ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2015) **13**(1); 27–35
doi:10.1017/S1479262114000598

Genetic characterization of some wild grape populations (*Vitis vinifera* subsp. *sylvestris*) of Zagros mountains (Iran) to indentify a conservation strategy

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Received 24 October 2013; Accepted 9 March 2014 – First published online 24 April 2014

Abstract

In this study, the genetic constitution of wild grape populations distributed in the Zagros Mountains in the north-west part of Iran was investigated to assess their risk of extinction and to identify the most suitable conservation strategy. A total of 65 wild grapevine samples from five different populations were analysed using 23 SSR loci. Consistent allelic richness was detected in all the analysed populations (from 4.01 to 5.35). The observed heterozygosity and expected heterozygosity were high with mean values of 0.67 and 0.69, respectively. A modest heterozygosity deficiency was detected only in the Piranshahr and Sardasht-Shalmash populations. The neighbour-joining dendrogram clustered the accessions into three groups corresponding to the geographical distribution of the populations. Molecular data combined with field evaluation revealed that an *ex situ* conservation approach is the most suitable strategy for preserving the genetic richness of Iranian wild grape populations with consistent rare alleles (about 20%). We propose *in situ* preservation only in the case of the Sardasht-Ghasmarash population to ensure that evolutionary dynamic forces continue to influence plant adaptation and survival in response to environmental alterations.

Keywords: genetic resources; germplasm collections; molecular markers; plant conservation; SSRs; wild grapevines

Introduction

Cultivated *Vitis vinifera* subsp. *sativa* has been domesticated from wild *Vitis vinifera* subsp. *sylvestris* (Lacombe *et al*., 2003), distributed from Middle East to European regions (Grassi *et al*., 2003a), for the development of genes tolerant to biotic and abiotic stresses that it is exposed to in new environments (Liu *et al*., 2012). Over the past few centuries, historical evidence combined with ampelographic data has frequently been used to characterize grape germplasm and to investigate the origin and relationships among wild and cultivated individuals (Labra *et al*., 2002; Barth *et al*., 2009; Ocete *et al*., 2011). Nowadays, molecular markers are commonly being used to study genetic relationships among *Vitis* accessions (Grassi *et al*., 2003b; Liu *et al*., 2012). The EU-PROJECT GENRES CT96 NO81

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has identified a universal set of simple sequence repeat (SSR) markers useful for univocally distinguishing each cultivar (This and Dettweiler, 2003). This marker set has also been used to investigate the evolution of wild grape populations, the gene flow among different areas, and the response of wild accessions to environmental impacts and human activities (De Mattia *et al.*, 2008; Ergül *et al.*, 2011; Imazio *et al.*, 2013). Moreover, from the start of grapevine genome analysis (Velasco *et al.*, 2007), several peculiar DNA traits have been identified in the European germplasm (Kuczmog *et al.*, 2012; Battilana *et al.*, 2013; Riahi *et al.*, 2013). Unfortunately until now, only a few DNA investigations have been carried out on the wild germplasm of the Middle East regions (Forneck *et al.*, 2003; Doulati-Baneh *et al.*, 2007; Ergül *et al.*, 2011). In these regions, wild grapes grow under environmental conditions that are different from those in Europe and plants are exposed to different stresses. Due to these reasons, the Middle East wild germplasm represents an important source of gene resistance traits suitable for improving cultivated grapevines. However, in the last few years, the natural habitats of wild grapevines have been altered by forest exploitation, overgrazing, soil drying, deforestation and other human activities. In Iran, wild grape populations are distributed in riparian wood habitats on river margins and in some regions a few of these populations can still be found near cultivated grape vineyards. The size of the Iranian wild grape populations has clearly decreased (Doulati-Baneh *et al.*, 2011) and the germplasm is at a risk of becoming extinct. Therefore, high priority should be given to the characterization and preservation of genetic variability. The present study analysed the genetic constitution of wild grape populations distributed in the Zagros Mountains located in West Azerbaijan and Kurdistan Provinces in the north-west region of Iran (Sabeti, 1976) to (1) assess the level of genetic diversity; (2) determine the risk of extinction; (3) identify the most suitable conservation strategy.

