

Volume 2, Issue 2

Research Article

Date of Submission: 03 Sep, 2026

Date of Acceptance: 03 Oct, 2026

Date of Publication: 13 Oct, 2026

Assessment of Genetic Diversity Landraces in *Onobrychis Sativa* Using SSR, RAPD, and SRAP Markers

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Citation: Sedeh, M. S. (2026). Assessment of Genetic Diversity Landraces in *Onobrychis sativa* Using SSR, RAPD, and SRAP Markers. *J Mol Genet Gene Res*, 2(2), 01-06.

Abstract

In order to evaluate genetic diversity of cultivated sainfoin accessions collected from different regions of the world, SSR markers from the *Medicago* genus were used. Polymorphism information content (PIC) values ranged from 0.20 to 0.43, with an average of 0.33. Cluster analysis based on SSR markers grouped the sainfoin populations into two main groups (Iranian and exotic cultivated sainfoin), with two sub-clusters within each group.

In another survey performed to determine the efficiency of ecological factors on genetic similarity, five populations of *Onobrychis viciifolia* from East Azerbaijan, Iran, were investigated using RAPD markers and bulked DNA analysis. The largest and smallest genetic distances were detected between Bonab and Heris (0.4904), and Amand and Khosroshahr (0.0572), respectively, on the UPGMA dendrogram. Two populations, Sarab and Heris, were nested within one cluster, while Amand and Khosroshahr were grouped in another cluster. Bonab was completely separated from all other populations.

Keywords: Sainfoin, Markers, Multiphase

Key Points

I survey Genetic Diversity in sainfoin to select best adjectives in sainfoin. After select best adjectives, you can produce best lines for sainfoin modify. And use them as food for animal.

Introduction

Please include line numbering in your manuscript (Wikipedia.org). According to its seed specification, it has been divided into 270 categories. Resistance to dryness is the most important characteristic of this plant, making it appropriate for dry farming and pastures. The larger content of tannins in common sainfoin leaves prevents bloat and protein hydrolysis in the livestock rumen and increases amino acid absorption in the intestine of ruminants [1,2].

Fabaceae provender is of great importance. Moreover, knowledge about the rate of polymorphism with differences between different examples is important in order to reduce the rate of hereditary resources kept in the gene bank and to analyze the purity of seeds in cross-pollinated plants. Studying genetic diversity via morphologic markers is accompanied by limitations such as low polymorphism, low heritability, and effectiveness due to environmental conditions [3,4].

The Molecular markers reveal genetic diversity at the DNA level and do not show the problems associated with morphologic indicators. Moreover, molecular markers are prominent tools for evaluating relational selection of better plants as well as for analyzing the similarities and differences among various species (Awasthi et al.). In this regard, micro-satellites or simple sequence repeats (SSR) are repeats of DNA sequences that were primarily thought to exist only in non-coding regions of DNA. However, with time, the occurrence of micro-satellites in coding sequences or translated sequences was discovered, and EST-SSRs were developed [5].

Micro-satellite markers usually show a higher level of polymorphism, and by considering the frequency of alleles at each gene locus, they can distinguish very similar species. Extracting SSR primers through genome libraries is an expensive and tedious method requiring proper skills. By having access to a number of ESTs and other information about DNA sequences, developing SSR markers through information extraction has become more effective and less costly for many plant species [6-8].

Although many EST databanks for modeled and non-modeled organisms are available in recent years and new generation technologies for finding sequences have created a great evolution in genetic research, the share available for a plant such as common sainfoin is still rather limited in the databanks.

Thus, a simple method for developing SSR primers in species that have not been extensively studied is the use of SSR primers from related species. Genomics has made apparent comparisons, and the order and contents of genes among closely related species are greatly conserved. Marker harmony shown in comparative mapping indicates that markers of the same material or type can exist in another material or type [6].

The number of 27 SSR markers from *Medicago sativa* and *Glycine max* was tested for transferability in *O. viciifolia* by (2011) [9]. After identifying markers with the potential to create polymorphisms, six pairs of SSR markers were used for fingerprinting and estimating genetic similarities in 23 cultivated sainfoin accessions and two wild types. Thus, cultivated sainfoin populations and two wild types were separated based on geographical origin [9].

