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Morphological and genetic diversity of *Senecio vulgaris* L. (Asteraceae) in Iran

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Running title: Intraspecific diversity of *Senecio vulgaris* L.

Abstract – *Senecio vulgaris* L., an annual herb belonging to the Asteraceae, is widely distributed in different regions of the world. There is no information on the intraspecific variations of the morphological and molecular features of this species. In the present investigation, we studied the morphological and genetic diversity of 81 accessions of *S. vulgaris* collected from 10 geographical populations. Eleven inter simple sequence repeat (ISSR) primers were used for the examination of genetic variations among the populations. Analysis of molecular variance (AMOVA) and G_{ST} analyses revealed significant differences among the investigated populations. A significant correlation between genetic distance and geographical distance was revealed by the Mantel test. However, reticulation analysis indicated the occurrence of gene flow among most of the populations studied. Principal component analysis (PCA) plot showed that the number of capitula, length of the cauline leaf and plant height were the most variable morphological characters. Principal coordinates analysis (PCoA) plot revealed two groups of populations, according to molecular and morphological data. The results suggested the existence of possible intraspecific taxonomic ranks within this species.

Keywords: gene flow, groundsel, morphology, Principal Component Analysis

Introduction

The genus *Senecio* L. (Asteraceae) includes ca. 1250 species and is one of the largest genera of flowering plants (Bremer 1994, Calvo et al. 2015). In Iran, some species of *Senecio* have a widespread distribution in the different geographical regions, such as *S. vulgaris* L., *S. glaucus* L., and *S. leucanthemifolius* subsp. *vernalis* (Waldst. et Kit.) Greuter.

Senecio vulgaris, commonly called groundsel or old-man-in-the-Spring, is an annual herb that grows in a variety of habitats from open grassland to woodland. It is usually regarded as a temperate weed and although it is almost cosmopolitan it appears to be more localized in temperate regions of America and Asia, Mediterranean areas and South Africa (Chater and Walters 1976).

Senecio vulgaris has a long history of herbal use and is widely used as an anthelmintic, antiscorbutic, diaphoretic, diuretic, emmenagogue and purgative (Hatfield 1977, Launert 1981, Lust 1983). A homeopathic remedy is made from aerial parts of the plant. The therapeutic activity of groundsel is related to a variety of biologically active substances such as phenols and flavonoids, essential oils, polysaccharides, triterpenes, amines, saponins, tannins and mucilage (Uzun et al. 2004, Conforti et al. 2006).

A population genetics survey plays the main role in the planning of genetic and breeding programs. It provides data about genetic diversity, gene migration, allelic drift, genetic fragmentation, genetic bottleneck and any other evolutionary forces acting on population divergence (Sheidai et al. 2013).

Intraspecific taxonomic entities can be identified after a detailed population-based investigation within plant species. In general, extensive morphological and genetic divergences among populations result in speciation events. Intraspecific variation occurs within both wild and cultivated crop plants (Maxted and Hunter 2011).

In Iran, *S. vulgaris* grows in different areas and forms numerous local geographical populations. There is no information about its genetic diversity and adaptation against population divergence. Population genetic studies not only can provide information on these aspects but can also unravel the speciation process of *Senecio* in general. One method for the study of genetic diversity employs morphological characteristics, but molecular markers are the potential tools to study genetic relations, phylogeny, population dynamics or gene- and genome mapping. .

The advent of molecular markers resulted in an improved ability to track evolution through a good understanding of genetic diversity among populations and new phylogenetic viewpoints (Muller-Scharer and Fischer 2001, Stuessy et al. 2014). Among molecular markers, inter simple sequence repeat (ISSR) deserves special attention as a tool for analyzing diversity. ISSR is easy to use, quick, simple and highly reproducible (Azizi et al. 2014, Eftekharian et al. 2016). ISSR markers usually show high polymorphism and have the very important advantage that no prior information about the genomic sequence is required (Kojima et al. 1998, Bornet and Branchard 2001).

This study aimed to investigate the genetic variation between *S. vulgaris* populations collected from different geographic regions by using ISSR and morphological characters. Also, gene flow and correlation of genetic and geographical distances were estimated.