The Zagros Mountains are considered to be an important site of biodiversity (Djamali *et al.*, 2009; Karami *et al.*, 2014); however, the recent landscape ecology evaluation (Karami *et al.*, 2014) has shown that human activities (grazing and agriculture) have led to a reduction of natural areas, especially of humid forests where wild grape populations grow. Therefore, accurate characterization and preservation of the existing grapevine germplasm of this area are urgently required to prevent potential genetic erosion.

Materials and methods

Plant material

In this study, a total of 65 wild grapevine samples were collected from five populations from the forest and wetland regions of the Zagros Mountains in Kurdistan and West Azerbaijan Provinces, Iran. In Fig. 1, the geographical coordinates and distribution of the analysed populations are shown. Each population is represented by a variable number of wild grape individuals (from 7 to 23) distributed in a limited area of about 500 m^2. A distance of at least 40 km separates each population from the other population. The Piranshahr (P) population consisted of 15 individuals (nine males and six females) distributed in the humid area of Piranshahr; the Baneh B population consisted of ten individuals (seven males and three famales) distributed in Baneh in Kurdistan Province. The other three populations were distributed in Sardasht: the Sardasht-Grjal (S-GR) population was the smallest one consisting of five males and two females, the Sardasht-Shalmash (S-SH) population consisted of ten individuals (eight males and two females), and the Sardasht-Ghasmarash (SG) population was the largest one consisting of 23 individuals (12 males and 11 females). Each individual was morphologically characterized based on the International Plant Genetic Resources Institute (IPGRI) descriptors (see Table S1; available online).

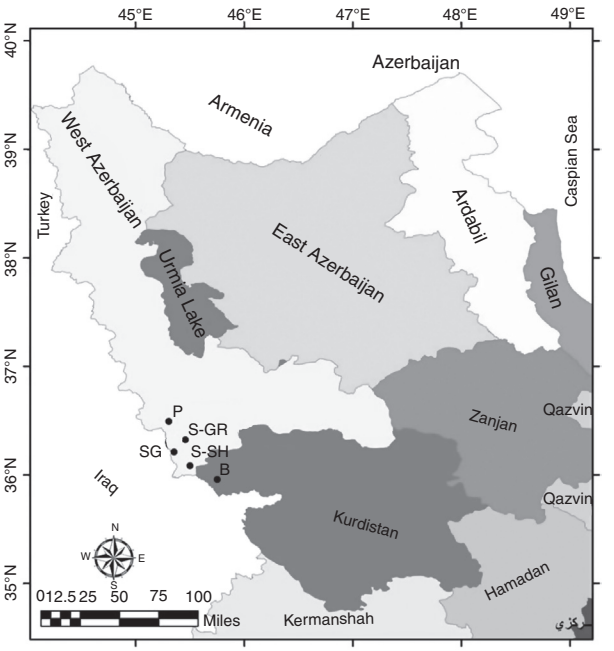

Fig. 1. Geographical coordinates and distribution of the analysed wild grape populations of the north-west region of Iran. P, Piranshahr; B, Baneh; S-SH, Sardasht-Shalmash; S-GR, Sardasht-Grjal; SG, Sardasht-Ghasmarash.

The populations are located at the altitude of 1100–1800 m above sea level and the region was characterized by the typical environmental conditions of wild grapevine habitats: wetlands and forests with a high degree of humidity due to rivers and brooks. Wild grape grows as a liana on different trees such as oak, hawthorn, willow, pear and terebinth.

https://doi.org/10.1017/S1479262114000598 Published online by Cambridge University Press

The plant sampling strategy followed in the study was the same for all the populations and was designed to prevent errors such as collection of clones or individuals of cultivated subspecies (*Vitis vinifera* subsp. *vinifera* L.). To avoid sampling of clones, young leaves from individuals spaced at least 30 m apart were collected. The risk of collecting cultivated varieties was very low for the P and B populations, as these were distributed in remote areas far away from vineyards (more than 30 km). In contrast, there were many vineyards in Sardasht. Rasha and Khoshnav (a clone of Rasha) are the main cultivated hermaphrodite varieties followed by Taifi, Angotka, Zardka and Kazav. Although the wild populations analysed in this study were located far from vineyards (almost 5 km), to reduce the risk of sampling of cultivated varieties only dioecious individuals were collected in the SG, S-SH and S-GR populations (Grassi *et al.*, 2003b). Moreover, to exclude sampling errors, the SSR profiles of the cultivars (Doulati-Baneh *et al.*, 2013) were compared with the molecular data obtained in our work related to wild accessions.

