ECO-GEOGRAPHICAL VARIATIONS OF ISSRS AMONG POPULATIONS OF *Onobrychis viciifolia* (SAINFOIN, FABACAE)

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Abstract. Studies on the genetic variation and structure of plant population are important for evaluation of dynamics population and conservation management. We studied the population genetic variation of *Onobrychis viciifolia* in five ecologically different regions in East-Azerbaijan, Iran using Inter-Simple Sequence Repeats (ISSR) markers. Populations' genetic diversity were estimated based on Nei' and Shannon's indices using polymorphic ISSRs loci. The relationship of populations' geographical and genetic distances and also population altitude and ISSRs variations were investigated. Similarity among populations was assessed by UPGMA clustering analysis. The results showed that polymorphic ISSRs loci varied from 38.75% to 61.25% among populations. Within-population Nei's diversity ranged from 0.1182 to 0.1790. Partitioning of total ISSRs diversity by AMOVA revealed that 67.45% diversity allocated to within- and 32.55% diversity to among-populations, indicating that *O. viciifolia* is predominantly an outcrossing species. This study showed that population geographical distances was not correlated with Nei distances (N=10, P *vale* $\geq$ 0.56) suggesting lack of gene flow among populations. Similarly, the altitude of populations had no impact on populations ISSRs variations (N=5, *P value* $\geq$ 0.227). The populations of *O. viciifolia* growing under salinity stress had both higher and lower ISSRs variability. This cast doubt on Nich-Width Theory, which expects higher variations from stressful populations. Therefore, it can be concluded that populations' genetic patterns in *O. viciifolia* might be affected by random change, rather than ecological selection. Comparison between the levels of ISSRs variations and those levels of RAPDs previous reported from the same populations showed that RAPDs detect significantly greater variation than ISSRs (N=5, P value $\leq$0.01, at $P \leq 0.05$ level).

Keywords: ecological selection, genetic diversity, genetic similarity, ISSRs, outcrossing, *Onobrychis viciifolia*, Sainfoin

INTRODUCTION

The human civilization has resulted in different environmental issues including the habitat fragmentation and destruction, environmental degradation pollution and over-exploitation, which consequently have dramatically changed the structure, distribution and function of natural ecosystems [25]. The habitat fragmentation has caused genetic consequences in terms of genetic drift [3, 12, 15], and therefore, loss of biodiversity [11, 25]. Studies on the effect of fragmentation on plants populations have largely focused on genetic structure diversity of plant populations [12, 15] and reproductive dynamics [2, 10]. In the small and fragmented populations these issues result in decreased genetic diversity and random genetic drift [11, 14]. Assessing the levels of genetic variations and structures of plant populations is important in conservation biology [13, 22].

On the basis of Nich-Width Theory, populations growing under the environmental stresses e.g. salinity should have higher genetic variation than those growing on environments with no stresses [7, 26]. This theory has been supported by several empirical evidences in plant populations [7, 8].

Different DNA markers have been used in assessing the population genetic variation in plant taxa. Among these markers, Randomly Amplified Polymorphic DNAs (RAPDs) and Inter-Simple Sequence Repeats (ISSRs) markers have been frequently used in evaluating the levels of population genetic variability. More recently, ISSRs have been the markers of choice for studies of population genetic studies in plants such as *Coffea arabica* [1], *Gossypium* [4], *Magnolia wufengensis* [9], *Ipomoea batatas* [18], *Allium* [19], *Secale cereal* [23], *Abelmoschus esculentus* [27] and *Michelia coriacea* [28]. ISSRs technique amplifies genomic DNA between two Simple Sequence Repeats (SSRs) with inverse orientations using primers with a single SSR motifs anchored at the 3' or 5'end by a few nucleotides. ISSRs allow detection of variation in inter-microsatellite loci. ISSRs are particularly suitable for evaluation of genetic diversity among plant populations of the same species [12]. Several comparative studies using both ISSRs and RAPDs in evaluating population genetic variation on different plant taxa have resulted in conflicting data. Some studies indicated that ISSRs have ability to reveal more variation than RAPDs, while other works showed the opposite.