In another study, identified the transferability of 95 markers from *Medicago truncatula* and *Phaseolus sativa* to sainfoin and selected 18 markers for analyzing the genetic diversity of sainfoin.

RAPD markers are widely used in studies concerning diversity, population structure, and genetic similarity among plant populations. These markers are suitable for genome studies and for investigating natural selection effects on the genetic structure of plant populations. Although RAPD markers have limitations, such as their dominant nature and inability to distinguish homozygous from heterozygous genotypes, SRAP markers are simple, highly reliable, provide broad genome coverage, and have the potential to amplify coding regions of the genome [10-12].

Materials and Methods

The population of the considered farming sainfoin included 5 masses of Iranian and 17 foreign sainfoin masses, provided by direct collection and sampling from gene banks (IPK/Germany and USDA/USA) (Table 1).

S.No	Population	HeightfromSeaSurface(m)	Geographicalwide	Weather
1	Banab	1290	37/3292	Warm
2	Amand	1400	38/1878	Warm
3	Khosroshahr	1500	38/0011	Semiwarm
4	Sarab	1680	37/9264	Cold
5	Haris	1900	38/2556	Cold

Table 1: Ecological Characteristic of Cultivated Sainfoin Population

A population of local *Medicago sativa* alfalfa was also used as the control sample. Two sets of seeds were cultivated in medium plastic pots in the research farm of Isfahan University of Technology in spring 2012. Two weeks after growing of seedlings, 10 samples were randomly selected from each population, and sampling was done from healthy and fresh leaves.

Extraction of DNA from the leaf samples of sainfoin was done by. To determine the quality and quantity of DNA, the gel (0.7%) of agarose in TBA 0.5× with ethidium bromide dye (10 mg/ml) and GelDoc device were used [13].

Since the micro-satellites (SSR) within *Onobrychis* are not known, screening the identified SSRs with *Medicago* provides the possibility of finding similar regions on the *Onobrychis* genome. In this study, 22 pairs of SSR and EST-SSR primers of *Medicago* containing high polymorphism information content were selected from different resources (Table 2).

S.No	Code	Country	Geographical Wide	Geographical length	Height
1	VICUNS8	Iran	53/00	32/00	1200
2	VICESF9	Iran	53/00	32/00	1200
3	VICUNS18	Iran	53/00	32/00	1200
4	VICUNS39	Iran	53/00	32/00	1200
5	VICUNS42	Iran	53/00	32/00	1200
6	VICRUS112	Russia	55/75	37/61	144/17
7	VICRUS113	Russia	55/75	37/61	144/17
8	VICRUS122	Russia	55/75	37/61	144/17
9	VICGES104	Kyrgyzstan	48/01	67/1	342/9
10	VICGES108	Kyrgyzstan	48/01	67/1	342/9
11	VICMAS134	Hungary	47/26	19/15	378/2
12	VICENS107	England	51/5	0/11	12/19
13	VICENS118	England	51/5	0/11	12/19
14	VICASS127	Slovakia	48/66	19/68	1081/12
15	VICGES119	Germany	51/15	10/45	459/94
16	VICHOS130	Netherland	52/11	5/28	7/92
17	VICSWS123	Switzerland	46/81	8/21	1777/59
18	VICSWS124	Switzerland	46/81	8/21	-
19	VICROS114	Romania	45/93	24/96	596/18
20	VICROS120	Romania	45/93	24/96	596/18
21	VICSPS115	Spain	40/27	3/73	895/8
22	VICUNS106	Unknown	-	-	-

Table 2: Various Pair of SSR and EST-SSR Primer of Medicago

Polymerase chain reaction (PCR) for the samples in 15 µL volume including 1.5 µL of buffer 10x, 1.5 µL of magnesium chloride, a unit of tag polymerase, 0.3 mmol of dNTP, 0.25 µmol of each starter, and 50 ng of genome DNA was prepared from each sample.

