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Changes in barley (*Hordeum vulgare* L. subsp. *vulgare*) genetic diversity and structure in Jordan over a period of 31 years

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Abstract

In many regions of the world, the cultivation of landraces is still common, in particular in centres of crop diversity. Significant effort has been put into *ex situ* conservation of landraces but limited data exist on the changes in genetic diversity that occur over time in farmers' fields. We assessed temporal changes in barley landrace diversity in Jordan using seed samples collected in 1981 and 2012 from the same locations. We did not observe significant changes in the amount of genetic diversity, but samples collected in 2012 were more homogenous and less locally distinct. In two sites, we observed replacement of the old material. We observed a change in phenotype, and phenotypes were found to be more homogeneous among sites in 2012. Climate changed significantly over the study period, becoming hotter and dryer, but we did not identify any correlation between the changes in climate and genetic and phenotypic variations. While the amount of genetic diversity in terms of allelic richness and number of multi-locus genotypes has been maintained, local distinctiveness among landrace barley populations in Jordan was reduced.

Keywords: genetic erosion, landrace, re-collection

Introduction

Landraces are defined as dynamic populations of a cultivated species associated with traditional farming systems (Jarvis *et al.*, 2000; Camacho Villa *et al.*, 2005). While locally adapted and genetically distinct, they usually lack a history of formal crop improvement. Landraces are a critical element of food security. In many regions of the world, the cultivation of landraces is still common, in particular in the centres of crop diversity (Ceccarelli, 1996; Mercer and Perales, 2010; Jarvis *et al.*, 2011). They are used in breeding because they are an important source of unique variability, in particular regarding adaptive traits (FAO, 2010; Bertoldo *et al.*, 2014; Dwivedi *et al.*, 2016). Although their conservation in *ex situ* collections is quite extensive – nearly half of the genebank material, for which the biological status of accessions (as defined in Alercia *et al.*, 2015) is known, are landraces (FAO, 2010) – gaps in *ex situ* collections are still being reported, and limited data exist on numbers and diversity of landraces currently grown in farmers' fields (FAO, 2010). On-farm management of landraces, i.e. maintenance of genetic diversity in production systems, is therefore an essential complement to *ex situ* conservation. Importantly, it allows adaptive processes to continue, which shape landrace genetic diversity and result in unique

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resources for farmers and breeders (Henry *et al.*, 1991). How landraces evolve under on-farm management and under the pressure of environmental change requires further research (Mercer and Perales, 2010; Dornelas *et al.*, 2013).

Barley is the fourth most important cereal crop worldwide in terms of production, yield and area harvested. It is adapted to marginal areas and stress-affected environments and is therefore important to small farmers in developing countries. In Jordan, barley is the predominant crop in areas with <300 mm of annual rainfall. In these areas, barley is mainly grown for animals; both grain and straw are utilised (Al-Tabbal and Al-Fraihat, 2012). Given that water scarcity is a major environmental challenge in Jordan, a crop such as barley will likely remain an essential component of the landscape, providing food security in Jordan's rain-fed agricultural system.

Genetic diversity in cultivated barley is known to be particularly high in the near East (Malysheva-Otto *et al.*, 2006; Varshney *et al.*, 2008). To examine how landrace barley diversity has changed in Jordan over a period of 31 years, we sourced landrace samples collected in Jordan in 1981 from a genebank and re-collected contemporary samples from the same sites in 2012. We examined genetic and phenotypic diversity at both time points to investigate the pattern of temporal changes in diversity and tested whether these changes were associated with geography or climate.

