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Research Article

Wild Grape Genetic Diversity in Iran with Germplasm Conservation and their Significance in Breeding Packages

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Abstract

Iran is one of the facilities for the emergence and distribution of grapes in the world. Iran has an excessive genetic and morphological diversity. These wild grapes are regarded as the earliest ancestors of modern-day grapevine that have been uncovered to extreme modifications in weather, diseases, and tensions over time. In order to investigate on the groundwork of sex, the plant life of wild grapevine vegetation has been recognized in three woodland areas of Sardasht, Baneh and Piranshahr. Then, in specific phonological stages, primarily based on the description of the World Gene Bank for Grapes, botanical qualities in all chosen plant life have been measured and recorded. Genetic variety of these genotypes and genetic relationship with neighborhood cultivars of the place was once investigated the usage of molecular markers like microsatellite and nucleoside (Chloroplast) chromosomes. In the subsequent step of research, the presence of viral ailments in these historical grapes was once studied. Tolerance of these wild genotypes to salinity and wintry weather bloodless stress and their comparisons with nearby cultivars was once carried out. Results of botanical research confirmed that there is an exceptionally excessive version amongst these genotypes. Also, the response of wild grapes to salinity and bloodless stresses was once different, however in each stresses, no excessive tolerance genotypes have been obtained. It is essential to learn about the tolerance of greater Iranian grapes to different stresses via investigating genotypes in different areas of Iran.

Keywords: Cold Stress; Genetic; Salinity and Vitis

Introduction

It is presumed that old cultivated grapevine (*Vitis vinifera* spp sativa) is thought to have been domesticated in Transcaucasia (Turkey, Iran and Armenia) from wild population of *Vitis vinifera* ssp sylvestris [1]. These centers represent the source for modern grapevine cultivation and the starting point of its spread throughout the European continent [2]. In the postglacial period, *Vitis sylvestris* escaped from its southern refugia, spread towards the north and became a common species in European forests, along riverbanks and on hillsides.

Wild grapes considered being an autochthonous and dioecious with anemophilous pollination. A principal distinction between *V. vinifera* subsp. sylvestris and cultivated *V. vinifera* subsp. vinifera is that the wild grape is dioecious with male and female plants, whilst the cultivated grape is monoecious with bisexual flora (i.e., flowers with ovary and anthers), which may also have been chosen for at some point of domestication [3]. During the procedure of domestication, severa adjustments happen in the genetic and physiological make up of crop plants. However, in domesticated grapevine genotypes, positive morphological characteristics have virtually end up fixed, which include hermaphrodite flowers, massive clusters and massive berries, alongside with greater sugar contents. Wild grapevines are predominantly forest climbers and prefer humid condition [4]. Populations of wild grapevine mostly distributed in different geographical environments [5]. Under diverse climates some desirable resistance genes to biotic and a biotic stress will be so their conservation is very important for breeding programs and biodiversity conservation exist [6]. Wild grape genetic diversity information and the evaluation of its distribution is useful to ensure that adapted genetic resources are available for use in breeding programs and to define the most suitable conservation strategies [7]. The usefulness of wild grape genotypes in breeding program as gene donor parent for some traits as salt tolerance [8], virus and crown gall and cold tolerance have been reported [9].

Over the past centuries historic evidence combined with ampelographic data have frequently been used to study the genetic variation, origin and relationships among wild and cultivated grapevines. Today's the availability of molecular markers such as simple sequence repeats (SSR) with the advantage of co-dominance, high polymorphic and multi allelic have been widely applied to analysis of genetic diversity among grape genotypes and relationship between wild and cultivated grapevines [10].

Haplotype diversity of 63 wild grape individuals originating have been studied in North-West of Iran by cp-SSR marker. Results showed a high genetic variation among these genotypes [11]. Wild grape populations in Danub river regions in Austria [12] and three Anatolin locations [13] were evaluated by molecular markers too and in both studies high genetic diversity among wild individuals were reported.

