PLOS ONE

RESEARCH ARTICLE

Geography of Genetic Structure in Barley Wild Relative *Hordeum vulgare* subsp. *spontaneum* in Jordan

Imke Thormann[1]*, Patrick Reeves[2], Ann Reilley[2], Johannes M. M. Engels[1], Ulrike Lohwasser[3], Andreas Börner[3], Klaus Pillen[4], Christopher M. Richards[2]

1 Bioversity International, Rome, Italy, **2** National Center for Genetic Resources Preservation, United States Department of Agriculture-Agricultural Research Service, Fort Collins, Colorado, United States of America, **3** Genebank Department, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, **4** Plant Breeding, Institute for Agricultural and Nutritional Science, Martin-Luther-University Halle-Wittenberg, Halle, Germany

* i.thormann@cgiar.org

Citation: Thormann I, Reeves P, Reilley A, Engels JMM, Lohwasser U, Börner A, et al. (2016) Geography of Genetic Structure in Barley Wild Relative *Hordeum vulgare* subsp. *spontaneum* in Jordan. PLoS ONE 11(8): e0160745. doi:10.1371/journal.pone.0160745

Editor: Dragan Perovic, GERMANY

Received: July 3, 2015

Accepted: July 25, 2016

Published: August 11, 2016

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Informed collecting, conservation, monitoring and utilization of genetic diversity requires knowledge of the distribution and structure of the variation occurring in a species. *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell., a primary wild relative of barley, is an important source of genetic diversity for barley improvement and co-occurs with the domesticate within the center of origin. We studied the current distribution of genetic diversity and population structure in *H*. *vulgare* subsp. *spontaneum* in Jordan and investigated whether it is correlated with either spatial or climatic variation inferred from publically available climate layers commonly used in conservation and ecogeographical studies. The genetic structure of 32 populations collected in 2012 was analyzed with 37 SSRs. Three distinct genetic clusters were identified. Populations were characterized by admixture and high allelic richness, and genetic diversity was concentrated in the northern part of the study area. Genetic structure, spatial location and climate were not correlated. This may point out a limitation in using large scale climatic data layers to predict genetic diversity, especially as it is applied to regional genetic resources collections in *H*. *vulgare* subsp. *spontaneum*.

Introduction

Crop wild relatives (CWR) are vital for food security because they provide novel alleles for crop improvement and adaptation [1–3]. Their diversity is threatened by global and climate change [4,5], and more knowledge about the geographical distribution of their genetic variation, and the processes that shape it, is required to more effectively collect, conserve, monitor and use this variation. Genetic data are still lacking for many CWR. Ecogeographical information, which combines environmental and spatial data, is increasingly used as a proxy for genetic diversity to improve collecting, conservation, monitoring and use of CWR [6–11]. This approach assumes that ecogeographical diversity among collecting sites is correlated with

genetic diversity because the distribution of genetic variation in wild plant species is affected by environment (via natural selection) and geographical separation (via isolation by distance). It follows that conserving populations sampled from the widest possible range of ecogeographical conditions is expected to maximize the genetic diversity conserved [12].

Ecogeographical data has been used to identify areas and populations for *in situ* conservation [7,10,13], to assemble core collections [14] and to identify germplasm potentially useful for crop improvement [15,16]. Habitat suitability modelling, also known as species distribution modelling or niche modelling, has been used to identify gaps in existing collections and to prioritize areas for collecting [8,17–19]. Habitat suitability modelling predicts the potential geographical distribution of a species using the known distribution and environmental data, which often come in the form of climatic, edaphic, geophysical and/or land use variables.

Maxted *et al.* [19] have cautioned that the expected correlation between genetic and ecogeographical diversity may not hold for all species and habitats. CWR are often found in ruderal areas and agricultural landscapes where natural, adaptive responses to climate might be altered through anthropogenic influences [20–23]. Of these, the breakdown of isolation by distance due to elevated gene flow may be particularly important.

Barley is the fourth most important cereal crop worldwide in terms of production, yield and area harvested, and is one of the crops in which CWR use in breeding programs is particularly prominent [24]. *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell. (hereafter *Spontaneum*) is the progenitor of barley and represents an important genetic resource in barley breeding for traits such as powdery mildew, leaf scald or leaf rust resistance [25–31], yield [32], drought and temperature tolerance [33,34] and agronomic traits such as malting quality [35,36]. Recently, a multi-parental nested association mapping population, using 24 *Spontaneum* donor accessions to induce genetic variation, was set up and tested to study regulation of flowering time in barley [37]. The Fertile Crescent has been considered the primary center of origin and domestication of barley [38,39]. Other studies suggest additional domestication events in areas east of the Fertile Crescent [40], Tibet [41], Ethiopia and the Western Mediterranean [42,43].

Efforts have been made since the 1970s to characterize wild barley germplasm across its distribution range using morphological characters, isozymes and molecular markers [44–55]. The highest genetic variation lies within the Fertile Crescent, and there specifically in Jordan and Israel [55,56]. A few studies have compared diversity in *Spontaneum* between Jordan and neighboring countries. Baek *et al.* [57] found that the number of alleles as well as the percentage of country specific alleles is significantly higher in Jordan than in Israel. Analysis of SNP diversity indicated Jordan and southern Syria as a likely site of domestication [54].

Past studies have investigated the correlation between genetic diversity and environment in *Spontaneum*. They have documented an association between genetic diversity, at single loci, and geography, acrosstemperature or rainfall gradients [44,49,52,58–61]. Genetic differentiation has also been shown to occur, in sympatry, between opposing slopes in the Evolution Canyon in Israel. This has been attributed to adaptation to different microclimates [62,63]. In Jordan, Jaradat [64] characterized kernel protein content and genetic diversity at four esterase loci in 12 wild populations. Ribosomal DNA (rDNA) polymorphism was used to study accessions from 27 collecting sites [65]. The distribution of alleles was found to be correlated with ecogeographical factors such as rainfall, temperature, and geographical location. Baek *et al.* [57] used 18 SSRs to study genetic diversity in accessions from 16 collecting sites and reported associations between ecogeographical variables and allele frequencies at individual loci. Hübner *et al.* [66] studied *Spontaneum* in Israel and attempted to correlate genetic population structure—as opposed to polymorphism or allele frequencies at individual loci—with climate variables. No studies of the correlation between environment and population structure of *Spontaneum* in Jordan have yet been published.

In this study, we sampled *Spontaneum* populations across their range in Jordan and analyzed this collection with a set of 37 SSRs. Our aim was to describe the patterns of genetic diversity and population structure of Jordanian *Spontaneum* and to determine the degree to which the genetic structure estimated with our markers is correlated with spatial and climatic variables derived from global data sources commonly used in conservation and ecogeographical studies [18,67–69].

Material and Methods

Plant material and germination

Single spikes of 12–15 individuals were collected from each of 42 *Spontaneum* populations during a barley collecting mission carried out in 2012, which covered the entire distribution of *Spontaneum* in Jordan. The collecting had been formalized in a letter of agreement between the Jordanian National Center for Agricultural Research and Extension (NCARE) and Bioversity International, which encompassed the permit to collect *Spontaneum* from all visited sites. The collecting was carried out with the continuous participation of NCARE staff and no rare or threatened species was collected. Seeds from each spike were germinated to produce leaf tissue for DNA extraction. Up to eight seeds per spike were rolled into germination paper and placed in an incubator at 25°C for germination. 50–100 mg of 3–5 day old leaf tissue was harvested from one germinated seed per spike. 32 populations (Table 1) (where leaf tissue was available from at least 11 individuals) were used for the study. This resulted in a total of 373 genotypes, with 8–13 individuals per population (S1 Table). The spatial distribution of populations is shown in Fig 1.

Ecogeographical and climate data of collecting sites

Geographical coordinates, altitude, slope, and aspect of the collecting sites were recorded with a GPS Garmin Emap device (datum: WGS84) and habitat type was recorded. Climate data was obtained from the WorldClim database version 1.4 (http://www.worldclim.org), a global and freely available source for climate data layers generated through interpolation of average monthly climate data from weather stations [70]. Layers for current climate conditions (1950–2000) for the 19 bioclimatic variables (Bioclim; see Table 2) were downloaded. Values for the 19 variables were extracted for each collecting site using DIVA-GIS. Collecting sites included ruderal habitats, barley field margins as well as nature reserves, covered an altitudinal range from 87 to 1680 m, a latitudinal range from 30.39875–32.70233 decimal degrees, a longitudinal range from 35.49686111–36.09266667 decimal degrees, an annual precipitation range from 229–491 mm and a mean annual temperature range from 12.5–21.5°C. Collecting site information is provided in S2 Table.