The current work investigated levels of genetic variability of five eco-geographically different populations of *Onobrychis viciifolia* (sainfoin, Fabaceae) and compared the obtained results with those levels, which we previously reported from the same populations using RAPDs [21].

MATERIAL AND METHODS

Plant species and location

The wild populations of *Onobrychis viciifolia* are distributed across East-Azerbaijan Province in Iran. The excess grazing limited the seed production and sexual propagation of the plants in the areas. This may consequently result in declining of genetic variability. Five different wild populations of *Onobrychis viciifolia* were investigated from eco-geographically different regions in East-Azerbaijan (Iran) by randomly sampling 10 individual plants from each population. Two populations located in warm area with saline soils

(Amand and Bonab), the other two populations situated in high altitude and cold climate (Sarab and Heris), and the fifth population located in the middle of the other populations (Khosroshahr). The populations were isolated from each other by either large geographical distance (over 50 km) or high mountains and large cities.

DNA extraction, PCR profile and ISSRs loci analysis

Nuclear DNA was extracted from the leaves following Nosrati et al. [21]. Concentration of the DNA samples was measured by spectrophotometry and subsequently adjusted at 10ng/ml. Ten different ISSRs primers were of which four primers produced the most polymorphic loci and therefore, were selected for study (Table 1). The ISSRs amplifications were repeated three times to ensure the reproducibility of the banding patterns. The ISSRs bands were scored as 1 for present and 0 for absent of band. A binary matrix was constructed and Nei and Shennon indices were calculated using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, ver. 2.02). The number and percentage of polymorphic ISSRs loci were obtained for each population. Genetic diversity within each population was estimated using Nei's and Shannon's information index (Popgen ver. 1.32). UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) dendrogram was generated based on matrix of Nei's distance in order to detect similarity among the populations, through the SHAN (sequential, hierarchical, agglomerative and nested clustering of the NTSYS-pc). Total genetic variation was partitioned into within- and among- populations based on analysis of molecular variance (AMOVA) using Arlequin ver. 3.11. The significance level for F-statistics analogous was determined using 1023 bootstrap replicates. Correlation between populations geographical and genetic Nei' distances was evaluated using Pearson Rank Correlation test. The levels of populations' genetic variations obtained by ISSRs and RAPDs [21] were compared using Mann-Whitney U test.

RESULTS

ISSRs polymorphism and genetic distance

Four ISSRs primers applied on 50 individuals sampled from five eco-geographically different populations of *Onobrychis viciifolia* produced 80 clear and reproducible polymorphic bands (Fig. 1). Percentage of polymorphic ISSRs loci and Nei' diversity ranged respectively from 38.75% and 0.1182 in Amand to 61.25% and 0.1790 in Heris populations. This range of population genetic variation was found to be from 0.1816 to 0.2779 based on Shannon's diversity for the above mentioned populations (Table 1).

Partitioning the total genetic variation into components of within and among populations by AMOVA indicated that 67.45% of total genetic variation belongs to within populations and 32.55% to among populations (Table 2).

The UPAGMA dendrogram constructed on the basis of Nei's distance (Table 3) clustered the ecologically different populations of Bonab and Heris in the same cluster, while separated the ecologically similar populations of Bonab and Amand from each ohter (Fig. 2).