In Sardasht, three female varieties, Mam Braima, Bol Mazu and Sarghola, are also distributed in a few vineyards. To differentiate the female wild grapevine varieties from the cultivated ones, we evaluated the berry characteristics. All the collected female wild grapevine varieties had small, round and black berries with sour taste, while the fruits of the three cultivars were big and sweet with yellow (Mam Braima and Bol Mazu) or red (Sarghola) colour.

DNA extraction and SSR analysis

For each sample, two to three young leaves were used for genomic DNA extraction according to the procedure described by Lodhi *et al.* (1994). Molecular characterization was carried out using 23 SSR markers for each sample (Table 1). The SSR loci and annealing temperatures used for polymerase chain reaction (PCR) analysis are listed in Table 1. Primer sequences were obtained from Thomas and Scott (1993); Bowers and Meredith (1996); Bowers *et al.* (1999); Sefc *et al.* (1999); Lefort *et al.* (2003). PCRs were carried out in a final volume of 10 µl, containing 15 ng of each primer, 1 U of Taq DNA polymerase (Sinagen Company, Teheran, Iran), 100 µM of each of the four dNTPs, 1 × PCR buffer (10 mM Tris–HCl, 50 mM KCl and 1.5 mM $MgCl_2$), 2.5 mM of $MgCl_2$, 50 ng of template DNA and distilled deionized water. Amplification reactions were carried out using the following cycling pro-

Table 1. Genetic parameters of SSR markers across the analysed wild grape genotypes

Markers	AT (°C)	AS (bp)	AF	AN	H_e	H_o	PIC
SsrVrZAG47	50	153–171	0.35	8	0.77	0.69	0.74
SsrVrZAG62	50	188–204	0.35	9	0.8	0.76	0.78
SsrVrZAG79	51	242–260	0.36	9	0.76	0.47	0.73
SsrVrZAG83	52	188–204	0.63	5	0.53	0.61	0.48
VVMD5	56	218–248	0.3	13	0.83	0.76	0.82
VVMD7	54	236–256	0.45	9	0.73	0.56	0.71
VVMD8	56	137–190	0.14	13	0.9	0.9	0.89
VVMD17	56	216–226	0.62	5	0.55	0.63	0.51
VVMD21	56	249–266	0.54	3	0.58	0.6	0.51
VVMD24	55.5	210–218	0.23	5	0.79	0.69	0.75
VVMD25	56	237–271	0.23	8	0.83	0.73	0.81
VVMD27	56	175–190	0.37	7	0.76	0.61	0.73
VVMD36	59	245–271	0.26	7	0.8	0.84	0.77
VVS2	52	125–145	0.22	9	0.85	0.75	0.83
VVS3	52	210–220	0.6	4	0.54	0.47	0.47
VVS4	58	166–180	0.58	7	0.58	0.44	0.53
ISV2	58	120–169	0.25	9	0.82	0.66	0.79
ISV3	58.5	131–151	0.22	8	0.83	0.78	0.81
ISV4	59	162–199	0.35	10	0.81	0.76	0.79
VMC6D12	58	132–184	0.27	10	0.83	0.83	0.81
VMC6G7	50	95–147	0.29	12	0.84	0.92	0.82
VMC6G10	55	140–182	0.57	4	0.59	0.6	0.53
UCH29	60	207–307	0.25	8	0.81	0.76	0.79
Mean			0.37	7.9	0.74	0.69	0.71

AT, annealing temperatures; AS, allele sizes; AF, allele frequency; AN, allele number; H_e, expected heterozygosity; H_o, observed heterozygosity; PIC, polymorphic information content.

https://doi.org/10.1017/S1479262114000598 Published online by Cambridge University Press

file: initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, 58–68°C for 1 min (see Table 1), and 72°C for 2 min, and a final extension step at 72°C for 7 min. The PCR products were separated on 6% (w/v) polyacrylamide gels and visualized by silver staining.

The allele sizes of the SSR bands were determined using internal size markers and by comparison with a standard set of microsatellite reference alleles (This *et al.*, 2004). For each SSR locus, allele number, allele frequency, expected heterozygosity (H_e), observed heterozygosity (H_o) and polymorphic information content (PIC) were calculated. Gene diversity, often referred to as expected heterozygosity, is the probability that two randomly chosen alleles at a locus within a set of genotypes will be different (Nei, 1987). The PIC values were estimated as described by Botstein *et al.* (1980). These genetic parameters were estimated using PowerMarker version 3.20 (Liu, 2002). For each population, genetic parameters, such as observed average allele number (N_a), allelic richness ($A_{[14]}$), H_o, H_e, coefficient of inbreeding (F_{is}) and private allele (P_a), were calculated using the program FSTAT (Goudet, 2001).