Material and methods

Plant specimens

Eighty-one plant specimens were collected from ten geographical regions (On-line Suppl. Fig. 1). Details of localities and voucher specimens are showed in Tab. 1. Morphological characteristics of *S. vulgaris* accessions were measured by a binocular microscope (Olympus BH2 ×400).

DNA extraction and PCR amplification

For the molecular study, a sub-sample of 0.5 g leaves from each population was taken and genomic DNA was extracted by the CTAB method (Sheidai et al. 2013).

Eleven ISSR primers were tested for PCR amplification; (AGC) 5GG, (AGC) 5GT, (CA) 7GT, (CA) 7AT, (GA) 9C, UBC807, UBC810, UBC811, UBC823, UBC834, and UBC847. PCR reactions were done in a total volume of 25 μL containing 1 μL primer (12.5 pmol), 0.5 μL dNTPs mix (10mM), 20 ng template DNA, 1.5 μL reaction buffer (10×), 1.2 μL $MgCl_2$, 1.5 U *Taq* DNA polymerase and 18.5 μL deionized water.

The PCR reaction was performed with a thermal cycler (Techne, Germany) with the following program: 94 °C for 5 min, 30 cycles at 94 °C for 30 sec, 55 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 10 min. The PCR products were electrophoresed at 85 V on 1.5% agarose gels and the resulting bands were observed under a UV transilluminator. The DNA fragment sizes were estimated after comparison with a 1-kb DNA ladder. Polymorphic ISSR markers were manually scored as binary data with presence as "1" and absence as "0".

Multivariate analysis

The one-way analysis of variance (ANOVA) was used to indicate a significant difference of morphological characters among the studied populations. Principal component analysis (PCA) was used to recognize important variable morphological characters in the studied populations. Principal coordinate analysis (PCoA) was carried out based on morphological and molecular data with Euclidean distance as a similarity index. Statistical analysis was performed using the program PAST v. 2.17c. (Hammer et al. 2001).

Genetic diversity and population variation parameters were performed by PopGene software v. 1.32 (Yeh et al. 1999). Mantel permutation test was used to check the correlation between genetic distances and corresponding geographical distances of the studied populations by GenAlex software v. 6.5. (Peakall and Smouse 2006).

Analysis of molecular variance (AMOVA) test was used to determine significant genetic differences among the studied populations with the use of GenAlex v. 6.5. Genetic differentiation was estimated by $D_{,ST}$ = Jost measure of differentiation (Jost 2008) and $G_{,ST}$ = standardized measure of genetic differentiation (Hedrick 2005).

The occurrence of allele flow among populations was estimated by reticulation analysis (Legendre and Makarenkov 2002).

Results

Morphological variability

Twenty-three qualitative and quantitative characters were selected for morphological evaluation of *S. vulgaris* populations (Tab. 2). ANOVA test showed that there are significant differences ($P \leq 0.01$) among the quantitative morphological characters of studied populations.

The most important morphological characters among the studied populations were identified by PCA-biplot. The bi-plot of the PCA based on quantitative morphological characters showed that the number of capitula, length of the cauline leaf and plant height are important markers in the distribution of the populations studied (Fig. 1). It showed two factors (PC1 and PC2) which together explained 82% of the total variance. The PC1 (51% of variance) had positive correlations with the number of capitula, and a negative significant correlation with length of the cauline leaf. PC2 (variance 31%) had positive correlations with plant height (Tab. 3).

The PCoA plot of both quantitative and qualitative characters divided the studied populations into two groups (groups I and II) based on morphological characters (Fig. 2). Populations No. 5, 7 and 9 were grouped into I and the rest of the studied populations were placed in Group II.

The capitula numbers of populations of the first group ranged between 6 to 9 and between 4 to 7 in populations of Group II. The means of the length of cauline leaf were 6 cm and 4 cm in Group I and II populations, respectively. Also, the mean of plant height of Group I populations was 25 cm whereas it was 18 cm in Group II populations.

Moreover, the stem type of Group I populations was often branched and the black tips of the bracts were more than 1 mm long whereas populations of another group mostly have an unbranched stem and the black tips of the bracts were less than 1 mm long (Fig. 3).

