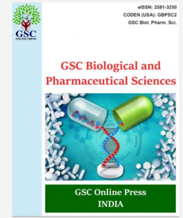
GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/



(Research Article)

Unravelling genetic diversity and genetic structure of *Senna italica* Mill. in Rabigh region, Saudi Arabia

Hassan Mansour *

Department of Biological Sciences, Rabigh-College of Science and Art, King Abdulaziz University, Rabigh 21911, Saudi Arabia.

GSC Biological and Pharmaceutical Sciences, 2024, 29(03), 183-189

Publication history: Received on 08 November 2024; revised on 18 December 2024; accepted on 20 December 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.3.0479

Abstract

Senna italica is a perennial plant distributed in desert and subtropical regions of the world. In Rabigh Province, western Saudi Arabia, the few persisting populations of this species are exposed to many threats, including overcutting and, recently, human habitation. These threats are predicted to be exacerbated with the advancement of aridification caused by climate change. The conservation and revival of the diminished populations of *S. italica* requires an assessment of their genetic diversity and genetic differentiation. To accomplish this objective, we applied 10 simple sequence repeat (SSR) primer pairs, with which 10 polymorphic loci were confirmed. These polymorphic loci were used to determine the population genetics of 60 plant accessions sampled from 5 populations of *S. italica* located in five sites in Rabigh Province: Wadi EL Khaneg, Wadi Al Johfa, Wadi Al Hakak and Wadi Khurieba and Wadi Wadi Albaidaa . Low to moderate levels of genetic diversity were found in all populations (the values of the PPL% ranged between 51.5% and 21.5%) along with a decreased value of H_T (0.451) and a considerable inbreeding value (F= 0.4552), which verified an obvious shortage of heterozygotes. High genetic differentiation among the populations and a low level of gene flow suggest isolation among the *S. italica* populations, which caused a severe deficiency in gene migration. The data obtained herein will inspire several recommendations for conservation the existing populations, including seed preservation, and management of human activities. All of these actions are urgently needed to prevent imminent extinction.

Keywords: *Senna Italica*; Conservation; Populations; Genetic Diversity; Differentiation; Rabigh

1. Introduction

Ashrek, *Senna italica* Mill*,* (Fabaceae) is a deciduous, perennial herb, or small shrub up to 60 cm tall with a prostrate stem. It is grown throughout tropical Africa, Arabia, Iraq, Iran, and southwestern Asia. Usually, it is found close to streams and in sandy and rocky valleys sides, and in disturbed habitats such as waste places about towns and country dwellings, abandoned gardens, roadsides. It. *S. italica* is cultivated and traded in many countries where it is originated for using its dried leaves and pods as a purgative, for skin problems, ulcers, liver complaints, gall bladder disorders, nausea, vomiting and dysmenorrhoea. The leaves and young pods are used as forage. The dried, powdered leaves for use as a hair conditioner [2-4]

S. italica aqueous extract shows anti-inflammatory, antimicrobial and pharmacological prospective due to its constituents like the anthraquinone glycosides(sennosides and their aglycone sennidin, emodin, aloe emodin, rhein and chrysophanol) which are responsible for the laxative properties [5].

The distribution of *S. italica* is limited to valleys and hills surrounding Rabigh, western Saudi Arabia. The apparently small populations of *S. italica* could be argued to the increasing aridity of this environment [6]. Anthropogenic influences are likely to lead to further decline in the population sizes of *S. italica* and other related species [7].

* Corresponding author: Hassan Mansour

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Individuals of the existing *S. italica* populations may suffer low genetic diversity because of co-occurring genetic drift, which is a leading factor in the low fitness and the capability of populations to acclimatize to environmental dynamics [8]. Therefore, interpreting the genetics and structure of the plant populations of *S. italica* is necessary to conserve and improve this plant species as well as, other plant species [9].

Plant populations in arid habitats are subject to ambient risks that threaten survival, including the loss of genetic diversity, genetic drift, and inbreeding [10].

Because of global climate change and human overutilization, many plants of the Rabigh valleys, including *S. italica*, are threatened with extinction, owing to reductions in population size and the subsequent erosion of genetic variation. For exploring genetic diversity and genetic structure, microsatellites DNA offer precise technology of detecting molecular basis for gene variation than any other marker system, because they target well variable repeat regions of the genome, because they can be deduced as co-dominant genetic markers [11].

In the present work, the distribution pattern, genetic diversity and genetic structure outlines among and within *S. italica* are considered using microsatellite loci. This research will provide information on the levels of inbreeding within *S. italica* populations prior to the development of conservation approaches. The proposed genetic analyses of this species will be helpful to improve its existing genetic diversity.