DNA extraction and genotyping

DNA was purified from 3–5 day old leaf tissue with the Qiagen DNeasy® 96 Plant Kit. Thirty-seven EST-derived SSR primers were used for genotyping [71–73] (Table 3). Loci were distributed across all 7 barley chromosomes. PCR was carried out in 5-μl reactions consisting of 2–10 ng genomic DNA, 1x Qiagen Multiplex PCR Master Mix, 225 nM of each primer pair. All fragments were amplified using MJ Research (Waltham, Massachusetts) PTC200 thermocyclers and the following PCR profile: an initial denaturing step of 15 min at 95°C followed by 40 cycles with denaturation at 94°C for 60 s, annealing at 60°C for 30 s and extension at 72°C for 15 s. After 40 cycles, a final extension step was performed at 72°C for 10 min. Amplification products were resolved by capillary electrophoresis on the ABI 3130xl Genetic Analyzer.

Table 1. Collecting site description.

Collecting site number	Latitude	Longitude	Elevation (m)	Number of individuals used in study
1	32.70233	35.72325	94	12
2	32.69611	35.737528	119	13
3	32.67656	35.804833	467	11
4	32.59239	35.666944	87	11
5	32.58686	35.998194	475	12
6	32.51183	35.645444	119	12
7	32.47747	35.969694	608	12
8	32.43733	35.691806	484	12
9	32.37594	35.7365	785	12
10	32.33386	35.91375	957	11
11	32.32931	36.092667	866	12
12	32.32144	35.750139	770	12
13	32.27547	35.891333	564	11
14	32.23847	35.889472	379	13
15	32.14508	35.856639	561	12
16	32.13858	35.646806	141	12
17	32.11461	35.86625	629	14
18	32.06989	35.715139	1044	11
19	32.06694	35.720583	1058	12
20	32.04581	35.775167	911	12
21	32.01156	35.733778	589	11
22	31.77919	35.798833	805	9
23	31.70953	35.960611	709	13
24	31.67036	35.785889	745	12
25	31.56639	35.791417	646	11
26	31.54019	35.773639	677	11
27	31.18831	35.696583	773	12
28	31.04872	35.708861	1222	12
29	30.68108	35.622222	1566	12
30	30.65542	35.610194	1257	12
31	30.42178	35.512	1580	8
32	30.39875	35.496861	1680	11

doi:10.1371/journal.pone.0160745.t001

Fragment sizes were calculated using GeneScan 400HD (ROX) internal size standards and scored with GeneMapper software (v. 5.0) (Life Technologies, Thermo Fisher Scientific Inc.).

Genetic diversity

Summary statistics of the marker data such as number of alleles, sample adjusted allelic richness, and observed heterozygosity were calculated with GDA [74] and FSTAT version 2.93.2 [75]. The number of multi-locus genotypes was determined with GeneticStudio (http://dyerlab.bio.vcu.edu). Polymorphism information content (PIC) per locus was calculated with PICcalc [76].

Population differentiation among sites

F_{ST} was used to measure differentiation between populations and was calculated with FSTAT. Inter-individual distances were calculated using a simple matching coefficient with DARwin

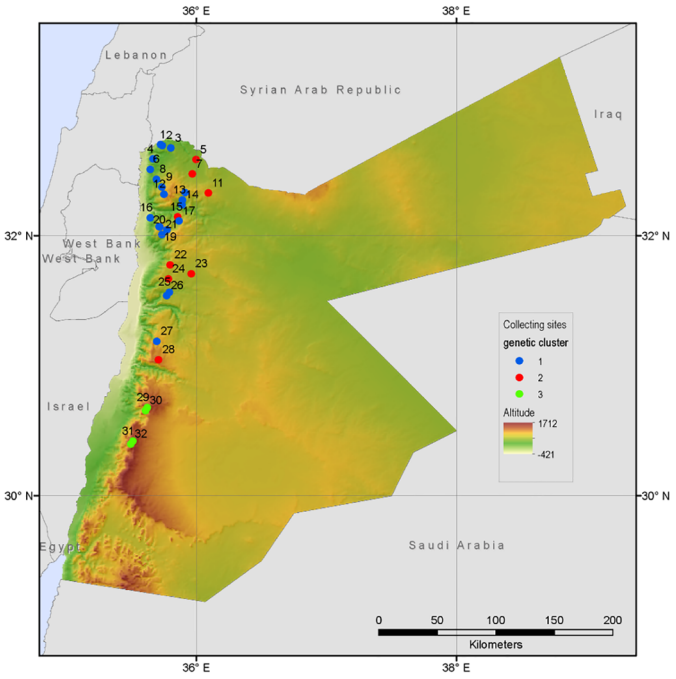

Fig 1. Collecting sites in Jordan.

doi:10.1371/journal.pone.0160745.g001

software version 5.0.158 [77] and used to build a neighbor-joining tree. Because *Spontaneum* is a highly selfing species, the program InStruct [78] was used to infer population structure. InStruct is an extension of the approach used in STRUCTURE [79] and can specifically account for self-pollination and inbreeding. InStruct was run in mode v = 3 (infer population

Table 2. Coding of bioclimatic variables according to WorldClim at http://www.worldclim.org/bioclim.

Code	Description
Bioclim 1	Annual Mean Temperature
Bioclim 2	Mean Diurnal Range (Mean of monthly (max temp—min temp))
Bioclim 3	Isothermality (Bioclim2/ Bioclim7) (* 100)
Bioclim 4	Temperature Seasonality (standard deviation *100)
Bioclim 5	Max Temperature of Warmest Month
Bioclim 6	Min Temperature of Coldest Month
Bioclim 7	Temperature Annual Range (Bioclim5- Bioclim6)
Bioclim 8	Mean Temperature of Wettest Quarter
Bioclim 9	Mean Temperature of Driest Quarter
Bioclim 10	Mean Temperature of Warmest Quarter
Bioclim 11	Mean Temperature of Coldest Quarter
Bioclim 12	Annual Precipitation
Bioclim 13	Precipitation of Wettest Month
Bioclim 14	Precipitation of Driest Month
Bioclim 15	Precipitation Seasonality (Coefficient of Variation)
Bioclim 16	Precipitation of Wettest Quarter
Bioclim 17	Precipitation of Driest Quarter
Bioclim 18	Precipitation of Warmest Quarter
Bioclim 19	Precipitation of Coldest Quarter

doi:10.1371/journal.pone.0160745.t002

Table 3. Characteristics of SSR markers.

Marker ID	Location	Ind no.	Allele no.	PIC	Allelic richness
GBM1002	1H	367	12	0.736	5.44
GBM1013	1H	373	7	0.383	3.194
GBM1029	1H	373	6	0.412	3.038
GBM1334	1H	372	5	0.538	3.317
GBM1461	1H	373	20	0.914	9.082
GBM1035	2H	373	6	0.66	4.264
GBM1036	2H	372	5	0.497	3.289
GBM1047	2H	364	7	0.664	4.269
GBM1208	2H	367	7	0.563	3.693
GBM1218	2H	369	4	0.591	3.593
GBM1459	2H	373	6	0.578	3.931
GBM1043	3H	372	5	0.495	3.735
GBM1110	3H	373	11	0.761	5.362
GBM1280	3H	372	5	0.661	4.038
GBM1405	3H	372	8	0.792	5.795
GBM1413	3H	372	5	0.517	3.171
GBM1003	4H	372	9	0.713	5.164
GBM1015	4H	373	19	0.834	7.016
GBM1020	4H	371	7	0.663	3.96
GBM1323	4H	371	7	0.642	4.535
GBM1026	5H	372	4	0.412	2.379
GBM1054	5H	368	7	0.682	4.822
GBM1064	5H	373	5	0.531	3.263
GBM1176	5H	373	7	0.58	3.892
GBM1363	5H	373	5	0.387	2.817
GBM1008	6H	366	9	0.75	5
GBM1021	6H	367	15	0.87	7.503
GBM1063	6H	373	10	0.746	5.26
GBM1075	6H	370	5	0.34	2.792
GBM1212	6H	372	4	0.376	2.208
GBM1404	6H	373	3	0.359	2.453
GBM1033	7H	373	6	0.687	4.268
GBM1060	7H	370	4	0.528	3.008
GBM1326	7H	373	10	0.816	5.926
GBM1419	7H	373	8	0.7	4.675
GBM1464	7H	365	22	0.888	8.074
GBM1516	7H	373	6	0.593	4.033
Mean		371	7.9	0.6	4.4