ISSR marker

In this study, 121 reproducible ISSR fragments (200-800 bp) were obtained utilizing the PCR amplification. ANOVA analysis revealed significant genetic differences among the studied populations (PhiPT = 0.83, $P \leq 0.01$). It also showed that most of the genetic variation (60%) occurred within populations, while 40% was due to inter-population genetic differences.

These results revealed the presence of a high level of genetic differentiation among *S. vulgaris* populations. This result was also supported by the G_{st} analysis and Hickory test.

Moreover, population differentiation parameters determined among the studied populations produced high values for Hedrick, standardized fixation index after 999 permutation (G'st = 0.886, P ≤ 0.001) and Jost, differentiation index (D-est = 0.275, P ≤ 0.001).

Various parameters of genetic diversity calculated in 10 geographical populations of *S. vulgaris* are shown in On-line Suppl. Tab. 1. The results obtained from the common genetic diversity indices are similar to the unbiased gene diversity parameter (which is free from the sampling size). The polymorphic loci percentage in each population varied from 10.54% to 55.61% (On-line suppl. Tab. 1). Kazerun population (Pop1) showed the highest percentage (55.61%) of polymorphic loci of all the populations while the Velenjak population (Pop 5) exhibited the lowest amount of polymorphism (10.54%). The values of Shannon's information index (I) varied from 0.062 to 0.298 in all the populations. The gene diversity (He) for all loci in each of the population ranged from 0.039 to 0.189.

PCoA analysis showed that there were genetic differences among studied populations of *S. vulgaris* based on ISSR data. Populations 5, 7 and 9 were categorized into separate groups while the rest of the studied populations were distanced from them (Fig. 4).

The Mantel test showed that there is a significant correlation (r = 0.5, P < 0.01) between genetic and geographic distance (On-line Suppl. Fig. 2). This result demonstrated that gene exchange occurred between populations that were close to each other.

A reticulogram revealed some degree of shared alleles among most of the populations. (Fig. 5) These shared alleles might be due to ancestral and or ongoing limited gene flow among the studied populations.

Discussion

Population genetic investigations are useful in understanding genetic variability, allele flow, inbreeding against outbreeding and effective population size. This information is valuable in choosing effective management in conservative plans and also throws light on the presence of intraspecific taxonomic forms and suggests an appropriate hybridization strategy (Freeland et al. 2011, Sheidai et al. 2013, 2014).

Genetic diversity of *S. vulgaris* was studied by Muller-Scharer and Fischer (2001). According to their investigation different geographical areas include different habitats and populations representing different habitats varied in their sizes.

In our recent study, the genetic structure of *S. glaucus* showed that population fragmentation, restricted gene flow, genetic drift, and local adaptation have played a role in the genetic divergence of *S. glaucus* populations in Iran (Eftekharian et al. 2016).

In the present investigation, *S. vulgaris* populations presented a high degree of genetic variability. Species that are dispersed over a wide area face different environmental conditions and therefore can possess a wide genetic and morphological variability to manage ecological challenges (Freeland et al. 2011, El-Amier et al. 2014, Eftekharian et al. 2015, 2016).

In this study, population fragmentation in *S. vulgaris* is revealed by PCoA. However, some degree of gene flow and genetic admixture occurred among populations as shown by a reticulogram. The probable loss of genetic variation within these populations can be prevented by this limited gene flow due to the action of genetic drift (Habibollahi et al. 2015).

According to the Mantel test, which presented a pattern of isolation-by-distance in the studied *S. vulgaris* populations, adjacent populations have more chance for gene exchange than more distant populations, which is why more genetic similarities have been shown in closely situated populations.

Comparison of the two datasets indicated that in some cases, the grouping of populations based on the morphological method was consistent with molecular groupings.

Therefore, the PCoA plot based on molecular and morphological data showed two groups of populations.

Genetic and morphological differences between the two groups may be related to different ecological conditions. Ecological and environmental factors (e.g., temperature, humidity, and soil factors) can be significantly effective in shaping genetic diversity patterns (Huang et al. 2016). According to Muller-Scharer and Fischer (2001) different habitats can affect the genetic structure of *S. vulgaris* populations. Tang et al. (2015) showed that there was a strong relationship between the soil factors (especially salinity and soil texture) and plant diversity. In our study, the habitat of studied populations varied from mountain meadows (Populations 5, 7 and 9) to roadsides (Populations 2, 3, 4, 6 and 10) and waste grounds (Populations 1 and 8). The mountain meadow habitats had gravelly soils while loamy soils were predominant in the waste ground and roadside habitats. However, the effects of some factors in plant population genetic diversity remain unclear and more investigations have to be done.