Intrapopulation genetic diversity was also determined in terms of A (El Mousadik and Petit, 1996) using a fixed sample size of seven (14 gene copies), corresponding to the number of individuals of the smaller population (S-GR). The F_{is} was calculated for polymorphic loci both to test the deviation from the Hardy–Weinberg equilibrium and to estimate the genetic differentiation among the populations (Weir and Cockerham, 1984). The significance of deviations from the Hardy–Weinberg equilibrium, as evidenced by the deviation of F_{is} from zero, was tested by randomization using the FSTAT software (Goudet, 2001). All the calculations were done using POPGENE version 3.2 (Yeh *et al.*, 1999) and FSTAT (Goudet, 2001).

Genetic distance was calculated by determining microsatellite allele frequencies using Nei's index (Nei, 1987). The resulting genetic distance matrices were used to construct a neighbour-joining (NJ) dendrogram. The dendrogram was constructed using the MEGA4 software (Tamura *et al.*, 2007). A hierarchical analysis of molecular variance (AMOVA) was carried out to separate total molecular variance into within-population and between-population variance using PowerMarker version 3.20 (Liu, 2002).

To exclude hybrids between local cultivated varieties and the analysed wild individuals, we used the Bayesian clustering method of Pritchard *et al.* (2000), implemented in the program STRUCTURE 2.1. In this case, the program was run using the 'admixture model', and all STRUCTURE analyses included no previous population information and correlated allele frequencies and were carried out using runs of one million interactions, following a burn-in period of 200,000 interactions. Clustering analyses were carried out in quadruplicate to ensure the convergence of parameters. The program New Hybrids (Anderson and Thompson, 2002) was also used to carry out a more detailed analysis of admixture proportions and hybrid ancestry, by inferring the posterior probability assignment of each sampled individual to six genotype frequency classes: pure wild (W), pure cultivated (C), F1, F2, wild backcross and cultivated backcross. Tests were carried out for all the 23 loci with uniform priors and with a run of 500,000 iterations after a burn-in period of 50,000 iterations. This approach reduces the influence of low-frequency alleles, preventing sampling and genotyping errors in closely related populations.

Results

DNA of good quality was observed in all the analysed samples. The amplification of the 23 SSR loci yielded 182 different alleles, ranging from three (VVMD21) to 13 (VVMD5 and VVMD8) per locus, with an average of 7.9 alleles/locus (Table 1 and Table S2, available online). The mean PIC value was consistent (0.71); the highest value was obtained for VVMD8 (0.89) and the lowest value for VVS3 (0.47).

The H_e values ranged from 0.53 (SsrVrZAG83) to 0.90 (VVMD8), while the H_o values ranged from 0.44 (VVS4) to 0.97 (VMC6G7).

The consistent polymorphisms detected among the used SSR markers allowed characterizing the genetic structure of the analysed populations. The results given in Table 2 reveal consistent A in all the analysed populations ranging from 4.01 for the B population to 5.35 for the S-SH population. The mean values of H_o and H_e were 0.67 and 0.69, respectively. A modest heterozygosity deficiency was detected only in the P and S-SH populations; the other populations had quite similar H_o and H_e. In accordance with the detected heterozygosity deficiency, the F_{is} values of the P (0.115) and S-SH (0.124) populations indicated slight inbreeding. In the B, S-GR and SG populations, the F_{is} values were close to 0, implying a randomly mating population.

The AMOVA (Table 3) revealed that 76.3% of the total molecular variation was within the populations and only 23.7% between the populations. The minimum within-population variation was recorded in the S-GR population, while the SG population exhibited the highest molecular variation (32%).