Studies on the residual wild grapevine locations in Europe have verified that the subsp. sylvestris is endangered [4]. In the 1980s, this subspecies was once introduced to the International Union for Conservation of Nature (IUCN) listing of endangered European plants. Since 2000, it has been declared a seriously endangered subspecies and it has been claimed as species to be strictly protected. In Iran wild grapes were found generally in riparian wood habitats on river margins located in Alborz and Zagros mountains in North and North-Western of country [14] and somewhere these remnants of populations can still be found almost nearby vineyard with cultivated grapes [15]. The natural habitats of wild grapevines are destroyed by forest exploitations, over grazing, soil drying, climate changing, diseases and deforestation decreased in size and leading to severe genetic erosion. Today only few individual plants can be found on their native habitats in Iran [16]. Therefore a high priority should be given to the collection and preservation of these valuable germplasm. The objective of this study was to use the microsatellite markers to reveal genetic diversity within and between wild grapevines populations collected from five forest locations of Zagross mountains in North-Western of Iran and provide information about their sanitary state and identify the some probably stress tolerate genes.

Materials and Methods

Based on morphological traits such as flower sexuality, dioeciously mating, leaf shape and fruit color, a total number of sixty three individuals of wild grapevine (*Vitis vinifera* ssp sylvestris) genotypes were identified and collected from forest and wetlands regions of Zagros Mountain in Kurdistan and West Azerbaijan province, Iran. The annual precipitation ranges of these regions are from 400 mm to 800 mm (16 to 30 inches) and falls mostly in the winter spring. The winters are severe, with low temperatures often below −25°C (-13°F). The region exemplifies the continental variation of the Mediterranean climate pattern, with a snowy, cold winter and mild rainy spring followed by a dry summer and autumn. Name, Identity code, number of individuals and geographical coordinates of each population was shown in table 1. The largest proportion of the samples was collected from the Sardasht- Qasma rash (Iran-Iraq borderline) habitat where the wild grapes grow as a liana near the river and springs.

Figure 1: The main Sites selection of wild grape populations from North-Western of Iran (closed squares).

Sanitary state

The incidence of Powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*) and GFLV symptoms on leaves and cluster and crown gall on trunk were evaluated.

Genetic diversity

To study the genetic variation among wild grape genotypes and their genetic relationship with some Iranian authectenous grape cultivars, 10 chloroplast microsatalite and 23 SSR markers were used.

Cold tolerance

After the leaf fall the four bud cuttings have been organized from the developing vines and then dealt with with freezing temperatures (-15, -18, -21, -23 and four °C as a control) in a cold chamber with 4 replications. The proportion of burst buds in greenhouse circumstance and the proportion of died foremost and secondary buds of every genotype in chilling temperature have been measured. Electrical conductivity (EC) of buds and cane tissue additionally measured. With lowering temperature, the price of predominant and secondary bud damage was once expanded

Table 1: List of wild grape populations, code, individual sex, population name and their geographic coordinate (* = Approx ± 3').