Reaction was made in a thermal cycler device in a primary blotting cycle in 94°C for 4 minutes, and 35 cycles of secondary blotting in 94°C for 1 minutes, with connection of the primer to DNA string in 50-55°C for 45 seconds, and expansion of synthesized DNA in 72°C for 2 minutes, as well as an al expansion cycle in 72°C for 10 minutes.

Polymerase chain product went under electrophoresis on acrylamide gel (12%) and dyeing was done by silver nitrate. A percentage of polymorph for each starter was obtained by division of polymorphic places and the number of alleles in the places. The polymorphic information content (PIC) was calculated for each band by the following relation [14,15].

$$PIC = 1 - \sum_i^n P_{ij}$$

P_{ij} is the abundance of amino allele in marker "i", population is "j", and "n" is the number of alleles. In this study, 5 different populations of farming sainfoin that are growing wild in different regions in East Azerbaijan province were analyzed. Ten (10) samples of the plant were randomly selected from each population.

Geographically, the individual plants in this population are away from each other, in 50 km distances. The studied populations are different with regards to the sea levels, geographical latitude, weather conditions, and soil salinity (Table 1).

Two populations in Amand and Khosroshahr are grown in rather salty soil, and in rather close geographical distances. Two populations in Heris and Sarab are growing in cold climates and higher sea levels, in closer distances, while the population in Bonab is comparatively in further distances than the other 4 populations, and ecologically, due to growing in properly salty soil, it is different to the other populations.

Regarding geographical latitudes, the populations in Sarab and Khosroshahr are closer to each other, and the populations in Heris and Amand are in almost equivalent geographical latitudes, while the latitude in Bonab has an enormous difference with others.

In this test, DNA from young seedlings and seeds were extracted by method, which is a faster and more economical method in comparison with other common methods in DNA extraction. The DNA of an individual in a population after extraction were mixed, in order to only prepare one DNA sample, i.e. mass DNA, as the representative of that population [16].

Estimating the density of extracting DNA was done by electrophoresis of agar gel (0.8%) and spectrophotometry in 260 nm, to adjust the final density of DNA samples in 10 ng/ml. Polymorphism of RAPD strings was tested for 5 studied populations by using 10 random starters. Hence, the contents of 10 ng of model DNA and primer with a density of 100 PM/ μ L in the final volume of 25 μ L were analyzed.

The number of 17 different bulks of sainfoin plant were collected that belongs to the four species: *O. viciifolia* (13 bulks), *O. viciifolia* (1 bulk), *O. viciifolia* (1 bulk), and *O. viciifolia* (1 bulk) from different regions in the country, and after extraction of DNA from their fresh leaves and determining their quality and quantity, the extracted DNA was used in PCR reactions, and 88 strings were obtained from the total of 10 primer compositions, 73 of which were polymorphic.

Eight pairs of SSR primer and one pair of EST-SSR primer in sainfoin out of 26 pairs of SSR and EST-SSR primers of medicago type (Table 2) indicated polymorphic forms (Table 3), which were used for analyzing genetic diversity in farming sainfoin populations.

Population	Amand	Bonab	Sarab	Khosroshahr	Haris
Amand	0	-	-	-	-
Bonab	0.3851	0	-	-	-
Sarab	0.1178	0.3851	0	-	-
Khosroshahr	0.0572	0.3363	0.1178	0	-
Haris	0.0870	0.4904	0.0870	0.1495	0

Table 3: Genetical Distance Matrix Between Five Population of *Onobrychis Sativa*

Nine (09) pairs of used micro-satellite primers could separate 44 alleles for evaluating 22 populations of sainfoins, and the number of alleles varied between 4 to 6, and the size of reproducing components were in the range of 100-400 pairs. Polymorphic information content was changing between 0.2 to 0.43 distances for studied micro-satellite places, having an average of 0.33. Only half of the 10 primers produced clear and gradable polymorphic strings. To ensure from repeatability of the produced strings, PCR was repeated for the studied samples with the 5 primers, for three times, and the patterns of strings were similar for the three repetitions, indicating the repeatability of the mentioned strings.

The five primers produced 12 polymorphic strings in total, and the highest rate of polymorphic strings was related to no.1 and no.2 primers, each with 3 (0.25) polymorphic strings and the lowest rate was related to no.2, no.3, and no.5 primers, each with 2 (16.7%) polymorphic strings.

