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Genetic variability of invasive species, *Fallopia convolvulus* in Iran

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Fallopia (Polygonaceae) as a noxious weed contains 17 species in the world out of which, three species occur in Iran with invasive distribution. *F. convolvulus* growing in wide range of soil types cause significant problems for native ecosystems of river banks. In this study we have examined genetic variability in *F. convolvulus* for the first time in Iran. Ten Inter Simple Sequence Repeats markers (ISSR) were used to study the genetic variability on 11 populations of this species. Genetic diversity parameters, genetic distance and gene flow were determined. Genetic variation at inter- and intra-populational level were evaluated by different methods. AMOVA and structure analyses revealed high genetic diversity within populations. Mantel test revealed a significant correlation between genetic and geographic distances. Between populations a limited gene flow was observed. It is concluded that local adaptation, low gene exchange and genetic drift can affect genetic diversity of *F. convolvulus*. Despite self-compatibility of this species, it is proposed that out-crossing may be occur because of higher genetic variation among populations of this taxon.

Keywords: *Fallopia convolvulus*; Genetic diversity; Gene flow; Invasion; ISSR

Introduction

Fallopia Adans. (Polygonaceae) has 17 species in different parts of the world. It was previously nested in *Polygonum* s.l. (The Plant List 2013). *Fallopia* species are widely distributed as a weed in cultivations or disturbed areas in temperate regions of the world (Qaiser 2001). It contains annual and perennial herbs, which can be distinguished by their racemose inflorescences with bisexual flowers, triangular, rounded or oval leaves, obvious petioles, short to sub-sessile fused styles (partial) with smooth or papillate stigmas, 8 stamens and 5 perianth clefts from other genera. It has erect or tendril stems and triangular achenes (Yasmin et al. 2009).

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Fallopia species are noxious weeds with invasive distribution (Tiébré et al. 2007b). In Iran, *Fallopia* is represented by three species as *F. convolvulus* (L.) Á. Löve, *F. dumetorum* L. and *F. baldshuanica* (Regel) Holub (Mozaffarian 2012), among which the latter is introduced and cultivated as ornamental species. *F. convolvulus* and *F. dumetorum* are known as weeds associated with crops and display anthropogenic behaviours (Tiébré et al. 2007a; FAO 2015).

F. convolvulus is an annual herb with prostrate or climbing stem up to 1m long, sometimes branched. Leaves are petiolate, rounded, ovate or oblong- ovate, 50 × 30 mm, with cordate or hastate base. Inflorescence has axillary flowers, perianth segments 5, 2-2.5 mm length, green with white margin, outer tepals bluntly angular without wings. Nuts are triangular, black, granulate (Rechinger and Schiman-Czeika 1968; Mozaffarian 2012).

Each plant of *F. convolvulus* can produce over 30000 seeds, which can remain dormant for many years (Forsberg and Best 1964; Roberts and Feast 1973; Conn and Deck 1995). Farming activities contribute in seed dispersal of *F. convolvulus*. Moreover, water can disperse seeds over short distances (Rutledge and McLendon 1996). Seed germination can occur in soil depth between 6 and 51 mm and temperature between 5°C and 15°C (Forsberg and Best 1964).

F. convolvulus grows in a wide range of soil types, causing significant problems for native ecosystems of river banks (Hume et al. 1983). This species could be considered as a problem for different cultivations in the temperate zone. It is problematic in case of cereals, and may decrease the production of potato, sugar beet and vegetables by competition. This weed occurs on different types of soils, lodging in

grain crops and causing harvesting problems (Hume et al. 1983). Its high densities can be considered as a contaminating agent for seed resources (Holm et al. 1991).

Tkachuk and Mellish (1977) stated that seeds of *F. convolvulus* are of edible importance as its amino acid content is very similar to buckwheat ones. It is believed that *Fallopia* seeds were used as a source of food from neolithic to medieval times (Hanf 1990). But They are not cultivated nowadays due to the low-yield. Its seeds and leaves are edible for birds (Wilson et al. 1999). It is also a host for some kinds of pathogenic agents as fungi and viruses (Royer and Dickinson 1999).

F. convolvulus has been observed to grow in polluted soils, so it can be considered as bio-accumulator agent with potential ability in phytoremediation (Pedersen et al. 2000).