2. Material and methods

2.1. Plant material

Five populations of *S. italica* were sampled form five different sites in Rabigh region, Western Saudi Arabia (Table 1). The largest population sampled, Wadi (Valley) Wadi Albaidaa, with 65 observed individuals. The sites in Wadi Al Johfa, Wadi Albaidaa, Wadi Al Hakak, and Wadi EL Khaneg were found in eastern Rabigh city, at a distance of at least 5 kilometres from each other. Twelve plants from each population were genotyped using 10 microsatellite loci. Portions of young leaves were collected form each individual were wrapped in filter paper and kept in silica gel to maintain complete dryness until DNA extraction.

Table 1 Sites and population information, Acronyms used to refer for populations, coordinates of sites, and population size of the twenty known populations of *S. italica* in Region of Rabigh

Population acronym	Population site	Longitude (E)	Latitude (N)	Total No. of individuals
Wkha	Wadi EL Khaneg	39° 9' 51.5232''	22° 45' 1.512''	21
Wjoh	Wadi Al Johfa	39° 8' 54.290''	22° 42' 37.336''	19
Whak	Wadi Al Hakak	39° 14' 31.3296''	22° 45' 14.7636''	23
Wkhb	Wadi Khurieba	39° 3' 12.6252''	23° 5' 21.2892''	55
Walbd	Wadi Albaidaa	39° 48' 54"	23° 21' 07"	65

2.2. Genomic DNA isolation and PCR amplification

DNA was isolated from dried leaf samples using a DNeasy Plant Mini Kit (Qiagen). For each individual, 10 loci were tested for polymorphisms using published primers [12] (Table 2). PCR reactions were conducted with a master mix of 25 µl containing 2.5 µl of 10× reaction buffer, 1 µl of $MgCl_2$ (50 mM), 0.5 µl of a dNTP mix, 0.2 µl of a forward primer (including the M13-tail (10 µM)), 0.5 µl of a reverse primer (10 µM), 0.5 µl of the universal M13 primer (10 µM) labelled with a fluorophore (FAM, NED, VIC, or PET), 0.1 µl of Taq DNA polymerase (Dream Tag, Fermentas; 50 U/µl), 1.0 µl of bovine serum albumin (20 mg/ml), 1.0 µl of 10 ng/µl genomic DNA, and sterilized water up to the final volume. All the PCRs were performed as singleplex assays using a C1000 Thermal Cycler (BioRad, USA) under the following conditions: initial denaturation at 94ºC for 5 minutes; 50 cycles of 94ºC for 30 seconds, 55ºC for 45 seconds and 72ºC for 1 minute; 8 cycles of 94ºC for 30 seconds, 53ºC for 45 seconds, and 72ºC for 1 minute; and a final extension step at 72ºC for 5 minutes. The fluorescently tagged PCR products were analysed in multiplexes on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using LIZ500 (Applied Biosystems, USA) as a size standard. The amplified fragments were scored using GeneMapper 4.0 (Applied Biosystems, USA), and the lengths of the amplified fragments ranged from 50 to 1050 bp according to [13].

Table 2 Information on the markers used for *S. italica* germplasm analysis

Marker	Primer Sequence (5'-3') (F: Forward; R: Reverse)	Ta(°C)	amplicion	SSR Motif	Ref.
SS_1	F: TTCTGAAGCAGACGGAAGGT R: CTCACCTTCCAAATTCAAAGC	58	132–142	$(AGAGA)_4$	[12]
SS_5	F: GTCCTTTTCACCGATTGA R: CTCCTGTCAAAACTCACAAA	59	136–148	$(AG)_9$	
SS_10	F: CACAGCCAAGAGGGAGAAAG R: CCTTCTCAATGGCCTTCTCTT	58	187–196	$(AGA)_5$	
SS_14	F: GAGTATGGGGAATACGAATC R: TGGGAAGTCAGATACCAT	56	305–307	$(TC)_3C(GT)_3$	
SS_15	F: GGTATCTGACTTCCCAAA R: AGATGTCCGGATTGCTAC	56	244–248	$(ATTT)_4$	
SS_18	F:GCGATGCTCAAGTTTCTCTT R:CAAACTAAACCAAAGGACGA	54	147–149	$(ATC)_3(TTC)_3(TC)_6$	
SS_24	F: GCCTGTGATCTGAAGGTGGA R: GGAGCAGTTTAAGCCAGCTTTATT	56	115–125	$(AG)_9$	
SS_26	F: GCGCCTCACTCATACATTGA R: GGTCCACCTCCGGGTAGTAT	56	124–132	$(CT)_4$	
SS_27	F: GAACTGGGAAGGCAGAAAAA R: GATTCTGGGCAGGCTCCTAT	54	314–316	$(TC)_4$	
SS_34	F: GAACTGGGAAGGCAGAAAAA R: ACTTTTCCCAACCTCCGTCT	58	153–157	$(AT)_4$	