For the matrix of calculated genetic distance, the greatest genetic distance (0.4904) was between the populations of Bonab and Heris, and the lowest distance (0.0572) was between the populations of Amand and Khosroshahr, and 0.087 in the next stage, between populations of Heris and Sarab and also between Heris and Amand, based on the genetic distance of "Nei" on a UPGMA dendrogram.

With the greatest genetic similarities, the populations of Amand and Khosroshahr were placed in a cluster, while the populations of Sarab and Heris were in another cluster, and the population of Bonab was placed in a separate cluster due to the least amount of genetic similarity as compared to the other mentioned populations.

Discussion

The genetic diversity of 48 individual plants from four cultivated populations of alfalfa (*Medicago sativa* L.) in Iran was assessed using sequence-linked amplified polymorphic markers (SRAP). Fourteen SRAP primer combinations produced 193 fragments, of which 95 were polymorphic. The number of polymorphic fragments identified in each primer combination ranged from 3 to 10 bands with an average of 6.78 bands. The average polymorphic information content (PIC) for all primer combinations was 0.343.

Although AMOVA (analysis of molecular variance) results showed significant differences in genetic diversity among populations ($P < 0.0001$), genetic diversity, which was mainly due to intrapopulation variation, accounted for 93.17% of the total genetic diversity. Analysis of marker data using unweighted pair-group arithmetic mean (UPGMA) method clearly separated subtropical (Yazdi) and subcold (Hamedani and Nikshahri) populations as well as the improved Kodi population.

It can be concluded that SRAP markers are useful for studying diversity and relationships between and within alfalfa populations. Overall, the results show that the obtained markers of medicago type can be suitable resources for identifying the place of micro-satellites in sainfoin. Micro-satellite markers are considered to have capacity for analyzing relationships and genetic diversity in sainfoin plants.

Moreover, the used SSR markers in the present study show high polymorphism. Populations of Iranian and foreign sainfoin plants were placed in separate populations, indicating a high genetic diversity in the considered germplasm. Moreover, high genetic diversity between Iranian and foreign sainfoin populations is suitable for modification programs in order to reach maximum heterosis.

On analyzing the genetic diversity, SRAP marker in sainfoin plant genetic diversity between the masses, the range of genetic distance between masses was found to be in between 124 and 349. Regarding the dispersion of bulks (masses), the lowest genetic distance was between the masses related to Russia (codes P and Q), and the greatest distance was in between the Damavand and Najafabad regions (codes G and M).

The numerical value (33.1) obtained for gene movements between groups shows genetic moves in the studied populations, but the move is not to such an extent to cause homogeneity in the populations. The range for information index for primer compositions was calculated to be in between 31 and 52. High overall heritability values for height, number of stems and percentage of stems indicate the major role of genetic effects in controlling these traits [17].

Given that these traits are also considered components of forage yield, indirect selection through them can increase the return from selection for improved forage yield. China was superior to Iran in terms of forage yield. Iranian varieties had a significant advantage over foreign varieties in terms of yield and its most important components. On the other hand, foreign varieties were superior only in terms of leaf percentage and leaf to stem ratio, which are considered as criteria for palatability and forage quality. It is

Results

The present study results on the farming of wild populations of sainfoin show that the populations growing in similar ecologic conditions (similar ecotype) and located at close distances to each other have more genetic similarities.

According to the present study findings using DNA RAPD, we can identify the ability of different ecotypes of the cultivated species. In cluster analysis of SRAP data, dendrograms drawn by the NTSYS VERSION 2 could not place the used genotypes in separate groups. In other words, mixture of masses with each other indicates little inter-mass genetic diversity.

Analysis of the main coordination also showed the first three components justified 62.95% of the changes. Thus, analysis of clusters by using SRAP data is a best method for evaluating inter-mass genetic diversity of sainfoin.

Declarations

Source of funding: None

Conflicts of Interest

There is no financial conflict with the subject matter discussed in the manuscript.

Additional Declarations

The authors declare no competing interests.

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