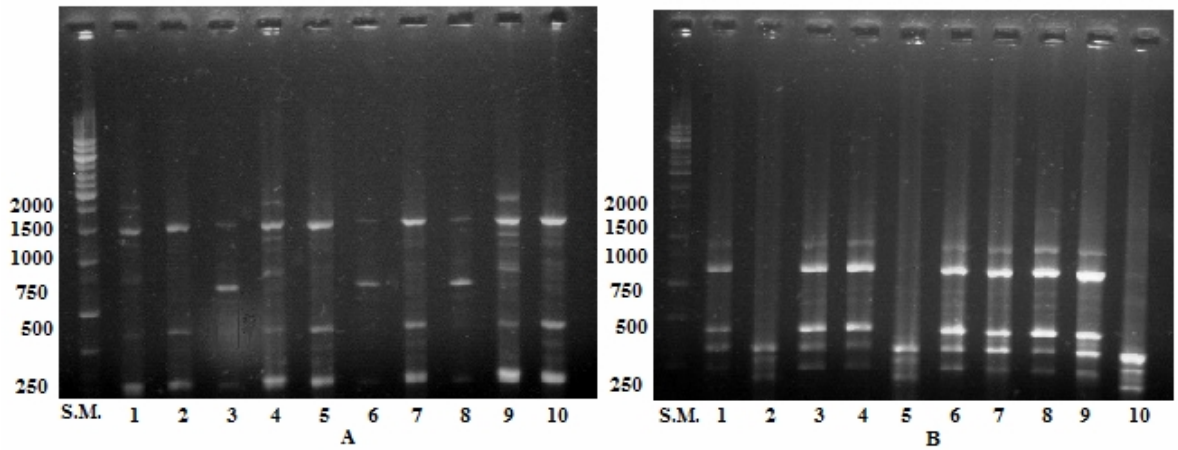

Figure 1. Samples of ISSRs gel electrophorese produced by primer E in two populations (A= Heris, B= Amand) of *Onobrychis viciifolia*

Table 1. Percentage of polymorphic ISSRs loci and Nei' and Shannon genetic diversity revealed in five populations of *Onobrychis viciifolia*

Population	Altitude	% ISSRs loci	Nei' diversity	Shannon diversity
Amand	1400	38.75	0.1182	0.1816
Sarab	1680	40	0.1212	0.1908
Khosrosha	1500	45	0.1237	0.1933
Bonab	1290	53.75	0.1393	0.2230
Heris	1900	61.25	0.1790	0.2779

Table 2. Partitioning of total ISSRs-based genetic variation into within and among populations of *Onobrychis viciifolia*

Components of total genetic variation	df	Sum squ. variation	Variance	% Variance	P value
Among populations	4	168.38	3.4868	32.55	< 0.00
Within populations	45	325.2	7.2267	67.45	< 0.01

Table 3. Matrix of Nei's genetic distance between population pairs of *Onobrychis viciifolia*.

Population	Amand	Bonab	Sarab	Khosrosha	Heris
Amand	0				
Bonab	0.1046				
Sarab	0.1133	0.0653			
Khosrosha	0.1475	0.0541	0.0933		
Heris	0.0897	0.0358	0.0741	0.0823	0

Correlation between Nei's-based genetic and geographical distance among ten population pairs (Table 4) revealed the lack of significant correlation between genetic and geographical distances (Pearson Rank Correlation Test, N=10, P vale ≥ 0.56, at $P \leq 0.05$ level) (Fig. 3).

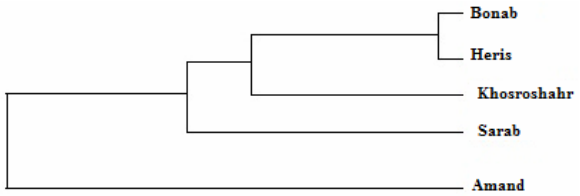

Figure 2. Nei's distances-based UPGMA dendrogram showing genetic similarly among populations of *Onobrychis viciifolia*.

Table 4. Geographical (Km) and ISSRs based Nei's genetic distances among population pairs of *Onobrychis viciifolia*

Population pair	Geographical distance	Genetic distance
Amand - Bonab	150	0.1046
Amand - Sarab	150	0.1133
Amand - Khosrosha	50	0.1475
Amand - Heris	70	0.0897
Bonab - Sarab	80	0.0653
Bonab - Khosrosha	110	0.0541
Bonab - Heris	160	0.0358
Sarab - Khosrosha	130	0.0933
Sarab - Heris	40	0.0741
Khosrosha - Heris	70	0.0823

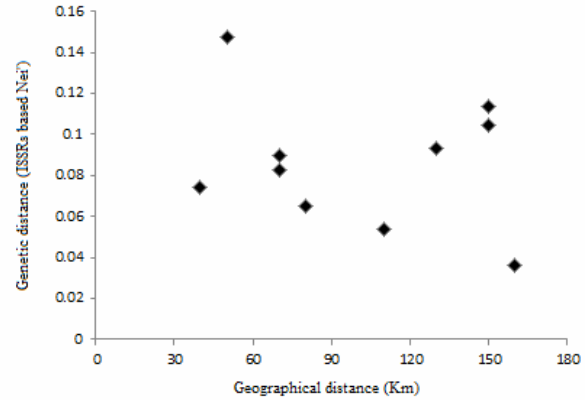

Figure 3. Lack of correlation between ISSRs based Nei's genetic and geographical distance among ten population pairs of *Onobrychis viciifolia* (Pearson Rank Correlation Test, N=10, P vale ≥ 0.56, at $P \leq 0.05$ level)