Individual code and sex	Coordinates	Population name	Individual code and sex	Coordinates	Population name
P1♂	36°14'; 45°5'	Piranshahr	S-SH60♂	36°5'; 45°30'	Sardasht- Shalmash
P2♂	36°14'; 45°5'	Piranshahr	S-SH61♂	36°5'; 45°30'	Sardasht- Shalmash
P3♂	36°14'; 45°5'	Piranshahr	S-GR33♂	36°25'; 45°22'	Sardasht- Grjal
P4♂	36°14'; 45°5'	Piranshahr	S-GR34♂	36°25'; 45°22'	Sardasht- Grjal
P5♂	36°14'; 45°5'	Piranshahr	S-GR35♀	36°25'; 45°22'	Sardasht- Grjal
P6♀	36°14'; 45°5'	Piranshahr	S-GR36♂	36°25'; 45°22'	Sardasht- Grjal
P7♀	36°14'; 45°5'	Piranshahr	S-GR37♀	36°25'; 45°22'	Sardasht- Grjal
P8♂	36°14'; 45°5'	Piranshahr	S-GR38♂	36°25'; 45°22'	Sardasht- Grjal
P9♀	36°14'; 45°5'	Piranshahr	S-GR39♂	36°25'; 45°22'	Sardasht- Grjal
P62♀	36°14'; 45°5'	Piranshahr	SG40♂	36°13'; 45°22'	Sardasht- Qasma rash
P63♀	36°14'; 45°5'	Piranshahr	SG42♀	36°13'; 45°22'	Sardasht- Qasma rash
P65♀	36°14'; 45°5'	Piranshahr	SG43♂	36°13'; 45°22'	Sardasht- Qasma rash
P10♂	36°14'; 45°5'	Piranshahr	SG44♀	36°13'; 45°22'	Sardasht- Qasma rash
P11♂	36°14'; 45°5'	Piranshahr	SG45♀	36°13'; 45°22'	Sardasht- Qasma rash
P12♂	36°14'; 45°11'	Piranshahr	SG46♂	36°13'; 45°22'	Sardasht- Qasma rash
B13♀	35°59'; 45°53'	Baneh	SG47♀	36°13'; 45°22'	Sardasht- Qasma rash
B14♂	35°59'; 45°53'	Baneh	SG48♂	36°13'; 45°22'	Sardasht- Qasma rash
B15♀	35°59'; 45°53'	Baneh	SG49♀	36°13'; 45°22'	Sardasht- Qasma rash
B16♂	35°59'; 45°53'	Baneh	SG50♀	36°13'; 45°22'	Sardasht- Qasma rash
B17♂	35°59'; 45°53'	Baneh	SG51♂	36°13'; 45°22'	Sardasht- Qasma rash
B18♂	35°59'; 45°53'	Baneh	SG52♂	36°13'; 45°22'	Sardasht- Qasma rash
B19♂	35°59'; 45°53'	Baneh	SG53♂	36°13'; 45°22'	Sardasht- Qasma rash
B21♂	35°59'; 45°53'	Baneh	SG54♀	36°13'; 45°22'	Sardasht- Qasma rash
B22♂	35°59'; 45°53'	Baneh	SG55♂	36°13'; 45°22'	Sardasht- Qasma rash
S-SH23♀	36°5'; 45°30'	Sardasht- Shalmash	SG56♀	36°13'; 45°22'	Sardasht- Qasma rash
S-SH24♂	36°5'; 45°30'	Sardasht- Shalmash	SG57♂	36°13'; 45°22'	Sardasht- Qasma rash
S-SH25♀	36°5'; 45°30'	Sardasht- Shalmash	SG58♀	36°13'; 45°22'	Sardasht- Qasma rash
S-SH26♂	36°5'; 45°30'	Sardasht- Shalmash	SG59♂	36°13'; 45°22'	Sardasht- Qasma rash
S-SH27♂	36°5'; 45°30'	Sardasht- Shalmash	SG64♂	36°13'; 45°22'	Sardasht- Qasma rash
S-SH28♂	36°5'; 45°30'	Sardasht- Shalmash	SG31♀	36°13'; 45°22'	Sardasht- Qasma rash
S-SH29♂	36°5'; 45°30'	Sardasht- Shalmash	SG32♀	36°13'; 45°22'	Sardasht- Qasma rash
S-SH30♂	36°5'; 45°30'	Sardasht- Shalmash			

and there used to be a massive distinction in bloodless tolerance amongst examined genotypes.

Salt tolerance evaluation

Vines of wild grapevine genotypes have been planted in pots containing a combination of soil (fine loamy, exquisite active, mixed, mesic typic calcixerepts) and sand (1:1 V/V). They have been grown for forty days and after the accurate institution (8 cm inexperienced shoot growth, 5 younger leaves), salinity redress started. The experimental design used to be a Factorial Complete Randomized Block format with two factors and 4 replications (salinity at 0, 50, 100 and 150 mM NaCl and genotypes which include eleven wild grapevines). This test used to be carried out at Agricultural and Natural Resource Research Center of West Azarbijan-Iran. At the give up of experimental duration awareness of K+, Na+, NO3- and Cl- of leaves was once measured and seen signs and symptoms of salt damage in vines scored as: 1-Plants with no necrotic tissues; 2- necrosis on 30% of blade and necrosis on the tip of the leaves; three - necrosis on 50% location of the leaves and necrosis on the stem; 4- necrosis on 60-80% of the leaves and necrosis on the stem; and 5- necrosis main to the loss of life of the plant.

Results

Among of 65 wild grape samples 24 of them female and 41 accessions were male. Number of male accessions in Baneh and Shalmash and Grjal- Sardasht were more than female once and partial balance between them was only existed in Piranshahr and Qasma rash-Sardasht regions. Clustering could not discriminate wild grapevines based on sexuality. In each group there were both genders with different frequency but in all groups almost all male grapevines located closed together and female grapevines too. In all areas, wild grapes are standing and rely on forest trees, often near rivers in the lower reaches of the valley or above the mountains along the water spring. The number of accessions in a population in these areas has fallen more than before for a variety of reasons such as unfavorable conditions in the environment, fire, excessive livestock and human involvement, and this decrease was higher in female grapes. The high differences in leaf shape were detected in wild genotypes (Figure 2).

