegg, A.L. Kahler, B.S. Weir (eds) Plant population genetics, breeding and genetic resources. Sinauer Associates, Sunderland 43–46 p.

Holm LG, Plunknett DL, Pancho JV, Herberger JP. 1977. Honolulu, Hawaii, USA: University Press of Hawaii pp. 98–104.

Li W, Wang B, Wang B. 2006. Lack of genetic variation of an invasive clonal plant *Eichornia crassipes* in China revealed by RAPD and ISSR markers. Aquatic Botany **84,** 176–180.

Li G, Quiros CF. 2001. Sequence-related amplified polymorphism (SRAP),a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theoretical and Applied Genetics **103,** 455–461.

Nianxi Z, Yubao G, Wang J, Ren A, Xu H. 2006. RAPD diversity of *Stipa grandis* populations and its relationship with some ecological factors. Acta Ecologica Sinica **26(5),** 1312–1319.

O'Hanlon PC, Peakal R, Briese DT. 2000. A review of new PCR-based genetic markers and their utility to weed ecology. Weed Research **40,** 239-254.

Qian ZQ, Xu L, Wang YL, Yang J, Zhao GF. 2008. Ecological genetics of *Reaumuria soongorica* Pall. Maxim. population in the oasis-desert ecotone in Fukang, Xinjiang, and its implications for molecular evolution. Biochemical Systematics and Ecology **36,** 593–601.

Reisch C, Anke A, Rohl M. 2005. Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps. Basic and Applied Ecology **6,** 35–45.

Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA. 1998. Phylogeographic studies in plants: problems and prospects. Molecular Ecology **7,** 465–474.

Schroeder D, MiiUer-Schihr H, Stinson CSA. 1993. A European weed surveyin 10 major crop systems to identrfy targets for biological control. Weed Research **33,** 449-458.

Sun K, Chen W, Ma RJ, Chen X, Ang L, Ge S. 2006. Genetic variation in *Hippophae rhamnoides ssp. sinensis* (Elaeagnaceae) revealed by RAPD markers. Biochemical Genetics **44(5–6),** 186–197.

Sunar S, Aksakal O, Yildirim N, Agar G, Gulluce M, Sahin F. 2009. Genetic diversity and relationships detected by FAME and RAPD analysis among *Thymus* species growing in eastern Anatolia region of Turkey. Romanian Biotechnological Letters **14(2),** 4313–4318.

Wright S. 1951. The genetical structure of populations. Annals of Eugenics **15,** 323–354.

Xia T, Chen SL, Chen SY, Ge XJ. 2005. Genetic variation within and among populations of *Rhodiola alsia* (Crassulaceae) native to the Tibetan Plateau as detected by ISSR markers. Biochemical Genetics **43(3–4),** 87–101.

Yeh FC, Yang RC, Boyle T. 1999. Popgene version 1.31. Microsoft windows-based freeware for population genetic analysis. University of Alberta, Canada Biochemical Genetics **48,** 603–611.

Vagelgsang S. 1998. Pre-emergence efficiacy of Phomopsis convolvulus Ormeno to control field bindweed (*Convolvulsu arvensis* L.). Ph. Thesis, Montreal, Canada.

Ward S, Jasieniuk M. 2009. Rewiev: Sampling weedy and invasive plant populations for genetic diversity analysis. Weed Science **57,** 593-602.