To better evaluate the relationships among different grape accessions, a dendrogram based on NJ algorithm was constructed (Fig. 2). The analysed samples were assigned to three major groups. Group 1 consisted of 16 samples, with most of the samples belonging to

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Table 2. Genetic characteristics of the analysed populations evaluated based on the polymorphisms of the 23 simple sequence repeat loci

Populations	No. of samples	N_a	$A_{[14]}$	H_o (SD)	H_e (SD)	F_{is}*
P	15	5.35	4.47	0.61 (0.25)	0.68 (0.17)	0.115
B	10	3.97	4.01	0.58 (0.29)	0.58 (0.13)	0.001
S-SH	10	5.48	5.35	0.67 (0.17)	0.73 (0.12)	0.124
S-GR	7	4.91	4.91	0.73 (0.25)	0.73 (0.15)	0.013
SG	23	6.30	5.08	0.75 (0.16)	0.75 (0.12)	−0.013
Mean	12.8	5.36	4.83	0.67 (0.13)	0.69 (0.12)	0.048

P, Piranshahr; B, Baneh; S-SH; Sardasht-Shalmash; S-GR, Sardasht-Grjal; SG, Sardasht-Ghasmarash; N_a, average number of alleles; A, allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity; SD, standard deviation; F_{is}, coefficient of inbreeding.
*Significant deviation of the F_{is} value from zero was tested with 1000 randomizations ($P < 0.05$).

the B population. Group 2 consisted of 28 genotypes belonging to the Sardasht populations (SG, S-SH and S-GR). All the samples of the S-GR population clustered in this group with the exclusion of the S-GR2 population, while the samples of the S-GR population exhibited a consistent genetic difference and clustered in all the three branches of the dendrogram. Group 3 consisted of 21 wild genotypes, with most of them belonging to the P population.

The analysis of rare alleles indicated that 34 (18.68%) of the 182 detected alleles at the 23 loci were private (Table 4). A total of 14 private alleles were observed in the SG population followed by the B and P populations with eight and seven private alleles, respectively.

To exclude the hybrid origin of wild accessions, the obtained SSR profiles of the cultivated and wild varieties were analysed with STRUCTURE 2.1 analysis (Zecca *et al.*, 2010). The results revealed that the W samples were genetically distinct from the C ones (Fig. S1, available online). At the same time, the analysis carried out using the New Hybrids program confirmed the sharp distinction between the W and C accessions, where each plant was assigned to each genotype frequency class with an average posterior probability >0.95 (Fig. S2, available online).

Discussion

Consistent genetic diversity was detected among the analysed wild grape populations distributed in the Zagros Mountains (Iran). Similar results have been obtained for Anatolia populations (Ergül *et al.*, 2011), and they indicate that the genetic diversity of wild grape populations in the Middle East regions is higher than that of populations distributed in other areas such as the Mediterranean basin (Grassi *et al.*, 2003a; Lopes *et al.*, 2009; Zinelabidine *et al.*, 2010). Moreover, no inbreeding depression events were observed in the analysed populations. Previously, studies had demonstrated a clear reduction in H_o in most of the European wild grape populations (Grassi *et al.*, 2003a; Di Vecchi-Staraz *et al.*, 2009; Lopes *et al.*, 2009), probably due to the reduction of population size by human activities. We can conclude that although in the last few years the Zagros Mountains have been subjected to intense deforestation activities (Karami *et al.*, 2014), the local wild grape germplasm has not yet undergone a genetic erosion. However, most of the analysed populations exhibited an unbalanced sex ratio. The modest number of female individuals in most of the Zagros Mountains populations (i.e. B, SG and S-SH) reduced the seed-dispersal ability of the species. This was also confirmed by our field evaluation during plant sampling activity; a large proportion of individuals were very old (more than 25–30 years old) and a few young plantlets were detected only in the SG population. We conclude that Iranian wild grape populations have a reduced regeneration ability probably due to modest seed productions or/and environmental alterations that lead to an inhospitable habitat for young seedlings (Arnold *et al.*, 2005; Ocete *et al.*, 2008).

In the last few years, the Zagros Mountains have been subject to consistent anthropic pressure, deforestation

Table 3. Analysis of molecular variance among and within the analysed populations

Sources	MS	Variance (%)
Among populations	176.2	23.7
Within populations	566.86	76.3
P	107.7	14.5
B	68.6	9.0
S-SH	89.15	12.0
S-GR	58.8	7.9
SG	242.5	32.0

P, Piranshahr; B, Baneh; S-SH; Sardasht-Shalmash; S-GR, Sardasht-Grjal; SG, Sardasht-Ghasmarash; MS, mean square.