The following values are presented for each marker: chromosome location (Location), number of individuals scored (Ind no.), number of alleles (Allele no.), polymorphism information content (PIC), allelic richness.

doi:10.1371/journal.pone.0160745.t003

structure and individual selfing rates) for K = 1–10. For each K, 5 chains were run, with 200,000 MCMC iterations, a burn-in of 100,000 and a thinning interval of 10 steps. Results from independent chains were summarized using CLUMPP [80] and graphical representations of cluster assignments were rendered with DISTRUCT [81]. The *ad hoc* measure of change in likelihood between successive K values, ΔK [82] was calculated to identify the appropriate

number of clusters. As recommended by Gao *et al.* [78], clustering results were compared with results obtained using STRUCTURE v. 2.3.3 [79] and Structure Harvester [83]. STRUCTURE was run with 5 independent runs for each value of K from 1 to 8, with a burn in period of 10^5 followed by 10^5 iterations.

Description of environmental variation in Jordan

We used a procedure developed by Newman and Rissler [84] to delineate distinct environments within the study area. A habitat suitability model was generated for *Spontaneum* with MaxEnt version 3.3.3k [85]. Occurrence data in Jordan was downloaded from Genesys (https://www.genesys-pgr.org). Occurrences showing a geographical coordinate quality rank > 70 [86] were included. The 19 Bioclim layers for current climate conditions (1950–2000), at a resolution of 2.5 arc-minutes, were used. Ten thousand sites were sampled pseudo-randomly from the study area, in proportion to their suitability, as estimated in the habitat suitability model. The environmental data associated with each site was then extracted from all Bioclim layers. Following normalization of each environmental variable, the data set was subjected to k-means clustering such that each pseudo-randomly selected site was assigned to one of k classes. By coloring each site according to habitat, regions within the study area that had similar mean environmental conditions could be visualized.

Association between genetic diversity, geography and environment

Correlations between allelic richness, InStruct clustering results and environmental data were tested using JMP 5.1 (SAS Institute, Cary, NC, USA). Means were compared using the Tukey-Kramer HSD test. Pearson product-moment and Spearman's Rho rank correlation coefficients were calculated. Isolation by distance (IBD) was estimated using R (http://www.r-project.org/). Geographic distances were calculated as straight-line distances with the GeographicDistanceMatrixGenerator version 1.2.3 [87] and log transformed. Genetic distances were calculated as $F_{ST}/(1- F_{ST})$ [88] and as population-wise allelic differences using the FPTEST [89]. Two-tailed Mantel tests were carried out with 10^5 permutations. To test isolation by environment (IBE), environmental distances between sites were estimated. A principal coordinate analysis (PCO) was performed using data from all Bioclim variables and altitude. Environmental distance was then approximated as the simple Euclidean distance between points on the first principal coordinate axis, which accounted for 49% of the environmental variation across sampling sites. The multivariate measure of environmental distance represented a conservative approach aiming to avoid overfitting, as many of the Bioclim parameters covaried significantly. Two-tailed Mantel tests were carried out to estimate IBE. As environmental and geographical distances were significantly correlated, IBD and IBE were also tested using a partial Mantel test. In addition, correlation of the distance matrix calculated with FPTEST to individual Bioclim variables was examined using appropriate Holm-Bonferroni correction [90] to avoid type I error inherent in multiple comparisons.

Results

Genetic diversity

A total of 291 alleles were identified. Alleles per locus ranged from 3 to 22, with an average of 7.9. The mean number of alleles per locus averaged across sites was 2.8. PIC varied from 0.34 to 0.914 with a mean of 0.62. Allelic richness per locus varied from 2.2–9.1. All populations showed low observed heterozygosity (H_o) ranging between 0–0.025. *Spontaneum* is a highly self-pollinating species and previous studies on *Spontaneum* reported similar levels of

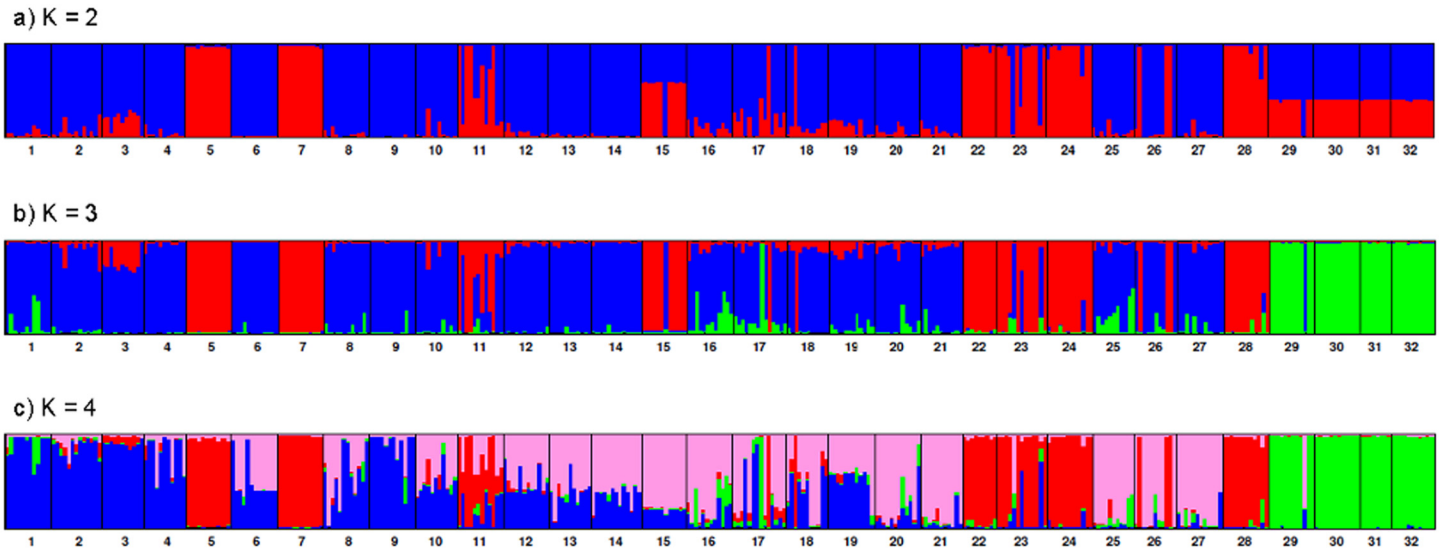

Fig 2. Assignment of individuals to genetic clusters identified by InStruct, for K = 2 to K = 4. Populations are sorted from left to right by decreasing latitude. Clusters are depicted in the following colours: cluster 1 = blue; cluster 2 = red; cluster 3 = green; cluster 4 = pink.

doi:10.1371/journal.pone.0160745.g002

heterozygosity [66,91]. A total of 370 multi-locus genotypes were identified. Only three populations (5, 15, 18) showed a single multi-locus genotype twice. Allelic richness per population ranged from 1.4 to 3.3 with a mean of 2.63.