Conclusion

Morphological characters revealed that two groups of the studied geographical populations differed from each other in two qualitative morphological features (black tips of calyculus bracts and type of stem), as evidenced in Fig. 3 and three quantitative characters (capitula number, length of the cauline leaf and plant height). Therefore, in these populations, morphological changes accompanied genetic differences and this pattern of arrangement has confirmed the existence of possible intraspecific taxonomic ranks within this species.

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Captions

Tab. 1. Geographical information concerning the studied populations of *Senecio vulgaris*.

Population	Locality	Habitat type	Altitude (m a.s.l.)	Longitude (E)	Latitude (N)	Voucher No.
Pop1	Fars, Kazerun	Waste ground	904	29.64	51.64	95109
Pop2	Khuzestan, Andimeshk	Roadsides	185	32.44	48.34	95105
Pop3	Khuzestan, Masjed soleiman	Roadsides	150	31.13	49.20	95104
Pop4	Fars, Nurabad	Roadsides	985	30.11	51.54	95110
Pop5	Tehran, Velenjak	Mountain meadows	1758	35.48	51.23	95101
Pop6	Lorestan, Khorramabad	Roadsides	1215	33.29	48.22	95103
Pop7	Alborz, Karaj	Mountain meadows	1327	35.38	50.92	95107
Pop8	Ilam, Ivan	Waste ground	1144	33.82	46.30	95106
Pop9	Tehran, Lavasan	Mountain meadows	1720	35.75	51.60	95102
Pop10	Khuzestan, Ahwaz	Roadsides	15	31.31	48.67	95108

Tab. 2. Quantitative and qualitative morphological characters of *Senecio vulgaris* accessions (length measured values are in cm). SD – standard deviation; N – number of analyzed samples.

Quantitative characters	**mean ± SD (N)**
Plant height	21.45 ± 8.71 (81)
Length of basal leaf	3.96 ± 1.08 (176)
Width of basal leaf	1.35 ± 0.53 (176)
Thickness in base of stem	3.21 ± 0.91 (81)
Length of cauline leaf	4.54 ± 1.16 (214)
Width of cauline leaf	1.97 ± 0.62 (214)
Number of capitula	6.13 ± 1.42 (81)
Length of peduncle	0.21 ± 0.08 (295)
Length of bract on a peduncle	0.37 ± 0.08 (205)
Number of involucral bracts	21.19 ± 0.59 (81)
Length of involucral bracts	0.64 ± 0.05 (232)
Number of calyculus bracts	10.1 ± 2.49 (81)
Length of corolla	0.67 ± 0.05 (217)
Length of corolla tube	0.40 ± 0.05 (217)
Length of corolla lamina	0.28 ± 0.04 (217)
Length of anther in disc florets	0.18 ± 0.01 (110)
Length of style in disc florets	0.09 ± 0.01 (192)
Length of papus	0.6 ± 0.08 (305)
Length of nutlet	0.31 ± 0.03 (273)
Qualitative characters	
Type of stem	Branched- unbranched
Stem indumentum	Yes - No
Blackness of calyculus bracts	Less than 1mm – More than 1mm
Blackness of involucral bracts	Less than 1mm – More than 1mm

Tab. 3. Correlation of morphological characteristics of the studied *Senecio vulgaris* populations with two components of principal component analysis (PCA).