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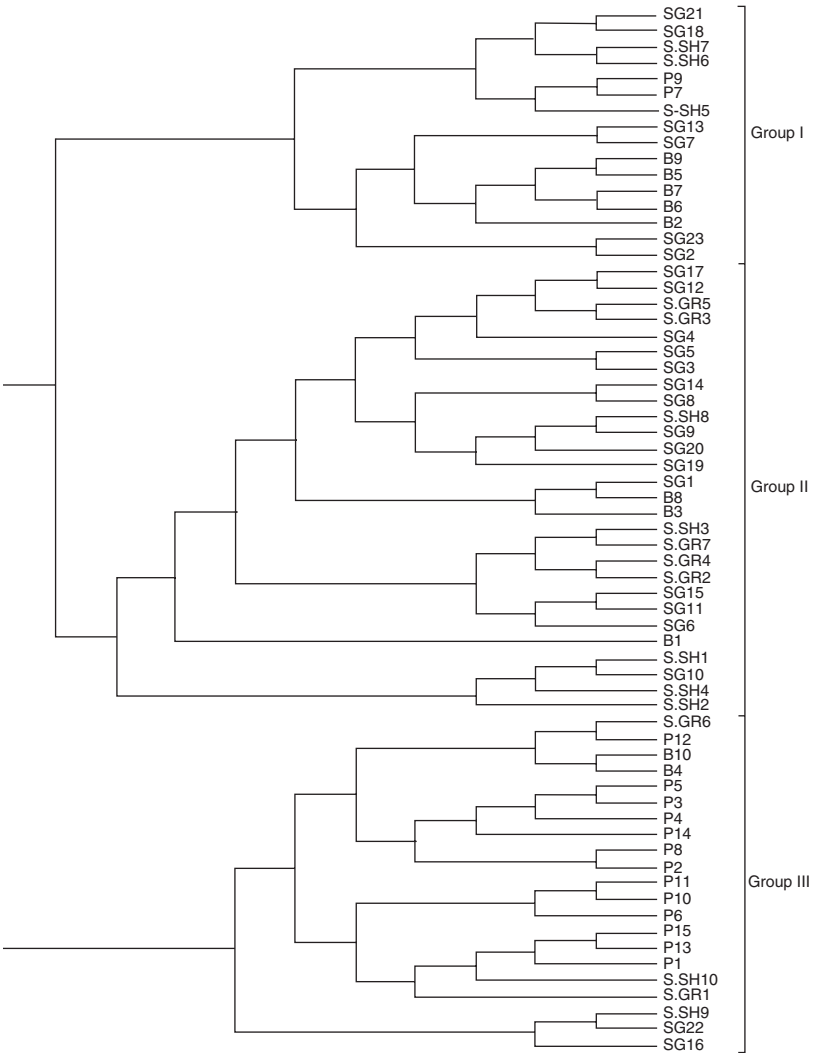

Fig. 2. Dendrogram depicting genetic relationships among the wild grape populations based on shared allele distance and neighbour-joining algorithm. P, Piranshahr; B, Baneh; S-SH, Sardasht-Shalmash; S-GR, Sardasht-Grjal; SG, Sardasht-Ghasmarash.

and overgrazing (Noroozi *et al.*, 2008) with the consequence being environmental alteration. Deforestation and river management have been indicated as the main threats to wild grape populations (Arnold *et al.*, 2005). The effect of habitat modifications was particularly evident in Sardasht region, where grazing, forest fire and agriculture (Haidari *et al.*, 2013) drastically reduced the riparian areas suitable for wild grape growth (Grassi *et al.*, 2003b). The S-GR and S-SH populations are relegated to a small area surrounded by cultivated fields and human activities, especially in Shalmash region. These rapid environmental alterations do not alter the genetic constitution of the existing wild grape individuals, as indicated by our molecular investigation, but induce population decline and prevent the development of new plantlets. In 2005, the UNDP (United Nations Development Programme) approved a ten-year project for biodiversity conservation in the central Zagros Mountains (http://www.undp.org.ir/index.php/component/content/article/77) with the main goal being the preservation of the endangered habitat including the humid forest and wetland areas. This is an essential step for preserving the wild grape and several riparial taxa distributed in the Zagros Mountains.