Population differentiation among sites

Differentiation among populations measured as F_{ST} was 0.33, i.e. 33% of variation was distributed between populations and 67% within populations, similar to previous studies [57,59,92,93]. The ΔK method [82] applied to InStruct and STRUCTURE results suggested subdivision into three clusters. Fig 2 shows the individual assignment coefficients for K = 2 to K = 4. Partitioning into three genetic clusters produced one group of populations predominantly located in the northwestern part of the collecting area and a second cluster which showed a longitudinal extension from the northeast southwards. A small third cluster was geographically separated in the southern part of the collecting area. The geographical distribution of the three clusters is shown in Fig 1. The InStruct assignment was compatible with the neighbor-joining tree based on inter-individual genetic distances (Fig 3). Assignment coefficients (q) varied across the study area. The average assignment coefficient of individuals to cluster 1 was significantly lower (q = 0.902; p = <0.0001) than those of cluster 2 (q = 0.966) and cluster 3 (q = 0.99). The assignment coefficient was inversely correlated with latitude (Pearson coefficient: r = -0.17; p = 0.001; Spearman's rank coefficient: r = -0.204; p<0.0001) and positively correlated with altitude (Pearson coefficient: r = 0.166; p = 0.0013; Spearman's rank coefficient: r = 0.235; p<0.0001) indicating that the level of admixture was higher in the north. While there were 10 populations whose respective individuals were all strongly assigned to the same genetic cluster ($q \geq 0.8$), the remaining populations contained some individuals either strongly assigned to a different cluster (physical admixture), and/or some genetically admixed individuals ($0.49 < q < 0.8$) (Table 4). Eight populations were physically admixed, with 1–6 individuals assigned to a different cluster. 18 populations contained 1–9 genetically admixed individuals (four of these populations were also physically admixed). In populations assigned to cluster 3, only the population in site 29 showed physical admixture (one individual assigned to cluster

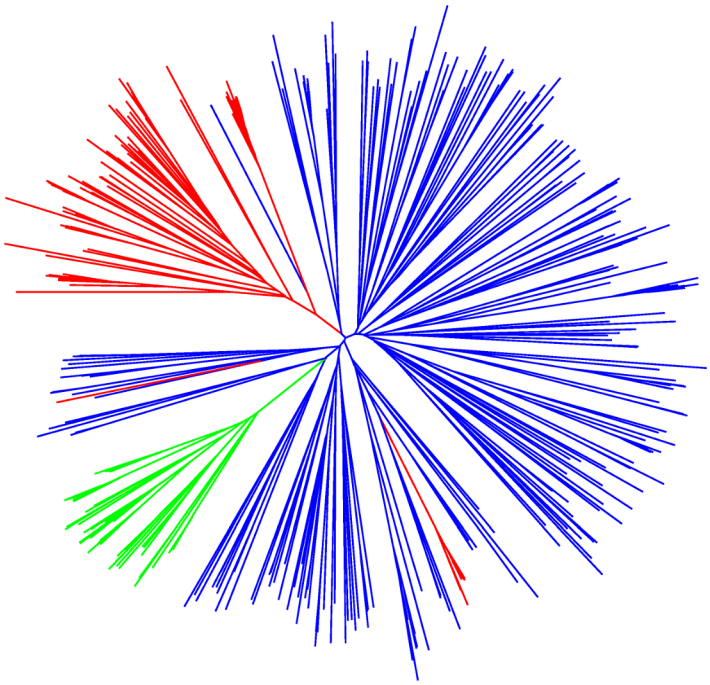

Fig 3. Neighbor-joining tree showing inter-individual genetic distances. Genetic clusters are depicted in the following colours: cluster 1 = blue; cluster 2 = red; cluster 3 = green.

doi:10.1371/journal.pone.0160745.g003

1), no genetic admixture was identified in any of the four populations of cluster 3. All other physical admixture stems from individuals either assigned to cluster 1 but growing within a site assigned to cluster 2 or vice versa. 88% of the 43 genetically admixed individuals belong to populations assigned to cluster 1, the remaining to cluster 2.

Association between genetic diversity, geography and environment

K-means clustering was used to delineate different environments that might be inhabited by *Spontaneum* in Jordan. Regions of the study area with distinct environmental conditions are depicted in Fig 4. They are predominantly arranged as north-south stripes corresponding to the three main topographical regions described by Al-Eisawi [94] (rift valley along the western border; mountain range extending from the north in Irbid to the south in Ras An-Naqab, and the eastern desert). Although the sampling scheme also followed a north-south transect, populations were sampled from the majority of the distinct environments identified (Fig 4). The geographical distribution of the genetic clusters did not match the geographical distribution of these environmental partitions.

When comparing populations collected from nature reserves and those collected from ruderal habitats, roadsides or field margins, no significant differences in genetic diversity

Table 4. Genetic and physical admixture.

	Physical admixture		Genetic admixture	
Cluster	**No. of populations**	**No. of individuals**	**No. of populations**	**No. of individuals**
1	4	11	14	38
2	3	5	4	5
3	1	1	0	0

doi:10.1371/journal.pone.0160745.t004

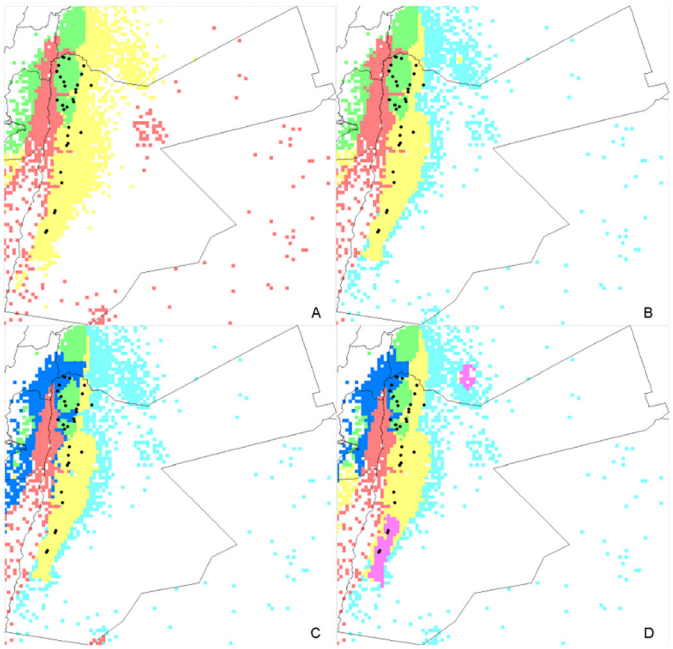

Fig 4. Habitat types in Jordan identified through k-means clustering. Black dots represent the *Spontaneum* collecting sites.

doi:10.1371/journal.pone.0160745.g004

measures were found. No physical admixture was detected in populations collected in reserves, while they do show genetic admixture. Plants collected in reserves were significantly smaller (37.0 cm) than those collected from ruderal areas or field margins (72.6 cm; p = 0.0047). Also population size observed in reserves was significantly smaller (p = 0.0112, Tukey-Kramer HSD test; based on observed size of all wild populations sampled during the 2012 barley collecting mission). The average habitat suitability, according to the habitat suitability model, was significantly lower in reserves than in the other sites (p = 0.0289).

Average values of geographical, geophysical and Bioclim variables, allelic richness, and selfing rates are compared among clusters in Table 5. Average longitude and Bioclim 6 were the only variables that were significantly different between all three clusters. Cluster 3 collecting sites were significantly different for several variables including: higher elevation, lower latitude, lower values for temperature-related Bioclim variables 1, 5, 8, 9, 10, 11 (these Bioclim variables are highly correlated, r>0.8) and lower selfing rates. Cluster 1 showed significantly higher allelic richness and higher values for Bioclim 13. No significant differences were found for habitat type, aspect, soil type and Bioclim 2, 3, 7, 15, 16 and 19. Bioclim 14, 17 and 18 were zero at all sites. Allelic richness of loci and of populations was weakly correlated with latitude (loci: Spearman's rank coefficient r = 0.079, p = 0.0065; Pearson's coefficient r = 0.147, p<0.0001; populations: Pearson's coefficient r = 0.36, p = 0.0432).

Genetic and geographical distance were significantly correlated (F_{ST} based distance: r = 0.3, p = 0.0003; FPTEST based distance: r = 0.2, p = 0.02), suggesting isolation by distance, when analyzed over all 32 populations, while the Mantel tests for isolation by environment were not significant. Environmental and geographic distance were strongly correlated (r = 0.4, p = 0.0001), indicating possible confounding effects. These were accounted for using a partial Mantel test which confirmed significant IBD among all studied populations (r = 0.25, p = 0.004), but did not find significant IBE (S1 Resource). Several climate variables

Table 5. Comparison of average values for geographical, geophysical and Bioclim variables, allelic richness and selfing rate at collecting sites among genetic clusters.