The correlation analysis among populations altitude and genetic Nei's distance based on ISSRs indicated that the altitude did not significantly influence the populations variation as revealed by ISSR in *Onobrychis viciifolia* (Pearson Rank correlation test, N=5, P value ≥ 0.227, *at* $P \leq 0.05$ level).

The levels of populations' genetic variation in *Onobrychis viciifolia* based on ISSRs-Nei's diversity ranged from 0.1182- 0.1790, while this range for the same populations was 0.2466- 0.3186 on the basis of RAPDs [21]. Comparing these results with our previous results regarding RAPD markers revealed significantly higher variation than ISSRs in *O. viciifolia* (Mann-Whitney U test, N=5, P value ≤0.01, at $P \leq 0.05$ level).

DISCUSSION

The results of the current study showed that levels of population genetic diversity revealed by ISSRs were dramatically lower than those variations previously detected by RAPDs in the same populations [21]. This is consistent with a general expectation that RAPDs have ability to detect more variation in plant population than ISSRs. For example, in comparative studies on *Cassia* [17] and *Allium porrum* [19] RAPDs detected more variations than ISSRs. The possible reason to justify the relatively higher variation detected by RAPDs is that RAPDs monitors the whole genome, while ISSR covers only the regions located between two microsatellites loci. However, in other species e.g. *Cassia* [17], *A. cepa* [19], *Brassica napus* [24] and *Cocos nucifera* [16] ISSRs variations were found to be higher than those of RAPDs. It should be noticed that in *Brassica napus* [24] higher ISSRs variation was observed using higher number of primers compared to RAPDs primers. In *Cocos nucifera* using the same number of ten primers of RAPDs and ISSRs resulted in respectively 86 and 97 polymorphic bands [16]. Studying on the levels of genetic variation in *Arthrocnemum macrostachyum* [5] using seven ISSRs and twenty RAPDs primers produced, on average, 11.4 ISSRs and 7.95 RAPDs polymorphic loci per primer. Similarly, in *Momordica charantia* [6] fifteen ISSRs primers produced, on average, 6.3 polymorphic bands per primer while twenty nine RAPDs primers gave 2.6 polymorphic bands per primer. Similar results were

reported from comparative study on *Picea mariana*, *P. glauca*, and *P. engelmannii* [20]. The higher levels of genetic variations detected by ISSRs compared to RAPDs indicate the occurrence of huge numbers of SSRs (Simple Sequence Repeats) loci in plant genomes, which are used by ISSRs primers as annealing site. Our study regarding the ability of ISSR and RAPD in detecting the genetic variation indicate that the patterns and levels of genetic variability vary and depends on the species of plant taxa.

Our results indicated that the populations of *Onobrychis viciifolia* growing under environmental stresses of soil salinity had either greater or lower ISSRs diversity. This finding is consistent with our previous results previously reported from the same populations using RAPDs [21]. Our both studies on populations of *O. viciifolia* disagree with the Niche-Width Variation Theory [26], which expects higher genetic variation in populations distributed in regions under the environmental stresses. Therefore, it can be concluded that populations' genetic patterns in *O. viciifolia* might be affected by random change, rather than ecological selection.

The AMOVA analysis showed that the majority of total ISSRs variations in populations of *O. viciifolia* belong to within-populations. This is consistent with AMOVA analysis based on RAPDs in the same populations. Both our current ISSRs and previous RAPDs [21] analyses indicated that *O. viciifolia* is predominantly an outcrossing species. Lack of correlation between geographical and genetic variations in populations of *O. viciifolia* indicates lack of gene flow among these populations.

Our studies alongside others show that the patterns of genetic variation estimated from ISSRs and RAPDs are more diverse and that both markers are useful for investigating population genetic variations and structure.

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