Today, plant invasion is globally increased and threat biodiversity, ecosystem and agriculture. Genetic analyses of invasive plant populations can provide us new insight about invasion process (Ward et al. 2008). These plants can adapt and expand in to new habitats. Plants grown in verity environmental conditions may show differences in terms of their genetic features as a result of adaptations (Anderson et al. 2011).

Inter Simple Sequence Repeats (ISSR) markers are simple, fast and reproducible, therefore can be a great choice for study of population genetic diversity (Wolfe et al. 1998). Moreover, since this technique has been previously used for genetic structure of other invasive plants (Innis et al. 2011; Ueno et al. 2015; Vicente et al. 2018), it is demonstrated to be suitable for such studies.

In this study, we would like to asses genetic diversity and structure among *Fallopia convolvulus* populations for the first time in Iran by ISSR markers. This study could provide new attitude to this species under genetic context. Also, it could produce

useful theoretical and experimental data for further control and management of plant invasion.

Material and methods

Plant materials

Eighty-five individuals of 11 populations of *F. convolvulus* were collected from different locations of Iran (Figure 1). Voucher details of populations are deposited in Herbarium of Alzahra University (ALUH) (Table1). [Figure 1 and Table 1 near here]

ISSR analysis

Fresh leaves of specimens dried in silica gel powder and leaves of herbarium vouchers were used. Genomic DNA was extracted by CTAB modified protocol (Križman et al. 2006). Ten ISSR primers comprising UBC 834, UBC 810, UBC 811, UBC 823, UBC 807, UBC $(GA)_9C$, UBC $(GA)_9A$, UBC $(GA)_9T$, UBC $(CA)_7AC$ and UBC $(CA)_7GT$ were used. Polymerase Chain Reaction (PCR) amplification was done by a Thermal Cycler (Bio-Rad, USA) using 25 µl reaction mixture containing 10 mM Tris-HCl buffer (pH=8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM single primer, 20 ng genomic DNA and 3 U Taq DNA polymerase (Bioron, Germany). PCR procedure was done as follows: 93°C for 3 min; 40 cycles of 93°C for 20 s, annealing at 55°C for 1 min, and extension at 72°C for 20 s; final extension at 72°C for 6 min. Agarose gel electrophoresis (1%w/v) was used for PCR product. Fragments were stained with Gel Red.

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Data analyses

Reproducible amplified ISSR bands were scored as binary codes: 1 for presence and 0 for absence. Different parameters of genetic diversity including number of effective alleles, Shannon's information index (I), gene diversity (He), unbiased genetic diversity (UHe) and Percentage of Polymorphic Bands (PPB) were resolved using PopGene software ver. 1.32 (Freeland et al. 2011). Nei's genetic distance matrix was used to construct UPGMA tree using Mega 7 software (Kumar et al. 2016).

The plant specimens were grouped by Multi-Dimensional Scaling (MDS) using PAST software ver. 2.17 (Hammer et al. 2001). Analysis of Molecular Variance test (AMOVA) with 1000 permutations was done by GenAlex software ver. 6.4, to illustrate the molecular difference among and within populations (Peakall and Smouse 2006). Mantel test was used to show the relationship between genetic and geographical distance by PAST software ver. 2.17 (Hammer et al. 2001).

Nei's genetic identity and distance were measured to show the genetic relationship between populations, (Nei 1978). The presence of gene flow at interpopulation level was tested by two different methods:

- Reticulation analysis (Legendre and Makarenkov 2002) by DARwin software ver. 6.
- Nm value (Nm = 0.5 (1 - GST)/GST) by PopGene software ver. 1.32.

Bayesian based model structure analysis was applied to consider the genetic admixture of populations (Pritchard et al. 2000). In order to find the correct number of k, Evanno test was applied on structure results (Evanno et al. 2005).

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Results

Ten ISSR primers produced 80 loci, among which 71 loci (88.75%) were polymorphic. Each primer amplified 9 to 19. The range of polymorphic bands was from 10 to 19. The size of bands was from 50 to 1500 bp.