Variable	Cluster 1			Cluster 2			Cluster 3		
	Level	Mean	p	Level	Mean	p	Level	Mean	p
Allelic richness	A	2.91		B	2.25	<0.0001	B	2.0	<0.0001
Selfing rate	A	0.831	0.0294	A	0.833	0.0220	B	0.0813	
Altitude (m)	B	564	<0.0001	B	749	0.0005	A	1521	
Latitude	A	32.195	<0.0001	A	31.968	<0.0001	B	30.539	
Longitude	A	35.76	0.0015 (1–3)	B	35.9	0.0038 (2–1)	C	35.56	<0.0001 (3–2)
Aspect	A	233.99	ns	A	185.07	ns	A	199.04	ns
Slope	A	3.54	0.0030	B	0.99		A	4.81	0.0024
Bioclim 1	A	18.03	<0.0001	A	16.67	0.0122	B	13.78	
Bioclim 2	A	11.68	ns	A	12.21	ns	A	11.60	ns
Bioclim 3	A	42.94	ns	A	44.10	ns	A	42.82	ns
Bioclim 4	A	625.73	0.0151	AB	620.55		B	598.47	
Bioclim 5	A	32.07	0.0005	A	30.94	0.0249	B	27.96	
Bioclim 6	A	4.9	<0.0001 (1–3)	B	3.25	0.03 (2–1)	C	0.86	0.0332 (3–2)
Bioclim 7	A	27.17	ns	A	27.69	ns	A	27.1	ns
Bioclim 8	A	10.1	0.0002	A	8.68	0.0287	B	6.06	
Bioclim 9	A	25.0	<0.0001	A	23.59	0.0145	B	20.65	
Bioclim 10	A	25.04	<0.0001	A	23.63	0.0130	B	20.65	
Bioclim 11	A	10.1	0.0002	A	8.68	0.0287	B	6.06	
Bioclim 12	A	388.0	0.0448 (1–3)	AB	323.88		B	289.75	
Bioclim 13	A	92.1		B	73.75	0.0314	B	67.0	0.0238
Bioclim 15	A	113.44	ns	A	113.02	ns	A	115.12	ns
Bioclim 16	A	250.1	ns	A	206.88	ns	A	187.0	ns
Bioclim 19	A	250.1	ns	A	206.88	ns	A	187.0	ns

Bioclim 1- Bioclim 19 = Bioclimatic variables as per definition on http://www.worldclim.org/bioclim (see Table 2); ns = non-significant; levels marked with different letters indicate significant difference among cluster averages (p<0.05) based on the Tukey HSD test. Numbers in brackets after p values indicate the two clusters being compared.

doi:10.1371/journal.pone.0160745.t005

were found to be different in cluster 3 compared to cluster 1 and 2 (Table 5). Cluster 3 was furthermore geographically separated from clusters 1 and 2, which are themselves partly overlapping. The correlation analyses were therefore repeated for populations belonging to clusters 1 and 2 only. No significant IBD or IBE was found between cluster 1 and 2, and neither were environmental and geographic distances significantly correlated. No significant correlations existed between single Bioclim variables and distance matrix calculated with FPTEST (S1 Resource).

Discussion

The present study examined the current geography of genetic structure and its correlation with landscape scale climatic and spatial variation in *Spontaneum* populations in Jordan. Correlation analyses showed large scale IBD across the study area but did not reveal a correspondence between climate and genetic structure. Analysis of population structure suggested that the 32 *Spontaneum* populations could be divided into three major, genetically differentiated clusters (Fig 1). Genetic diversity was concentrated in the northern part of the study area, across a range of environments, where populations are characterized by physical and genetic admixture, and high

allelic richness. Allelic richness and admixture decrease towards the south; the southernmost populations are not admixed, exhibit low allelic richness and contain physically smaller plants.

Genetic structure is not correlated with climatic variation inferred from global layers

Three genetic clusters were distributed along a longitudinal gradient in the North (clusters 1 and 2), with a distinct cluster (cluster 3) in the South. The study area was characterized by a longitudinal distribution of distinct habitat types as shown in Fig 4, of which the central mountain range was the most variable. At the large scale across the entire study area, where geographical and environmental distances were strongly correlated, significant IBD implied that physical distance was important for genetic differentiation among populations, but environmental variation was found to have no effect. Results were different at a slightly smaller scale, across the central and northern part of the study area, where clusters 1 and 2 spread across an environmentally heterogeneous landscape. Here, geographical and environmental distances were both uncorrelated with genetic distance either measured by F_{ST} or by population-wise allelic differences.

Spontaneum prefers disturbed, human-made or influenced habitats [20,22], sympatric with its domesticate [95–98]. These habitats favor anthropogenic movement of material—inclusion and transport with cultivated barley seed lots or hitchhiking on livestock fur or human clothing—which interferes with natural diffusion and selection processes. This may alter the expected distribution of genetic diversity across the landscape and lead to weak or nonexistent correlations between ecogeographical and genetic diversity as found in our study. Natural dispersal and selection processes may not have been the principle force shaping genetic structure in some regions of Jordan.

Spontaneum is a highly self-pollinating species. In self-pollinating species much genetic diversity is distributed among populations rather than within populations, population to population variation is greater than in out-crossing species and the genetic structure is more variable [99]. Given their low gene flow and very localized gene transfer, genetic structure has been found at local scale [63,100,101]. This local variation is unlikely to be detected by globally available layers commonly used to represent landscape scale spatial and climatic variation.

Global climate data such as the Bioclim layers provided by WorldClim climatic data are used in a range of studies and applications [11,19,67–69,85,86,102], and the inherent assumption is that they are robust proxies for genetic data, which is often not available. Our results suggest that there may be some limitations on this assumption. Our study did not find a correlation between climate, as represented by commonly used global, interpolated data layers, and genetic structure for *Spontaneum*. Thus global climatic data would not be especially useful for predicting existing genetic diversity in Jordan. A ruderal habitat preference and high self-pollination might explain why the general expectation of tight correlation between genetic and ecogeographical diversity does not hold. If collecting and conservation actions are designed without previous knowledge of genetic structure, it will be important to consider species biology and habitat preferences when using ecogeographical diversity to predict genetic diversity.

Sampling and monitoring genetic diversity within *Spontaneum* populations

All *Spontaneum* populations sampled here, irrespective of cluster assignment, contained many unique multi-locus genotypes. Only three populations showed a single multi-locus genotype twice, and no multi-locus genotype was repeated among populations. Allelic richness, which is a good metric to assess and monitor genetic diversity [103], increased significantly towards the

northern part of the study area. Here, populations were also characterized by admixture. More than half of the populations in clusters 1 and 2 showed considerable genetic admixture as well as physical admixture, a characteristic that was also found by Hübner *et al.* [66] in Israel. Hübner *et al.* [93] observed a fairly high rate of gene flow in *Spontaneum* attributed to sporadic outcrossing events [104] and gene flow through seed dispersal. These mechanisms likely contribute to physical and genetic admixture in Jordan as well.

Due to the reduced level of diversity expected within populations of highly selfing species, germplasm collections are often limited to a few samples per population. The heterogeneity found within populations in this study cautions against such sampling strategies. Modeling studies have shown that collections of highly selfing species need substantially more samples than are commonly recommended to capture existing diversity [105]. The distribution of genetic structure we have described for *Spontaneum* in Jordan prescribes further collecting and monitoring in the northern part of the country, in particular the area occupied by cluster 1.

Ex situ and *in situ* conservation of *Spontaneum*

Natural populations of *Spontaneum* have been reported to harbour large neutral genetic diversity, and also show considerable diversity in disease resistance and quantitative traits of agronomic importance [45,106–108]. Despite evidence of high genetic, adaptive and quantitative diversity in Jordanian *Spontaneum* populations, the number of *ex situ* barley accessions from Jordan in global collections is lower than those from neighboring countries. Although in general the number of *Spontaneum* accessions in *ex situ* collections seems relatively high compared with other CWR samples in genebanks, they are derived from a limited number of populations [109]. Maxted and Kell [24] suggest that, although *Spontaneum* is widespread and locally common [110], individual populations might contain important adaptive traits, thus populations should be actively conserved throughout the geographical range. Vincent *et al.* [111] identified Jordan as one of the countries where wild *Hordeum* should be conserved and suggested the establishment of a network of several reserves in the Israel/Jordan region to more effectively conserve the genetic diversity of wild *Hordeum*. These assessments describe the obvious need to promote *in situ* conservation of *Spontaneum* in Jordan and to enlarge *ex situ* collections. Our description of the distribution of genetic diversity across the Jordanian landscape provides a tool to evaluate the propriety of existing *in situ* conservation activities and supports the application of proper sampling techniques for future *ex situ* acquisitions.