2.3. Population genetic analysis

The determination of the parameters of genetic diversity, genetic structure and inbreeding was performed using GenAlEx 6.1 [14]. The genetic differentiation among the populations was assessed with R_{ST}, an equivalent to F_{ST} developed for microsatellite loci [15]. The genetic structure of the *S. italica* populations was determined by analysis of molecular variance (AMOVA; 999 permutations) [16]. The number of reproductively successful migrants per generation (Nm) was estimated by the private allele method [17]. The established heterozygosity (H_o), the expected heterozygosity (H_e) under Hardy-Weinberg equilibrium, and Wright fixation index ($F = 1 - H_o/H_e$) were assessed for each locus in each population to test deviations from the Hardy-Weinberg equilibrium, which determines inbreeding. UPGMA dendrogram using PAST 4.02 [18] based on genetic diversity varibles: number of alleles N_a, number of effective alleles N_e, shanon information index I, number of private alleles, the expected heterozygosity (H_e), heterozygosity (H_o), and Percentage of Polymorphic Loci $P\%$

3. Results

A total of 10 loci revealed polymorphisms. The percentage of polymorphic loci (Table 3) was the highest value (51.5%) in Wadi EL Khaneg population, while the lowest percentage of polymorphic loci (21.5%) was found in Wadi Khurieba population in. High selfing is suggested by our results for *S. italica*, as the average fixation index (F) was equal to 0.455, confirming an explicit deficit of heterozygotes (Table 3).

The mean number of alleles per locus (N_a) ranged between 1.650 (Wadi EL Khaneg population) and 1.3 (in Wadi Khurieba population). The means of the effective number of alleles per locus (N_e), Shannon's Index (I), and expected heterozygosity (H_e) varied between 1.492, 0.369, and 0.229in the Wadi Khurieba population and 1.180, 0.144, and 0.088 in Wadi Khurieba population, respectively (Table 3.).

Table 3 The mean values of the of genetic diversity variables across the studied populations of *S. italica* Na (No. of Different Alleles), Ne (No. of Effective Alleles), I (Shannon's Information Index), No. Private Alleles (No. of Alleles Unique to a Single Population), He (Expected Heterozygosity), P (the percentage of polymorphic loci), and F (fixation index) in the twenty studied populations of *S. italica*

Population	Na	Ne	I	No. of Private Alleles	He	P%	F
Wkha	1.650	1.392	0.369	0.240	0.229	51.50	0.455
Wjoh	1.625	1.377	0.261	0.025	0.151	29.00	0.719
Whak	1.725	1.430	0.330	0.175	0.201	46.50	0.094
Wkhb	1.300	1.180	0.144	0.050	0.088	21.50	0.464
Walbd	1.350	1.187	0.167	0.050	0.105	29.50	0.544
Overall mean	1.55	1.3332	0.2542	0.11	0.1548	36	0.4552

The average total heterozygosity (H_T) for all the loci and populations was 0.451.

The cluster analysis was shown on UPGMA dendrogram (Figure 2) subdivided into three main clusters, the first cluster including two populations one population from Al Hakak and one population from Wadi EL Khaneg, which were exhibited the highest means of genetic variables; the remaining three populations were distributed in the second cluster which contained two populations from Al Johfa site, and Wadi in one sub-cluster and one population from Wadi Khurieba site where this site revealed a lowest values of genetic diversity variables and geographically isolated.