The SG population is distributed in a large humid area located at the Iraq–Iran border. This area is subject to conspicuous rainfall that guarantees full rivers and humidity. The consistent number of individuals and a proper balance between males and females and the presence of young plantlets indicate that the SG population is not at a risk of becoming extinct. Moreover, due to the war between Iran and Iraq, in the last 8 years, very few

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Table 4. Distribution of rare SSR alleles in the wild grape populations

PO	AF	AS (bp)	SSR locus	PO	AF	AS (bp)	SSR locus
S-SH	0.05	151	ISV2	SG	0.04	143	VVS2
SG	0.17	131	ISV3	SG	0.08	198	ZAG62
SG	0.06	151	ISV3	S-GR	0.14	188	ZAG62
P	0.03	199	ISV4	S-GR	0.07	228	VVMD5
B	0.05	220	VVS3	S-SH	0.05	118	VVMD5
SG	0.04	172	VVS4	B	0.1	248	VVMD5
SG	0.1	169	VVS4	P	0.03	222	VVMD5
SG	0.6	180	VVS4	SG	0.04	254	ZAG79
B	0.05	170	VVS4	SG	0.02	250	ZAG79
P	0.03	159	ZAG47	B	0.05	145	G7
B	0.05	256	VVMD7	B	0.05	116	G7
P	0.1	141	VVMD8	P	0.03	147	G7
SG	0.1	259	VVMD25	SG	0.04	168	D12
SG	0.158	269	VVMD25	S-SH	0.15	182	D12
P	0.13	181	VVMD27	P	0.26	132	D12
SG	0.03	175	VVMD27	SG	0.02	159	ISV2

PO, name of the population; AF, allele frequency; AS, allele size; S-SH; Sardasht-Shalmash; SG, Sardasht-Ghasmarash; P, Piranshahr; S-GR, Sardasht-Grjal; B, Baneh.

people have travelled to this area and thus the anthropic pressure on biodiversity is modest.

The evident effect of population erosion was also observed in the B population. In this area, local communities mainly depend on ranching and a serious forage shortage has occurred in the last few years. Local people began to exploit trees such as oak to feed cattle (Fatahi, 1994; Moradi *et al*., 2010) and deforestation has led to drastic effects on climber species such as wild grape.

The P population is distributed in Prdanan tourist area and it is distributed far from the other populations. Although this is the second largest analysed population, the increase in tourism, overgrazing, seasonal river floods and agricultural activities has led to negative effects on riparian plants including wild grape. To preserve the genetic diversity of this population, some individuals have been propagated and transferred to the germplasm collection of the Kahriz Horticultural Research Station in Ourmia.

The results of our molecular investigation combined with field evaluation suggested that wild grape populations distributed in the Zagros Mountains have consistent genetic richness, but are at a risk becoming extinct due to human activities, especially deforestation, and increase in agricultural activities.

Ex situ conservation approaches are convenient and rapid for preserving genetic traits (Barth *et al*., 2009). Most of the wild grape accessions had peculiar alleles (see Table S2, available online); these samples should be the first to be preserved in germplasm collections. Moreover, preliminary evaluation carried out by a team of researchers of the Agricultural Research Center of West Azerbaijan provides evidence that characteristics such as powdery mildew and fanleaf virus resistance and salinity tolerance are present in some accessions in the germplasm (H. Doulati-Baneh, pers. commun.). This is in agreement with the results of a recently conducted analysis highlighting the presence of pest resistance traits in the wild grape germplasm (Riaz *et al*., 2013). We underline that such evaluations are very important for future breeding programmes of table and wine grape.

Plants propagated using *ex situ* strategies should also be also used for restoration ecology programmes (i.e. replanting and reinforcement). Reintroduction programmes that have been very successful have been implemented using local genotypes; for this reason, we suggest that the wild grape collection be separated into three distinct groups corresponding to the dendrogram groups that reflect the geographical distribution of the populations.

Unfortunately, plants conserved by *ex situ* strategies do not evolve under natural forces and processes. By contrast, the conservation of genetic resources under *in situ* conditions ensures that evolutionary dynamic forces continue to influence plant adaptation and survival. Based on the results of the present study, we suggest that the SG population be maintained in its natural habitat through the establishment of a local protected area where the river margins are preserved and tourism activity is regulated to reduce the negative effects of human activities. *In situ* conservation is a practical way to complement *ex situ* conservation and meets the need of expanding germplasm collections with new genetic traits coming from the natural evolutionary processes (Pavek *et al*., 2003).

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Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000598

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