Material and methods

Barley germplasm collecting missions

Barley landrace samples were collected in Jordan in 1981 (18 May–2 June) during a collecting trip carried out under a regional FAO project operated by the IBPGR (International Board for Plant Genetic Resources) (Witcombe *et al.*, 1982) and were re-sampled from the same sites in 2012 (21 May–3 June) in collaboration with the Jordanian National Center for Agricultural Research and Extension (NCARE). Using map coordinates and location descriptions from the 1981 collecting reports, and information provided by the original collector, precise site locations were determined for resampling in 2012. Seed samples in 1981 were collected from farmers' fields by walking a transect across the field, collecting up to 200 spikes, and trying to maximize variability expressed in phenotypes while collecting. After threshing, seeds of each sample were divided at random between the three participating organizations (J. Witcombe, pers. commun.). Samples of up to 100 spikes were randomly collected from each site in 2012 and conserved at NCARE and the German federal *ex situ* genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK).

Seeds from the 1981 collecting mission were obtained from the Nordic Genetic Resource Center (NordGen), where the original seed samples had been stored for the long term in sealed aluminium foil bags, in standard household deep freezers at −18°C. The collecting years are referred to in the following also as time point t_1 (1981) and time point t_2 (2012).

Field trial

Accessions were grown in a standard field trial at IPK during the 2013 growing season. Seeds were sown on 19 April 2013. Each sample was sown in two rows of 1 m each, separated by a row of wheat, in 1.0 × 1.5 m^2 plots. No irrigation was provided and hand weeding occurred as necessary. Plants were bagged before flowering to avoid any cross fertilization with adjacent wild barley (*Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell.) plots. A total of 40 accessions, 20 from each collecting year, were used for the study (Fig. 1). Sixteen individual plants per accession were randomly chosen for phenotypic and genotypic data collection. Thinning was carried out between these plants to equalize plant density within plots. Leaf tissue was collected from all labelled plants, dried at 37°C, and then frozen for later DNA extraction. Twenty phenotypic traits were measured during the growing season, at harvest and post-harvest (online Supplementary Table S1) to assess relative phenotypic variation.

DNA extraction and genotyping

DNA was purified using the Qiagen DNeasy® 96 Plant Kit. Thirty-eight EST-derived SSR primers were used for genotyping (Thiel *et al.*, 2003; Stein *et al.*, 2007; Varshney *et al.*, 2007) (online Supplementary Table S2). Loci were distributed across all seven barley chromosomes. DNA amplification and fragment size analysis was completed by the Genomics and Bioinformatics Research Group, USDA-ARS, Stoneville, Mississippi. PCR was carried out in 5-µl reactions consisting of 2–10 ng genomic DNA, 1× Qiagen Multiplex PCR Master Mix, 225 nM of each primer pair. Fragments were amplified using the following PCR profile: an initial denaturing step of 15 min at 95°C followed by 40 cycles with denaturation at 94°C for 30 s, annealing at 55°C for 60 s and extension at 72°C for 60 s. After 40 cycles, a final extension step was performed at 60°C for 20 min. Amplification products were resolved by capillary electrophoresis on an ABI 3730XL Genetic Analyzer. Fragment sizes were calculated using GeneScan 500 (ROX) internal size standards and scored with GeneMapper Software (version 5.0) (Life Technologies, Thermo Fisher Scientific Inc.).

Care was taken to mitigate scoring errors in the microsatellite data. Out of the 45 loci examined, the 38 used were