Character	Component	
	PC1	PC2
Number of capitula	0.908	0.132
Length of the cauline leaf	-0.895	0.115
Length of peduncle	0.531	0.043
Plant height	0.144	0.951

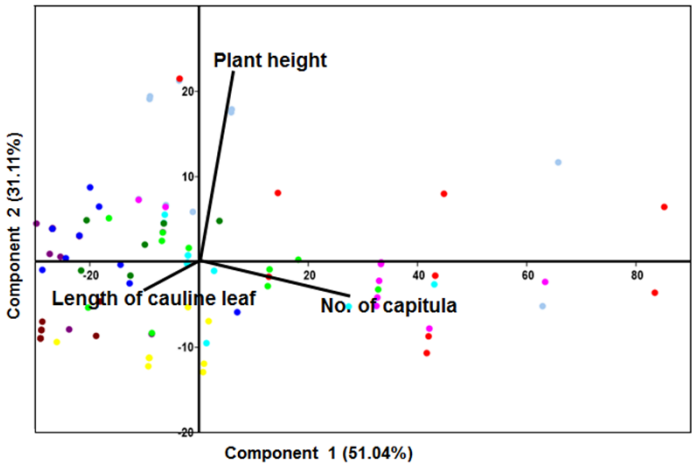


Fig. 1. Principal component analysis (PCA) among studied population based on quantitative morphological characters. Component 1 and component 2 refer to the first and second principal components, respectively. Arrows represent the correlations between the independent variables and the two principal components represented. Each point represents an individual sample.

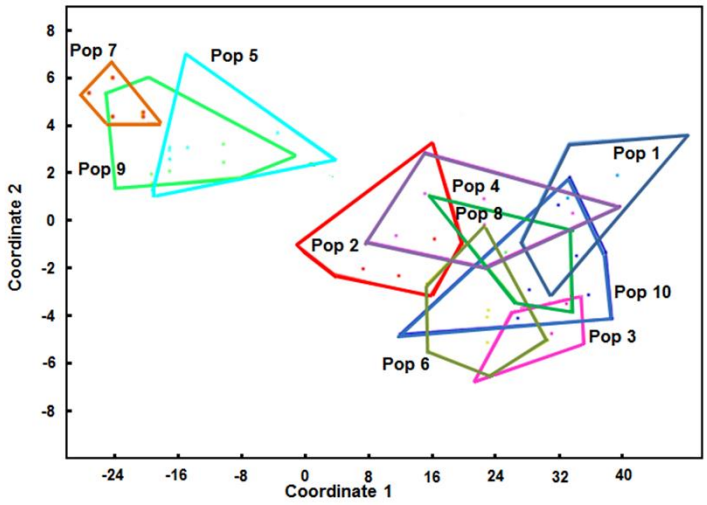


Fig. 2. Two dimensional plot of principal coordinate analysis (PCoA) of the studied *Senecio vulgaris* populations based on morphological characters. Different colors indicate the plant specimens from each geographical population. Group 1: the populations in north Iran (Populations 5, 7 and 9); Group 2: the populations in west and south west Iran (Populations 1, 2, 3, 4, 6, 8 and 10).

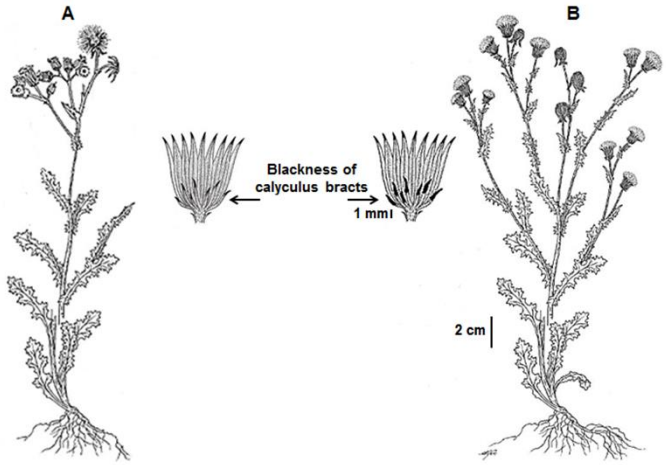


Fig. 3. Morphological characters of two different types of *Senecio vulgaris*. A – unbranched stem and bract black tip less than 1 mm long, B – branched stem and bract black tip more than 1 mm long (adapted and modified from Klinkenberg 2019).