Different features of genetic diversity are listed in Table 2. The highest gene diversity (He) was observed (0.2) in Velenjak population (no. 7), while the lowest one was detected in Khanbebin population (no. 5) (0.066). [Table 2 near here]

The highest and the lowest values of Shannon's information index and unbiased genetic diversity were also observed in Velenjak population (no. 7) and Khanbebin population (no. 5), respectively. Velenjak (no. 7) and Shafa Rud (no. 11) populations had the highest and the lowest percentage of polymorphism, respectively. Number of alleles ranged from 0.493 to 1.425, and effective alleles ranged from 1.106 to 1.317 in Velenjak population (no. 7) and Khanbebin population (no. 5).

AMOVA test showed that, there was a significant difference among the genetic of populations studied (P=0.01). It revealed that most of variations were among populations (71%) versus 29% within populations (Table 3). [Table 3 near here]

Nei's genetic identity compared to genetic distance of populations studied showed that, Vanak (no. 1) and Dehnar (no. 9) populations had the highest genetic similarity (0.9855), but Firuzkuh (no. 4) and Shafa Rud (no. 11) populations showed the lowest similarity (0.8003) (Table 4). [Table 4 near here]

The UPGMA tree based on Nei's genetic distance (Figure 2) produced four branches. Populations 1, 9, 5, 7, 2, 3 (Vanak, Dehnar, Khanbebin, Velenjak, Doab, Veresk) and 6 (Kalak-e- bala) formed the first group. Within this cluster, populations 1 (Vanak), 9 (Dehnar) and 5 (Khanbebin) showed more affinity, while other populations

were placed in separate sub-clusters. Populations 8 (Karaj-Qazvin) and 10 (Asara) formed the second branches. Populations 4 (Firuzkuh) and 11 (Shafa Rud) were placed in two other clusters. [Figure 2 near here]

Both intra- and inter-populational genetic diversity was shown by MDS plots of ISSR results (Figure 3). The highest amount of genetic variation at intra-population level was observed in populations 7 (Velenjak) and 9 (Dehnar). Populations 5 (Khanbebin) and 9 (Dehnar), and populations 1 (Vanak), 2 (Doab), 3(Veresk) showed inter-populational genetic similarity. Shafa Rud (no. 11) and Firuzkuh (no. 4) populations showed the highest inter-population variation. [Figure 3 near here]

Mantel test with 5000 permutations illustrated a significant relationship between genetic and geographic distances ($r = 0.2026$, $P = 0.0002$). This shows the importance of geographic isolation in shaping the genetic structure of each population.

The mean $Nm = 0.97$, acquired for total ISSR loci, revealed a limited amount of gene flow between populations. Additionally, the reticulograms showed low degree of inter-populational gene flow among populations studied (Figure 4). [Figure 4 near here]

Clustering based on populations grouped by Bayesian method ($k = 2–11$) was done to show the genetic composition of populations. Evanno method showed that, the best number of populations is $K=7$ (Figure 5). The structure plot showed (Figure 5) that each population contained special allelic mixture (The most frequent colour) and smaller differently coloured segments, pointing to the degree of gene flow with other populations. According to this figure, Firuzkuh (no. 4), Khanbebin (no. 5), Karaj-Qazvin (no. 8) and Shafa Rud populations (no. 11) showed low level of genetic variation in their allelic composition, while populations of Vanak (no. 1), Doab (no. 2), Kalak-e-bala (no. 6) and Dehnar (no. 9) had the highest genetic variation. Moreover,

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Vanak (no. 1) and Dehnar (no. 9), and Doab (no. 2) and Veresk (no. 3) had an almost similar allelic composition. Generally, the structure plot showed within-population genetic differentiation, supporting AMOVA results. [Figure 5 near here]

Discussion

Invasive species are species of interest due to their ability in colonizing and distribution into new environments. Despite this interest, the influencing parameters in successful invasion are undetermined or poorly studied (Rollins et al. 2013; Ueno et al. 2015). Molecular markers provide valuable procedures to clarify evolutionary forces contributing to genetic diversity of invaders (Matesanz et al. 2014).

Invasion of other *Fallopia* species has been documented by different approaches in Europe and North America (Conolly 1977; Bailey and Conolly 2000; Hollingsworth and Bailey 2000; Gammon et al. 2007; Grimsby et al. 2007; Pashley et al. 2007; Tiébré et al. 2007b). Although, *F. convolvulus* has shown invasion in some parts of Southern hemisphere (Webb et al. 1988; Mackee 1994; Wilson 2008; Acevedo-Rodríguez and Strong 2012) and has been reported as an introduced taxon in North America and some parts of Europe (CABI 2019), there is no record on genetic structure of *F. convolvulus*.