Figure 2: Leaves shape diversity among some wild grapevine genotypes. above, left to right: S-SH30♂, S-SH27♂, SG31♀ ; Below, left to right: S-GR37♀, S-SH61♂, S-SH60♂

Sanitary state (Disease tolerance)

Symptoms caused by powdery mildew (*Uncinula necator*) are often observed on leaves and shoots in growing season. Typical symptoms of downy mildew (*Plasmopara viticola*) did not recognize. None of the analysed specimens suffered from crown gall and phylloxera. Only some GFLV symptoms are observed in some wild genotypes especially in Sardasht region. Thus the wild vines do not constitute a risk for the surrounding commercial Rasha cultivar vineyards. On the other hand, diseases spread from cultivated grapevines may seriously harm the wild vine population. Rate of infection are quite different among populations within the same site, from one accession to the other and regions. Based on all morphological data it was revealed that there is a high genetic diversity among wild grape genotypes which is the vital key to avoid genetic erosion, to start biotic an abiotic tolerance breeding programs.

Haplotype diversity

Results confirmed that amongst analyzed cpssr loci solely ccmp3 and ccmp10 had been polymorphic inside cultivars and solely ccmp3 used to be polymorphic in wild grape individuals. The measurement versions of each loci mix in a whole of four distinct haplotypes. All the four haplotypes are displayed in the cultivars whilst solely two are introduced in wild grapes. Sultani or Keshmeshi Bidaneh cultivar has the haplotype III that there is now not this haplotype amongst the wild grapes of studied regions. Concerning to existence of each haplotypes I and II in the quantity of Iranian cultivated and wild grapes, it is viable to think about that wild grape are ancestor of some of native cultivars.

Genetic diversity

Twenty three SSR primers with clear bands and high polymorphism were screened from 30 primer pairs selected for this study

Table 2: Number and frequency of haplotype in wild grapevines (Doulati baneh., *et al.* 2007).

Populatin	Haplotype I	Haplotype II	Sample number
Baneh	0.03	0.11	10
Piranshahr	0.2	0.01	15
Sardasht-shalmash	0.11	0.05	10
Sardasht-grjal	0.00	0.11	7
Sardasht-ghasmarash	0.14	0.22	23
	0.49	0.51	65

All selected primers amplified polymorphic fragments in the panel of wild grape accessions. The number of alleles ranging from 3 (VVMD21) to 13 (VVMD5 and VVMD8) with a total of 182 alleles and an average of 7.9 alleles/locus (Table 3). The PIC mean value was 0.71 and VVMD8 locus with value of 0.89 and VVS3 markers with value of 0.47 showed the maximum and minimum PIC, respectively.

Table 3: Genetic parameters of SSR markers across all wild grapevines.

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

Specific alleles for wild grapes in different regions

Out of 182 alleles detected at 23 loci, 33 were specific alleles (i.e. occurred only in populations from one of the regions) in wild grape populations of five regions (Table 4). The percentage of these alleles in our populations was 18.13%. The allele H locus VVS2 only in Qasma rash-Sardasht grapes population (g5), allele A locus ISV4, Allele F locus ZAG47 and allele I locus VVMD8 in Piranshahr grapes population (g1) and allele A locus VVMD7 in Baneh populations (g2) was found.

Locus	Allele	Frequency	Population found	Locus	Allele	Frequency	Population found
VVS2	H	0.04	g5	ISV3	J	0.17	g5
ZAG62	D	0.08	g5	ISV3	A	0.06	g5
ZAG62	I	0.14	g4	ISV4	A	0.03	g1
VVMD5	I	0.07	g4	VVS3	A	0.05	g2
VVMD5	M	0.05	g3	VVS4	C	0.04	g5
VVMD5	A	0.1	g2	VVS4	E	0.1	g5
VVMD5	L	0.03	g1	VVS4	A	0.6	g5
ZAG79	D	0.04	g5	VVS4	D	0.05	g2
ZAG79	B	0.02	g5	ZAG47	F	0.03	g1
G7	B	0.05	g2	VVMD7	A	0.05	g2
G7	E	0.05	g2	VVMD8	I	0.1	g1
G7	A	0.03	g1	VVMD25	D	0.1	g5
D12	D	0.04	g5	VVMD25	B	0.158	g5
D12	B	0.15	g3	VVMD27	G	0.13	g5
D12	J	0.26	g1	VVMD27	E	0.03	g1
ISV2	E	0.02	g5	VVMD36	G	0.05	g2
ISV2	F	0.05	g3	VVMD36	D	0.25	g2

Table 4: Specific alleles for wild grapevines in studied regions.