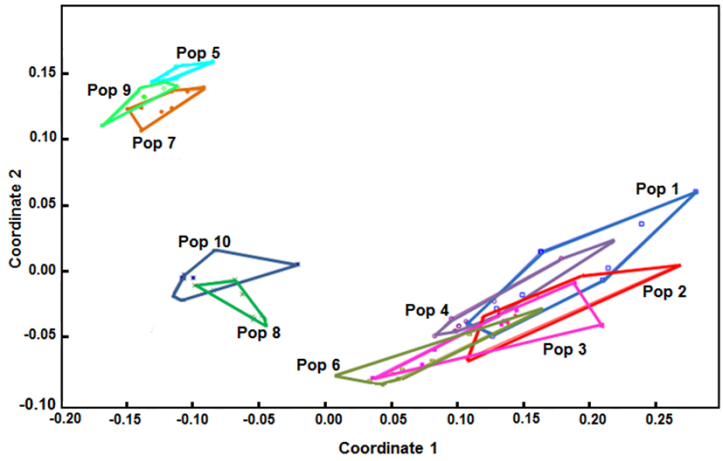


Fig. 4. Two dimensional plot of principal coordinate analysis (PCoA) of the studied *Senecio vulgaris* populations based on ISSR data. Different colors indicate the plant specimens from each geographical population. Group 1: the populations in north Iran (Populations 5, 7 and 9); Group 2: the populations in west and south west Iran (Popu;atopms 1, 2, 3, 4, 6, 8 and 10).

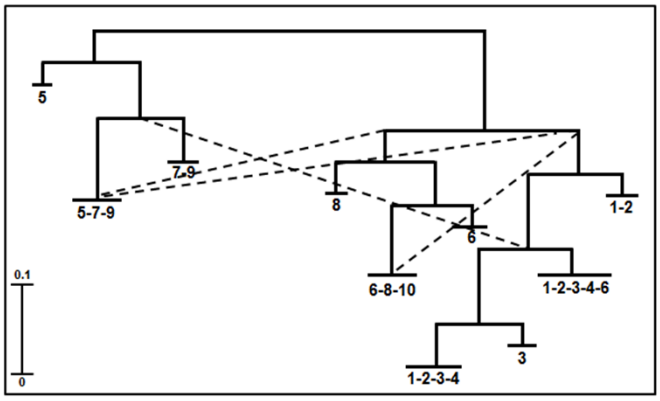
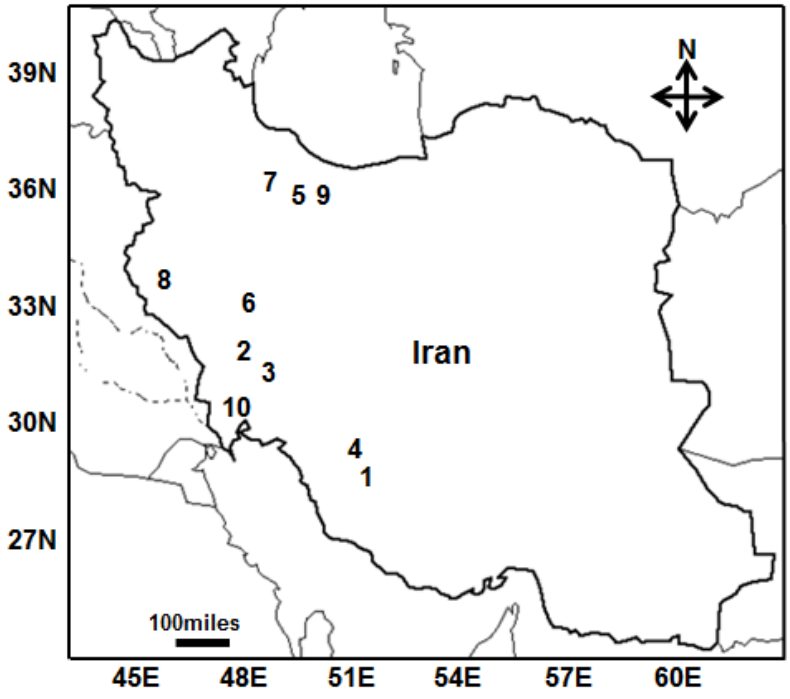


Fig. 5. Reticulogram of the studied *Senecio vulgaris* populations based on a neighbour joining tree of ISSR data. Populations are marked with numbers from 1-10 according to Tab. 1. Dashed lines indicate gene flow. Reticulation analysis revealed that limited amount of shared alleles or gene.