Supporting Information

S1 Resource. Correlation analyses between genetic, geographic and environmental data.
(PDF)

S1 Table. Microsatellite data for 373 *Spontaneum* individuals.
(PDF)

S2 Table. Ecogeographical and genetic data for 32 *Spontaneum* populations collected in Jordan in 2012.
(PDF)

Author Contributions

Conceptualization: IT JMME UL AB KP CMR.

Data curation: IT PR AR CMR.

Formal analysis: IT PR AR CMR.

Funding acquisition: IT.

Methodology: IT PR AR JMME UL AB KP CMR.

Project administration: IT.

Writing - original draft: IT.

Writing - review & editing: IT PR AR JMME UL AB KP CMR.

References

1. Gur A, Zamir D. Unused natural variation can lift yield barriers in plant breeding. PLoS Biology. 2004; 2(10):e245. doi: 10.1371/journal.pbio.0020245 PMID: 15328532
2. Hajjar R, Hodgkin T. The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica. 2007; 156:1–13.
3. McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E, et al. Feeding the future. Nature. 2013; 499:23–24. doi: 10.1038/499023a PMID: 23823779
4. Jarvis A, Lane A, Hijmans RJ. The effect of climate change on crop wild relatives. *Agriculture*, Ecosystems & Environment. 2008; 126:13–23.
5. FAO. Report of the Fourteenth Regular Session of the Commission on Genetic Resources for Food and Agriculture. CGRFA 14/13/report. 2013. Available: http://www.fao.org/nr/cgrfa/cgrfa-meetings/cgrfa-comm/fourteenth-reg/en/.
6. Parra-Quijano M, Draper D, Torres E, Iriondo JM. Ecogeographical representativeness in crop wild relative *ex situ* collections. In: Maxted N, Ford-Lloyd BV, Kell SP, Iriondo JM, Dulloo E, Turok J, editors. Crop wild relative conservation and use. CAB International, Wallingford, UK; 2008. pp. 249–273.
7. Parra-Quijano M, Iriondo JM, Frese L, Torres E. Spatial and ecogeographical approaches for selecting genetic reserves in Europe. In: Maxted N, Dulloo ME, Ford-Lloyd BV, Frese L, Iriondo JM, Pinheiro de Carvalho MAA, editors. Agrobiodiversity Conservation: securing the diversity of crop wild relatives and landraces. CAB International, Wallingford, UK; 2012. pp. 20–28.
8. Parra-Quijano M, Iriondo JM, Torres E. Improving representativeness of genebank collections through species distribution models, gap analysis and ecogeographical maps. Biodivers Conserv. 2012; 21:79–96.
9. Steiner JJ. Exploring the relationship of plant genotype and phenotype to ecogeography. In: Greene SL, Guarino L, editors. Linking genetic resources and geography: Emerging strategies for conserving and using crop biodiversity. Crop Science Society of America Inc., Madison, WI; 1999. pp. 39–50.
10. Dulloo ME, Labokas J, Iriondo JM, Maxted N, Lane A, Laguna E, et al. Genetic reserve location and design. In: Iriondo JM, Maxted N, Dulloo ME, editors. Conserving plant genetic diversity in protected areas. CAB International; 2008. pp. 23–64.
11. Parra-Quijano M, Iriondo JM, Torres E. Review. Applications of ecogeography and geographic information systems in conservation and utilization of plant genetic resources. Spanish J Agric Res. 2012; 10(2):419–429.
12. Maxted N, Van Slageren MW, Rihan J. Ecogeographic surveys. In: Guarino L, Ramanatha Rao V, Reid R, editors. Collecting plant genetic diversity: Technical guidelines. CAB International, Wallingford, UK; 1995. pp. 255–286.
13. Maxted N, Magos Brehm J, Kell S. Resource book for preparation of national conservation plans for crop wild relatives and landraces. Food and Agriculture Organization of the United Nations, Italy; 2013.
14. Parra-Quijano M, Iriondo JM, de la Cruz M, Torres E. Strategies for the development of core collections based on ecogeographical data. Crop Sci. 2011; 51:656–666.
15. Khazaei H, Street K, Bari A, Mackay M, Stoddard FL. The FIGS (focused identification of germplasm strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. PLoS ONE 2013; 8(5):e63107. doi: 10.1371/journal.pone.0063107 PMID: 23667581
16. Thormann I, Parra-Quijano M, Endresen DTF, Rubio-Teso ML, Iriondo MJ, Maxted N. Predictive characterization of crop wild relatives and landraces. Technical guidelines version 1. Bioversity International, Rome, Italy; 2014.

17. Jarvis A, Williams K, Williams D, Guarino L, Caballero PJ, Mottram G. Use of GIS for optimizing a collecting mission for a rare wild pepper (*Capsicum flexuosum* Sendtn.) in Paraguay. Genet Resour Crop Evol. 2005; 52:671–682.
18. Ramirez-Villegas J, Khoury C, Jarvis A, Debouck DG, Guarino L. A gap analysis methodology for collecting crop genepools: A case study with *Phaseolus* beans. PLoS ONE. 2010; 5(10):e13497. doi: 10.1371/journal.pone.0013497 PMID: 20976009
19. Maxted N, Dulloo ME, Ford-Lloyd BV, Iriondo MJ, Jarvis A. Gap analysis: a tool for complementary genetic conservation assessment. Divers Distrib. 2008; 14:1018–1030.
20. Ellstrand NC. Dangerous liaisons? When cultivated plants mate with their wild relatives. Johns Hopkins University Press, Baltimore, USA; 2003.
21. Andersen MS, de Vicente CM. Gene flow between crops and their wild relatives. Johns Hopkins University Press, Baltimore, USA; 2010.
22. Jain SK. Genetic reserves. In: Frankel OH, Hawkes JG, editors. Crop genetic resources for today and tomorrow. Cambridge University Press, UK; 1975. pp. 379–396.
23. Jarvis S, Fielder H, Hopkins J, Maxted N, Smart S. Distribution of crop wild relatives of conservation priority in the UK landscape. Biol. Cons. 2015; 191:444–451.
24. Maxted N, Kell SP. Establishment of a global network for the in situ conservation of crop wild relatives: status and needs. FAO Commission on Genetic Resources for Food and Agriculture, Rome, Italy; 2009.
25. Fischbeck G, Schwarzbach E, Sobel F, Wahl I. Mehltauresistenz aus Israelischen Populationen der zweizeiligen Wildgerste (*Hordeum spontaneum*). Z. Pflanzenzüchtung. 1976; 76:163–166.
26. Ivandic V, Walther U, Graner A. Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theor Appl Genet. 1998; 97:1235–1239.
27. Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, et al. Localization of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and wild barley (*Hordeum vulgare* subsp. *spontaneum*) line. Theor Appl Genet. 2003; 106:353–362. PMID: 12582863
28. Dreiseitl A, Bockelman HE. Sources of powdery mildew resistance in a wild barley collection. Genet Resour Crop Evol. 2003; 50:345–350.
29. Genger RK, Williams KJ, Raman H, Read BJ, Wallwork H, Burdon JJ, et al. Leaf scald resistance genes in *Hordeum vulgare* and *Hordeum vulgare* ssp. *spontaneum*: parallels between cultivated and wild barley. Aust J Agric Res. 2003; 54(12):1335–1342.
30. von Korff M, Wang H, Léon J, Pillen K. AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. Theor Appl Genet. 2005; 111(3):583–90. PMID: 15902395
31. Repkova J, Dreiseitl A, Lizal P, Kyjovska Z, Teturova K, Psotkova R, et al. Identification of resistance genes against powdery mildew in four accessions of *Hordeum vulgare* ssp. *spontaneum*. Euphytica. 2006; 151:23–30.
32. von Korff M, Wang H, Leon J, Pillen K. AB-QTL analysis in spring barley. II. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*Hordeum vulgare* ssp. *spontaneum*). Theor Appl Genet. 2006; 112:1221–1231. PMID: 16477429
33. Chen G, Li C, Shi Y, Nevo E. Wild barley, *Hordeum spontaneum*, a genetic resource for crop improvement in cold and arid regions. Sciences in cold and arid regions. 2008; 1:0115–0124.
34. Lakew B, Henry RJ, Eglinton J, Baum M, Ceccarelli S, Grando S. SSR analysis of introgression of drought tolerance from the genome of Hordeum spontaneum into cultivated barley (*Hordeum vulgare* ssp *vulgare*). Euphytica. 2013; 191(2):231–243.
35. Erkkila MJ, Leah R, Ahokas H, Cameron-Mills V. Allele-dependent barley grain β-Amylase activity. Plant Physiol. 1998; 117:679–685. PMID: 9625721
36. von Korff M, Wang H, Léon J, Pillen K. AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). Mol Breed. 2008; 21(1):81–93.
37. Maurer A, Draba V, Jiang Y, Schnaithmann F, Sharma R, Schumann E, et al. Modeling the genetic architecture of flowering time control in barley through nested association mapping. BMC Genomics. 2015; 16:290. doi: 10.1186/s12864-015-1459-7 PMID: 25887319
38. Zohary D, Hopf M. Domestication of plants in the Old World, 2nd edition. Oxford University Press; 1993.