Previous studies on populations of two other taxa of Polygonaceae in Iran, *Polygonum aviculare* and *Persicaria minor*, showed high amount of genetic variation. In both taxa, genetic diversity was higher within populations than among populations (Mosaferi et al. 2015; Sheidai et al. 2016). High intra-population polymorphism was also reported in other *Fallopia* species such as *F. japonica* (Bzdęga et al. 2012). In this study, AMOVA showed high within population variation. This genetic diversity can be

seen in structure results. Our results support genetic polymorphism and were in agreement with them.

The value for gene flow is a key factor that affects genetic structure among populations. When Nm> 1, the gene flow is an important factor to increase genetic similarity between groups and resist genetic drift within populations. If 1>Nm>0.5, the gene flow is weak and when 0.5>Nm>0, the groups are totally isolated (Zhang et al. 2013). In the present study, the value for Nm is 0.97 which showed some degrees of gene flow between populations, which was in accordance with genetic admixture in structure analysis.

The results of the present study demonstrated a significant positive relation between genetic difference and geographic distance, so isolation by distance (IBD) occurred in *F. convolvulus* populations.

Previous studies on invasive species with self-mating systems such as *Alliaria petiolata* (M. Bieb.) Cavara & Grande, *Centaurea diffusa* Lam., *Miconia calvescens* DC. and *Polygonum cespitosum* Blume showed heterozygote deficiencies in populations (Durka et al. 2005; Marrs et al. 2008; Hardesty et al. 2012; Matesanz et al. 2014). In this study, all populations showed low heterozygosity.

Despite of the findings of Mulligan and Findlay (1970) who mentioned *F. convolvulus* as a self-compatible species, in present study, a high level of intra-population genetic diversity was observed. This level of diversity shows that *F. convolvulus* is not a completely self-compatible species and some degree of outcrossing may be occurred in this taxon in studied area.

Plant populations can invade different habitats geographically through local adaptation (Matesanz et al. 2014; Sheidai et al. 2016). *F. convolvulus* can grow in wild

range of soil types and has been adapted to wide range of habitats (Hume et al. 1983; Rashed Mohassel et al. 2001). Generally, it is concluded that factors such as local adaptation, confined gene exchange and genetic drift can influence genetic diversity of *F. convolvulus*. The results of the present study were in agreement with those of previous studies (Leinonen et al. 2013; Oduor et al. 2016).

In conclusion, this is the first study of genetic diversity of *F. convolvulus* in Iran. Generally, self-mating species have lower genetic variation at intra-population level than inter-population one (Durka et al. 2005; Novak and Mack 2005; Matesanz et al. 2014). Higher within populations genetic diversity was found in *F. convolvulus*. This assumption that the taxon is not completely self-compatible needs more evidence to be proved.

As an invasive weed, number of specimens of *F. convolvulus* has been significantly increased, and it causes a serious problem in cereal fields in North of Iran (Gherekhloo and Sohrabi 2014). Effective planning regarding the invasive plant control requires information on reproduction, population dynamics and genetic structure of invaders. Molecular markers can provide reliable data on genetic population of plants. Knowing more about biology, seed dispersal and habitat preferences of these plants, can help us control and manage the plant invasion.

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Table 1. Populations, their localities and voucher Numbers.

Table 2. Genetic diversity parameters in *Fallopia* populations studied (N: number of samples, Na: Mean number of alleles, Ne: number of effective alleles, I: Shannon's information index, He: expected heterozygosity, UHe: unbiased expected heterozygosity, PPB: Percentage of polymorphic bands).

Table 3. Analysis of Molecular Variance (AMOVA) in *Fallopia* populations studied.

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Figure 1. Geographical distribution of studied populations.

Figure 2. UPGMA tree in studied populations.

Figure 3. MDS plots of genetic differences among studied populations.

Figure 4. Reticulograms of studied populations.

Figure 5. Structure analysis of *Fallopia convolvulus* populations (a: the relationship between K and LnP(D); b: the relationship between K and Delta K; c: the grouping when K = 7).