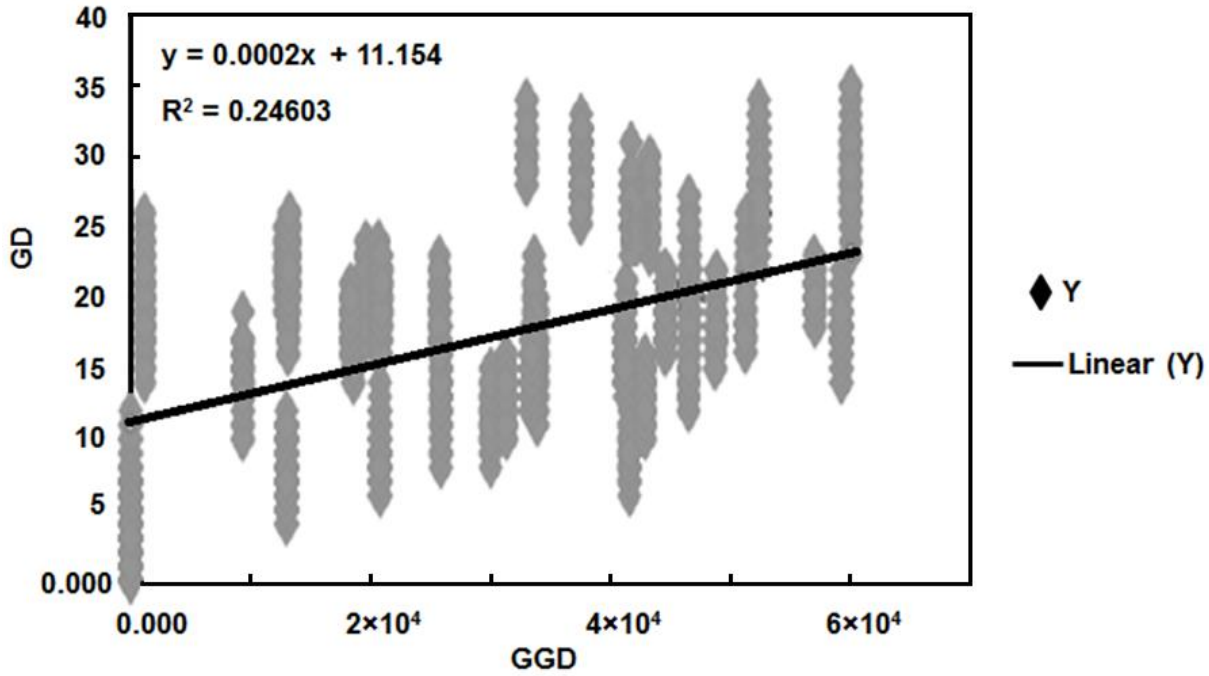
On-line supplemental materials

On-line suppl. Tab. 1. Genetic diversity parameters estimated within the populations of *Senecio vulgaris*. N – number of specimens, P – polymorphism percentage, Na – number of different alleles, Ne – number of effective alleles, I – Shannon information index, He – gene diversity, UHe – unbiased gene diversity.

Population	N	P (%)	Na	Ne	I	He	UHe
Pop1	9	55.61	1.187	1.289	0.298	0.189	0.195
Pop2	9	41.50	0.903	1.219	0.221	0.137	0.142
Pop3	7	49.76	1.063	1.241	0.246	0.143	0.151
Pop4	8	32.31	0.701	1.158	0.166	0.098	0.102
Pop5	6	10.54	0.351	1.023	0. 062	0.039	0.044
Pop6	10	39.14	0.876	1.189	0.197	0.128	0.133
Pop7	9	12.51	0.398	1.041	0.074	0.046	0.051
Pop8	8	13.24	0.419	1.054	0.078	0.053	0.059
Pop9	7	15.31	0.442	1.067	0.095	0.072	0.076
Pop10	8	22.32	0.628	1.098	0.125	0.097	0.102



On-line suppl. Fig. 1. Map of Iran, showing localities of the collected populations of *Senecio vulgaris*.



On-line suppl. Fig. 2. Mantel test plot of genetic distance (GD) allied with geographical distance (GGD) in the studied *Senecio vulgaris* populations. A significant positive correlation exist between Nei's genetic distance and geographic distance (km) ($r = 0.5$, $P < 0.01$).