39. Badr A, Mueller K, Schaefer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, et al. On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol. 2000; 17(4):499–510. PMID: 10742042

40. Morrell PL, Clegg MT. Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent. Proc Natl Acad Sci USA. 2007; 104:3289–3294. PMID: 17360640

41. Dai F, Nevo E, Wu D, Comadran J, Zhou M, Qiu L, et al. Tibet is one of the centers of domestication of cultivated barley. Proc Natl Acad Sci USA. 2012; 109(42):16969–16973. doi: 10.1073/pnas.1215265109 PMID: 23033493

42. Molina-Cano JL, Russell JR, Moralejo MA, Excacena JL, Arias G, Powell W. Chloroplast DNA microsatellite analysis supports a polyphyletic origin for barley. Theor Appl Genet. 2005; 110:613–619. PMID: 15723272

43. Orabi J, Backes G, Wolday A, Yahyaoui A, Jahoor A. The Horn of Africa as a center of barley diversification and a potential domestication site. Theor Appl Genet. 2007; 114(6):1117–27. PMID: 17279366

44. Nevo E, Zohary D, Brown AHD, Haber M. Genetic diversity and environmental associations of wild barley, *Hordeum sponateneum*, in Israel. Evolution. 1979; 33(3):815–833.

45. Nevo E, Beiles A, Gutterman Y, Storch N, Kaplan D. Genetic resources of wild cereals in Israel and vicinity. II. Phenotypic variation within and between populations of wild barley, *Hordeum spontaneum*. Euphytica. 1984; 33(3):737–756.

46. Baum BR, Nevo E, Johnson DA, Beiles A. Genetic diversity in wild barley (*Hordeum spontaneum C*. Koch) in the Near East: a molecular analysis using Random Amplified Polymorphic DNA (RAPD) markers. Genet Resour Crop Evol. 1997; 44(2):147–157.

47. Pakniyat H, Powell W, Baird E, Handley LL, Robinson D, Scrimgeour CM, et al. AFLP variation in wild barley (*Hordeum spontaneum C*. Koch) with reference to salt tolerance and associated ecogeography. Genome 1997; 40:332–341. PMID: 18464832

48. Gupta PK, Sharma PK, Balyan HS, Roy JK, Sharma S, Beharav A, et al. Polymorphism at rDNA loci in barley and its relation with climatic variables. Theor Appl Genet 2002; 104:473–481. PMID: 12582721

49. Liviero L, Maestri E, Gulli M, Nevo E, Marmiroli N. Ecogeographic adaptation and genetic variation in wild barley, application of molecular markers targeted to environmentally regulated genes. Genet Resour Crop Evol. 2002; 49:133–144.

50. Ivandic V, Hackett CA, Nevo E, Keith R, Thomas WTB, Forster BP. Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time. Plant Mol Biol. 2002; 48(5–6):511–527. PMID: 11999832

51. Ozkan H, Kafkas S, Sertac Ozer M, Brandolini A. Genetic relationships among South-East Turkey wild barley populations and sampling strategies of *Hordeum spontaneum*. Theor Appl Genet. 2005; 112(1):12–20. PMID: 16283231

52. Cronin JK, Bundock PC, Henry RJ, Nevo E. Adaptive climatic molecular evolution in wild barley at the Isa defense locus. Proc Nat Acad Sci USA. 2007; 104:2773–2778. PMID: 17301230

53. Wang A, Yu Z, Ding Y. Genetic diversity analysis of wild close relatives of barley from Tibet and the Middle East by ISSR and SSR markers. Comptes Rendus Biologies. 2009; 332:393–403. doi: 10.1016/j.crvi.2008.11.007 PMID: 19304270

54. Russell J, Dawson IK, Flavell AJ, Steffenson B, Weltzien E, Booth A, et al. Analysis of 1000 single nucleotide polymorphisms in geographically matched samples of landrace and wild barley indicates secondary contact and chromosome-level differences in diversity around domestication genes. New Phytologist. 2011; 191(2):564–578. doi: 10.1111/j.1469-8137.2011.03704.x PMID: 21443695

55. Jakob SS, Roedder D, Engler JO, Shaaf S, Oezkan H, Blattner FR, et al. Evolutionary history of wild barley (*Hordeum vulgare* subsp. *spontaneum*) analyzed using multilocus sequence data and paleodistribution modeling. Genome Biol Evol. 2014; 6(3):685–702. doi: 10.1093/gbe/evu047 PMID: 24586028

56. Fu YB, Horbach C. Genetic diversity in a core subset of wild barley germplasm. Diversity. 2012; 4 (2):239–257.

57. Baek HJ, Beharav A, Nevo E. Ecological-genomic diversity of microsatellites in wild barley, *Hordeum spontaneum*, populations in Jordan. Theor Appl Genet. 2003; 106(3):397–410. PMID: 12589539

58. Lin JZ, Brown AHD, Clegg MT. Heterogeneous geographic patterns of nucleotide sequence diversity between two alcohol dehydrogenase genes in wild barley (*Hordeum vulgare* subsp. *spontaneum*). Proc Nat Acad Sci USA. 2001; 98:531–536. PMID: 11149938

59. Turpeinen T, Tenhola T, Manninen O, Nevo E, Nissilä E. Microsatellite diversity associated with ecological factors in *Hordeum spontaneum* populations in Israel. Mol Ecol. 2001; 10(6):1577–1591. PMID: 11412377

60. Chen G, Suprunova T, Krugman T, Fahima T, Nevo E. Ecogeographic and genetic determinants of kernel weight and colour of wild barley (*Hordeum spontaneum*) populations in Israel. Seed Sci. Res. 2004; 14:137–146.

61. Batchu AK, Zimmermann D, Schulze-Lefert P, Koprek T. Correlation between hordatine accumulation, environmental factors and genetic diversity in wild barley (*Hordeum spontaneum C*. Koch) accessions from the Near East Fertile Crescent. Genetica. 2006; 127(1–3):87–99. PMID: 16850216

62. Yang Z, Zhang T, Bolshoy A, Beharav A, Nevo E. Adaptive microclimatic structural and expressional dehydrin 1 evolution in wild barley, *Hordeum spontaneum*, at 'Evolution Canyon', Mount Carmel, Israel. Mol Ecol. 2009; 18:2063–2075. doi: 10.1111/j.1365-294X.2009.04140.x PMID: 19344351

63. Nevo E. Evolution of wild barley at "Evolution Canyon": adaptation, speciation, pre-agricultural collection, and barley improvement. Israel J Plant Sci. 2014; 62(1–2):22–32.

64. Jaradat AA. Genetic diversity of four esterase loci in natural populations of *Hordeum spontaneum* C. Koch from Jordan. Theor Appl Genet. 1992; 84:725–729. doi: 10.1007/BF00224176 PMID: 24201365

65. Sharma S, Beharav A, Balyan HS, Nevo E, Gupta PK. Ribosomal DNA polymorphism and its association with geographical and climatic variables in 27 wild barley populations from Jordan. Plant Sci. 2004; 166:467–477.