Analysis of Molecular variance (AMOVA)

To discriminate the distribution of molecular variations of wild grape samples both among and within populations variance we used the Molecular variance analysis (AMOVA) methods. Results showed that 76.3% of the total variance being attributable within-population (among individuals) and 23.7% of residual variation was related to differences among-populations. The minimum within-population variance was recorded in wild grape populations in Grjal-Sardasht that showed the homogenous of samples in this region. The maximum within-population variation (32% total variation) was observed in Qasma rash wild grapevines (Table 5) suggesting the existence of a high level of genetic differences among individuals.

Table 5: AMOVA method to reveal genetic variability among and within populations of wild grapevines.

Source	Mean square (MS)	Percent
Among population	176.2	0.237
Within population	566.86	0.763
Piranshahr population (g1)	107.7	0.145
Baneh population (g2)	68.6	0.09
Shalmash-Sardasht population (g3)	89.15	0.12
Grjal- Sardasht population (g4)	58.8	0.079
Qasma rash- Sardasht (g5)	242.5	0.32
Total	743.06	1

Cluster analysis

The genetic distance between individuals was calculated based on the proportion of shered alleles. Cluster analysis was performed on the basis of the Neighbor-Joining and UPGMA algorithms. A Dendrogram was constructed using MEGA-4 software [17]. A hierarchical analysis of molecular variance (AMOVA) was performed on different dendrogram cutting points to determine the optimum number of clusters using PowerMarker Ver. 3.20 [6]. The dendrogram showed the relationship among Iranian wild grape genotypes (Figure 3). Cluster analysis divided wild grapevines to three major groups that partially correlated with their geographical distribution.Group 1, in this cluster there were 16 wild accessions from four regions (g1, g2, g3 and g5) that 5 collected samples from Baneh region (g2) was located in this group. There were not any wild accessions of Grjal- Sardasht (g4) in this cluster. Group 2, including 28 genotypes mostly contained Qasma rash-sardasht (g5) individuals with some individuals of g3, g4 and g2 were included respectively. In this cluster there are not any wild samples of piranshahr (g1) region. A total of 21 wild genotypes taken from five studied regions were grouped in cluster 3. From 15 wild grape samples belong to Piranshahr, 13 accessions were located in this group. In this clustering, regardless the grouped of some genotypes of all regions together but partially related with geographic origin of the accessions was proved.

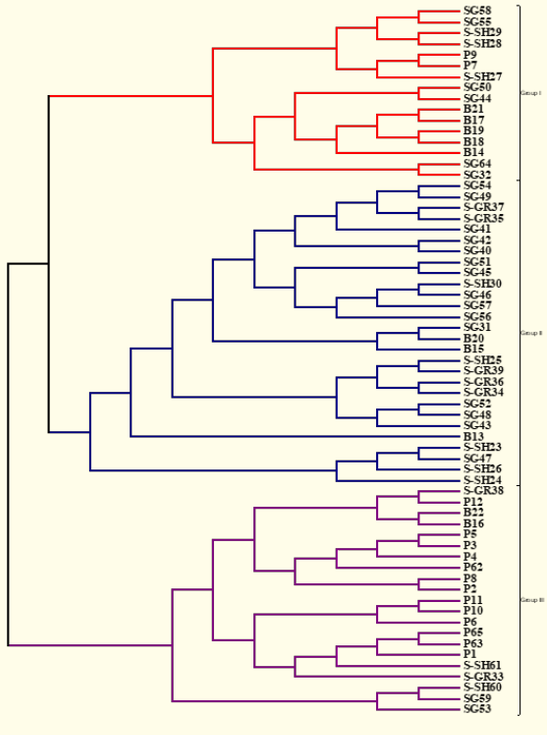

Figure 3: Dendrogram depicting genetic relations of wild grapevines based on shared allele distance and Neighbor-Joining algorithm.