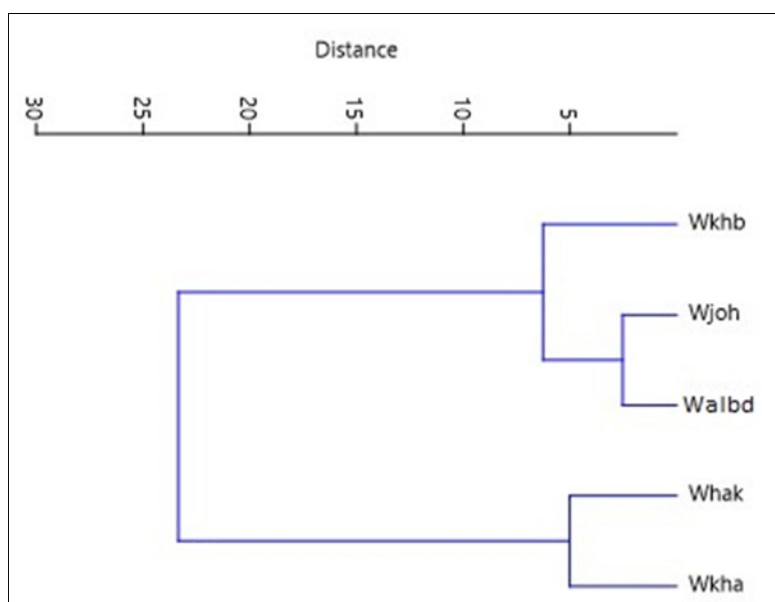

Figure 1 UPGMA dendrogram for the 5 populations of *S. italica*

The AMOVA revealed considerable genetic differentiation among the studied *S. italica* populations (F_{ST} = 0.363, R_{ST} = 0.791). The highest genetic differentiation occurred among the populations (77%, P = 0.010), whereas the lowest value (17%, P = 0.010) was detected among individuals within the populations.

4. Discussion

The analysis of 10 polymorphic loci, representing the mean number of alleles per locus (N_a), means of the effective number of alleles per locus (N_e), Shannon's Index (I), expected heterozygosity (H_e), and total genetic diversity of *S. italica*, implied moderate to low genetic diversity in all the populations of *S. italica*. Likewise, other researches have outlined that low genetic diversity has been determined for other species with isolated and small populations [19-24].

Low polymorphism rates can be a result of a reduction in population size; therefore, genetic drift and inbreeding will aggravate the risk of genetic diversity depletion in grown species in extreme environmental conditions [25- 27]. The random allele scarcity and thus the moderate-low genetic variation found in the current research could be due to the significant recent decline in the population size of *S. italica*; the number of individuals ranged between 12 and 15.

The main factors for the considerable reduction in the population size of *S. italica* include over cutting and overgrazing by camels and sheep herds which are common in these locations. Last, overconsumption of underground water reserves by local residents and for recent growing industrial projects in the Rabigh region great threat for water resources [28; 6]. Because of these anthropogenic factors, together with the predictions for upcoming higher temperature and drier conditions as a consequence of global climate change [7], *S. italica* is facing future extinction due to reductions in population size and the following erosion of genetic diversity.

Moreover, the direct consequence of water scarcity on plant survival, the rising in temperatures could harm the flowers of the species and subsequently impair the pollination fitness [30]. Finally, the decline of genetic diversity and gene flow among the populations might ultimately increase selfing and enhance the negative impacts of aridity. The extraordinary values of differentiation among the populations were in accordance with the extremely low value measured for gene flow among the populations (Nm = 0.090). These findings in accordance with the high value of genetic differentiation between populations of some other rare species, including other plant species grown in arid habitats [25-32].

The deficient gene migration among the considered populations could be accredited to the inability of *S. italica* to spread its seeds over other distant locations.

Therefore, the main factor of gene migration is pollen transfer in other plant species that reproduce sexually [32]. However, the negative effects of aridity on pollinator service could cause the presence of many unviable seeds. The measured value of gene flow was lower than the limit required to prevent genetic drift [33] The joint effect of genetic drift and gene flow could worsen future drop in genetic diversity of the remaining populations of *S. italica*.

5. Conclusions and Recommendations

Our present research is considered as a first insight into the population genetics and genetic structure of *S. italica* in one of the most prominent regions in the western region of Saudi Arabia. Our findings specified a mild to severe loss of polymorphic genes joined with noticeable genetic differentiation and high inbreeding. Firm actions should be engaged to achieve sustainable controlling and conservation of the existing populations of *S. italica* in Rabigh. The restoration of populations are fundamental actions for long-term conservation programs, and they can be concluded as follows. First, cutting and removal of existing populations should be checked and managed in regions with highly endangered populations with low genetic diversity parameters, e.g., Johfa and Wadi Albaidaa valleys. Second, comprehensive programmes to reduce water consumption will be suggested, and these programmes will be publicized through media and educational institutes. Their aim will be to effectively manage underground water.

Third, the evident reduction in the genetic diversity and high genetic differentiation sustain the idea that the populations of *S. italica* in Rabigh could be mended mainly by inclusive seed collection from the *S. italica* plants of all the existing populations [33].

The collected seeds would primarily be incorporated into *S. italica* restoration programmes, in which the seeds will be germinated in greenhouse, and then the seedlings will be planted in highly endangered sites. The new *S. italica* plants will be reintroduced into habitats resembling to those of its original populations in order to inhibit consequences including forthcoming inbreeding and a severe drop in gene flow. Some of the collected seeds should be presreved using seed bank procedures; these will be supportive to continue future plans to conserve *S. italica* in its original habitats.

Compliance with ethical standards

Acknowledgments

The author thank Rami El-jahdali (Rabigh-College of Science & Art) for valuable guidance and help during sampling of *Senna italica* from the study sites in Rabigh region.

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