66. Hübner S, Hüffken M, Oren E, Haseneyer G, Stein N, Graner A, et al. Strong correlation of wild barley (*Hordeum spontaneum*) population structure with temperature and precipitation variation. Mol Ecol. 2009; 18:1523–1536. doi: 10.1111/j.1365-294X.2009.04106.x PMID: 19368652

67. Castañeda-Álvarez NP, de Haan S, Juárez H, Khoury CK, Achicanoy HA, Sosa CC, et al. Ex Situ Conservation Priorities for the Wild Relatives of Potato (*Solanum* L. Section Petota). PLoS ONE. 2014; 10(4):e0122599. doi: 10.1371/journal.pone.0122599

68. Khoury CK, Castañeda-Alvarez NP, Achicanoy HA, Sosa CC, Bernau V, Kassa MT, et al. Crop wild relatives of pigeonpea [*Cajanus cajan* (L.) Millsp.]: Distributions, ex situ conservation status, and potential genetic resources for abiotic stress tolerance. Biol Conserv 2015; 184:259–270.

69. Kantar MB, Sosa CC, Khoury CK, Castañeda-Álvarez NP, Achicanoy HA, Bernau V, et al. Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (*Helianthus annuus* L.). Frontiers in Plant Sci. 2015; 6:841.

70. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. Int J Climat. 2005; 25(15):1965–1978.

71. Thiel T, Michalek W, Varshney RK, Graner A. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet. 2003; 106:411–422. PMID: 12589540

72. Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, et al. A 1000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet. 2007; 114:823–839. PMID: 17219208

73. Varshney RK, Marcel TC, Ramsay L, Russell J, Roder MS, Stein N, et al. A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet. 2007; 114(6):1091–1103. PMID: 17345060

74. Lewis PO, Zaykin D. Genetic Data Analysis: Computer Program for the Analysis of Allelic Data (version 1.1). 2001; Available: http://hydrodictyon.eeb.uconn.edu/people/plewis/downloads/gda-1.1.win32.zip.

75. Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). 2001; Available: http://www.unil.ch/popgen/softwares/fstat.htm.

76. Nagy S, Poczai P, Cernák I, Gorji AM, Hegedűs G, Taller J. PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. Biochemical Genet. 2012; 50(9–10):670–672.

77. Perrier X, Jacquemoud-Collet JP. DARwin software. 2006; Available: http://darwin.cirad.fr/.

78. Gao H, Williamson S, Bustamante CD. A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. Genetics. 2007; 176 (3):1635–1651. PMID: 17483417

79. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–959. PMID: 10835412

80. Jakobsson M, Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 2007; 23 (14):1801–1806. PMID: 17485429

81. Rosenberg NA. Distruct: a program for the graphical display of population structure. Mol Ecol Notes. 2004; 4:137–138.

82. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 2005; 14(8):2611–2620. PMID: 15969739

83. Earl DA, von Holdt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2012; 4(2):359–361.

84. Newman CE, Rissler LJ. Phylogeographic analyses of the southern leopard frog: the impact of geography and climate on the distribution of genetic lineages vs. subspecies. Mol Ecol. 2011; 20:5295–5312. doi: 10.1111/j.1365-294X.2011.05353.x PMID: 22066968

85. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. Ecol Modeling. 2006; 190:231–259.

86. Parra-Quijano M, Torres E, Iriondo JM and López F. CAPFITOGEN Tools User Manual Version 1.2. International Treaty on Plant Genetic Resources for Food and Agriculture, FAO, Rome, Italy; 2014.

87. Ersts PJ. Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. Available: http://biodiversityinformatics.amnh.org/open_source/gdmg.

88. Rousset F. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics. 1997; 145(4):1219–28. PMID: 9093870

89. Fu YB. FPTEST: a SAS routine for testing differences in allelic count. Mol. Ecol. Res. 2010; 10 (2):389–392.

90. Holm S. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics. 1979; 6:65–70.

91. Morrell PL, Toleno DM, Lundy KE, Clegg MT. Low levels of linkage disequilibrium in wild barley (*Hordeum vulgare* ssp. *spontaneum*) despite high rates of self-fertilization. Proc Nat Acad Sci USA. 2005; 102(7):2442–2447. PMID: 15699350

92. Turpeinen T, Vanhala T, Nevo E, Nissilä E. AFLP genetic polymorphism in wild barley (*Hordeum spontaneum*) populations in Israel. Theor Appl Genet. 2003; 106:1333–1339. PMID: 12748785

93. Hübner S, Günther T, Flavell A, Fridman E, Graner A, Korol A, et al. Islands and streams: clusters and gene flow in wild barley populations from the Levant. Mol Ecol. 2012; 21:1115–1129. doi: 10.1111/j.1365-294X.2011.05434.x PMID: 22256891

94. Al-Eisawi D. Vegetation of Jordan. UNESCO—Cairo office. Regional office for science and technology for the Arab States; 1996.

95. Witcombe JR, Bourgois JJ, Rifaie R. Germplasm collections from Syria & Jordan. Plant Genet Resour Newsletter. 1982; 50:2–8.

96. Jaradat AA. Diversity within and between populations of two sympatrically distributed *Hordeum* species in Jordan. Theor Appl Genet. 1989; 78:653–656. doi: 10.1007/BF00262560 PMID: 24225825

97. Jaradat AA. Ecotypes and genetic divergence among sympatrically distributed *Hordeum vulgare* and *Hordeum spontaneum* from the xeric region of Jordan. Theor Appl Genet. 1989; 78:857–862. doi: 10.1007/BF00266671 PMID: 24226019

98. Nevo E. Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the Fertile Crescent. In: Shewry PR, editor. Barley: Genetics, Biochemistry, molecular biology and biotechnology. CAB International, Wallingford, UK. 1992. pp 19–43.

99. Schoen DJ, Brown AHD. Intraspecific variation in population gene diversity and effective population size correlated with the mating system in plants. Proc Nat Acad Sci USA. 1991; 88:4494–4497. PMID: 11607182

100. Nevo E. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum* in Turkey. Genetica. 1986; 68:203–213.

101. Volis S, Mendlinger S, Ward D. Adaptive traits of wild barley plants of Mediterranean and desert origin. Oecologia. 2002; 133:131–138.

102. Parra-Quijano M., Iriondo J.M., Torres E. Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. Genet Resour Crop Evol. 2012; 59(2):205–217.

103. Hoban S, Arntzen JA, Bruford MW, Godoy JA, Hoelzel R, Segelbacher G, et al. Comparative evaluation of potential indicators and temporal sampling protocols for monitoring genetic erosion. Evol Appl. 2014; 7(9):984–998. doi: 10.1111/eva.12197 PMID: 25553062

104. Abdel-Ghani AH, Parzies HK, Omary A, Geiger HH. Estimating the outcrossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. Theor Appl Genet. 2004; 109:588–595. PMID: 15083273

105. Hoban S, Strand A. *Ex situ* seed collections will benefit from considering spatial sampling design and species' reproductive biology. Biol Conserv. 2015; 187:182–191.

106. Nevo E. Genetic diversity in wild cereals: regional and local studies and their bearing on conservation *ex situ* and *in situ*. Genet Resour Crop Evol. 1998; 45:355–370.

107. Al-Saghir MG, Malkawi HI, El-Oqlah A. Morphological Diversity in *Hordeum spontaneum* C. Koch of Northern Jordan (Ajloun Area). Middle-East J Sci Res. 2009; 4(1):24–27.

108. Shakhatreh Y, Haddad N, Alrababah M, Grando S, Ceccarelli S. Phenotypic diversity in wild barley (*Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell.) accessions collected in Jordan. Genet Resour Crop Evol. 2010; 57(1):131–146.

109. van Hintum T, Menting F. Diversity in *ex situ* genebank collections of barley. In: von Bothmer R, van Hintum T, Knüpffer H, Sato K, editors. Diversity in barley (*Hordeum vulgare*). Elsevier Science B. V., Amsterdam, the Netherlands; 2003. pp. 247–257.

110. von Bothmer R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I. An ecogeographical study of the genus *Hordeum*. 2nd edition. Systematic and Ecogeographic Studies on Crop Genepools 7. International Plant Genetic Resources Institute, Rome, Italy; 1995.

111. Vincent H, von Bothmer R, Knüpffer H, Amri A, Konopka J, Maxted N. Genetic gap analysis of wild *Hordeum* taxa. Plant Genet Resour-C. 2013; 10(3):242–253.