In this study, the genetic charter of wild grape populations allotted in the Zagros Mountains in the north-west section of Iran was once investigated to determine their chance of extinction and to discover the most appropriate conservation strategy. Consistent allelic richness was once detected in all the analysed populations (from 4.01 to 5.35). The located heterozygosity and predicted heterozygosity have been excessive with imply values of 0.67 and 0.69, respectively. A modest heterozygosity deficiency was once detected solely in the Piranshahr and Sardasht-Shalmash populations. Molecular facts mixed with area assessment published that an ex situ conservation method is the most appropriate method for retaining the genetic richness of Iranian wild grape populations with regular uncommon alleles (about 20%). We advocate in situ protection solely in the case of the Sardasht-Ghasmarash populace to make certain that evolutionary dynamic forces proceed to have an impact on plant adaptation and survival in response to environmental alterations.

Cold tolerance

All grape cultivars belong to Vinifera species are cold touchy and rely on cultivar they can be broken via the temperature round -15 to -20 °C. In this research, the bloodless tolerance of some Iranian wild grape (*Vitis vinifera* ssp sylvestris) genotypes used to be evaluated. Approximately all Iranian wild grape genotypes have been touchy to bloodless stress above -15°C however amongst them, there had been reasonable bloodless tolerant genotypes too. H6 and H4 interspecies hybrids with Labrusca species confirmed greater bloodless tolerance in contrast to wild grape genotypes (Table 6).

Salt tolerance results

To evaluate the suitability of wild grapevine genotypes to saline conditions leaves ions and salt injury symptoms were measured. Na^+ and Cl^- content in all samples increased with increasing salinity but rate of content was different among genotypes. Based on Na^+, Cl^- ions content and salt symptoms, genotypes 4 and 7 showed less symptoms in moderate (50-100 mM) NaCl concentration but no one could tolerate high salt (150 mM) concentration. Under saline conditions, the ions accumulated in the leaves significantly varied among wild genotypes and some of them will be recommending as saline tolerant genotypes. By increasing the salt concentrations, injury symptoms were extended in leaves and shoots of all genotypes. At 50 mM NaCl wild genotypes only genotypes 4 and 7 showed lowest injury symptoms and they continue to tolerate 100 mM NaCl. Although at 150 mM NaCl genotypes 2 and 7 did not completely dry but other genotypes were died (Table 7).

Genotypes	4°c		-15 °c		-18°c		-21 °c		-24 °c	
	Primary bud (%)	Secondary bud (%)	Primary bud (%)	Secondary bud (%)	Primary bud (%)	Secondary bud (%)	Primary bud (%)	Secondary bud (%)	Primary bud (%)	Secondary bud (%)
H4	0 p	0 l	0 p	0 l	0 p	0 l	21.3 mn	0 l	16.7 nop	33.3 d-i
H6	0 p	0 l	0 p	0 l	0 p	0 l	0 p	0 l	0 p	0 l
Labrusca	0 p	0 l	0 p	0 l	0 p	0 l	0 p	0 l	16.7 nop	0 l
PR1B11	0 p	0 l	41.7 jkl	25 g-k	65.7 e-h	29.2 e-j	83.5 a-d	45.8 c-f	98.7 a	60 b
PR1B12	0 p	0 l	46.6 jkl	27.8 f-k-q	68.2 d-g	39.5 c-g	89.2 ab	49.9 bcd	100 a	60 b
PR1B5	0 p	0 l	41.1 jkl	47.5 n-q	37.2 j-m	26.7 f-k	67 d-g	9.5 kl	88 ab	50.2 bcd
PR1B8	0 p	0 l	59.2 f-i	23.2 b-e	69.6 c-e	35.8 c-h	88.9 ab	77.8 a	100 a	93.3 a
R1B1	0 p	0 l	33.3 k-n	25 g-k	47.5 ijk	18.7 h-l	64.8 e-h	38.1 c-g	80.8 b-e	53.3 bc
R1B16	0 p	0 l	8.9 op	0 l	25.6 lmn	8.3 kl	51.8 g-j	32 d-j	100 a	50 bcd
R1B2	0 p	0 l	0 p	0 l	25 l-o	12.8 jkl	50 h-k	4 l	81.7 b-e	42.8 b-g
R1B7	0 p	0 l	25 l-o	8.3 kl	29.2 lmn	13.1jkl	67.3 d-g	16.3 h-l	86.2 abc	50 bcd
R2B2	0 p	0 l	0 p	0 l	40.7 jkl	17 h-k	73.7 b-f	49.2 bcd	74.9 b-f	53.3 bc

Table 6: The effects of genotype and freezing temperature on primary and secondary bud mortality.

Table 7: Effect of salinity levels on salt injury in the leaves of wild grape genotypes.

Genotype	NaCl Levels			
	0 mM	50 mM	100 mM	150 mM
G1	1e	5a	5a	5a
G2	1e	4b	5a	4b
G3	1e	4b	3c	5a
G4	1e	2d	2d	5a
G5	1e	5a	5a	5a
G6	1e	5a	5a	5a
G7	1e	3c	2d	4b
G8	1e	5a	5a	5a
G9	1e	4b	4b	5a

Discussion

Based on studies of Sabeti, 1976 [14] and Doulati Baneh., *et al*. 2007 [11] wild grapevines (*vitis vinifera* ssp. silvestrys) identified in forests of north and north western of Iran. Average of genetic diversity (He) observed in Iranian wild grapes are lower than wild types reported from Anatolia [13] but equal to Hungarian types [18]. The percentage of unique alleles in Iranian populations (18.13%) was lower than Anatolian populations (22.36%) and approximately equal to Iberian populations (17.9%) that reported by Ergul., *et al*. 2011 [13]. This findings was not expected since Iran one of the main center of grape diversity. This lower genetic diversity values that observes in our grapevine populations may be showed that they were suffered from inbreeding depression. Studies of other wild specimens especially in Caspian Sea (North of Iran) will enable us to have a more precise view on genetic diversity of wild grapevines in Iran and compare this information with Asian and European databases.

Results of clustering showed the three genetic groups of wild grapevines that partially correlated with their geographical regions. However, some genotypes from the same population were genetically closer to genotypes from different populations than those of the same population. The gene flow may have occurred among the populations of these five regions but in some cases such as population of Baneh and PIranshahr with long distance it seems that gene flow by wind is not efficient. Seed dispersal by birds would be one of the main candidate to separate seeds of wild grapes from different regions. Once new plant are introduced into a new location through seed dispersal, future breeding is expected to occur among the these new plants and native populations that leading to the gene flow from one population to other and a rate of genetic difference among populations could back to different history of their relationship with the domesticated group [13].

The molecular analysis of variance revealed that genetic diversity within the wild populations (76.3%) was greater than between populations (23.7%). These results suggest the moderate genetic differences between populations. It proved that gene flow between populations in five regions limited by some unknown barriers or the origins of them are different due to historical events in primary center of diversity. The variance of between wild grape populations obtained in this study was greater than that reported by Ergul., *et al.* [13] in Anatolian. The low level of between population variance indicative of high levels of gene migration among populations [13]. It seems that gene flow around Iranian wild grapevine populations lower than that reported around world or there is unique population with acceptable diversity.

The great variation was measured within populations especially in Sardasht population that suggests the high biodiversity and existence of distinct gene stock. This might be attributed to geographically position that far away of human impacts. This observed unique genetic diversity represents a strong attempt for preservation of wild grapevines *in situ* and *ex situ* conservation as desirable genetic resources for breeding programs.

Wild Grape germplasm resources in Iran can be found almost in temperate climates with cold winter. It was assumed that in this situation several cold hardy genotypes have been developed by natural selection. In this study, approximately all Iranian wild grape genotypes were sensitive to salt stress, but among them there was moderate cold tolerance as well, but this tolerance were not more tolerable than to the local cold cultivars. Cold tolerance in grapevine not only determines by genetics, but also involves a complex process of physiological changes in which climatic and ecological factors have an important effect. It was reported that altitude and latitude have a significant effect on cold tolerance of accessions of *V. amurensis* and *V. adstricta* [9]. In future studies, it will be better to conclude both latitude and altitude factors in evolution of cold tolerance and other more important traits of wide accessions of Iranian wild grape genotypes.

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

H.D.B. and F.T. conceived, designed, performed the experiments, analyzed the sequencing data and wrote the manuscript. F.T. submitted the manuscript.

Statement

Results presented in this paper are original, not published before and paper is not sent to the publication in another Journal.

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