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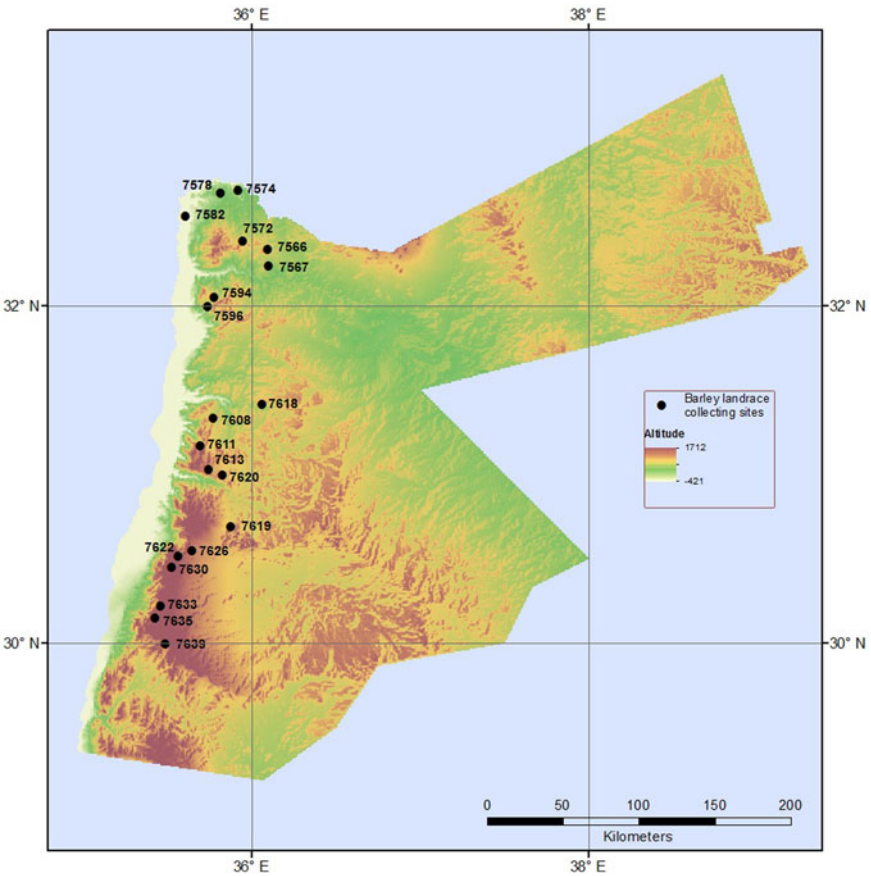

Fig. 1. Collecting sites of barley landraces for temporal comparison.

chosen based on polymorphism, low drop-out rates and scoring/amplification consistency. Every sample was inspected manually for allele call fidelity. DNA from four *H. vulgare* L. accessions from IPK (BCC844, BCC1500, BCC1411 and BCC1390) were used as internal controls for genotype scoring. Unique positioning of controls and blanks on each 96-well plate provided checks for plate identification and orientation as well as for scoring consistency. In the rare case discrepancies were observed, the entire plate was rerun. Samples with ambiguous peaks were also rerun. Because barley is an inbreeding species, and exhibits extreme heterozygote deficiency, deviations from Hardy–Weinberg equilibrium could not be used to indicate scoring errors. However, loci with a high incidence of heterozygotes were suspect and were re-examined. There were 40 out of 24,320 (0.17%) missing data points in the SSR data set.

Statistical analyses

Genetic diversity, population differentiation and structure

Summary statistics, such as number of alleles, sample adjusted allelic richness and observed heterozygosity, were calculated with GDA (Lewis and Zaykin, 2001) and FSTAT version 2.93.2 (Goudet, 2001). The PIC (polymorphism information content) and the number of multilocus (ML) genotypes was determined using GenAlEx 6.502 (Peakall and Smouse, 2006, 2012). Population subdivision was quantified using F_{ST} and Jost's D (Jost, 2008), which is an alternative to F_{ST} that is unaffected by within-population diversity (Meirmans and Hedrick, 2011). Both were calculated with the R package *diveRsity* (Keenan *et al.*, 2013). Differentiation among populations within a collecting year as well as differentiation within each site between collecting years was calculated.

Population structure was inferred using InStruct (Gao *et al.*, 2007). InStruct extends the algorithm used in STRUCTURE (Pritchard *et al.*, 2000) to account for self-pollination and inbreeding, common in barley. InStruct was run in mode $v = 2$ (infer population structure and population selfing rates) for $K = 1$–10. For each K, five chains were run, with 200,000 Markov chain Monte Carlo iterations, a burn-in of 100,000 and a thinning interval of 10 steps. Results from independent chains were summarized using CLUMPP (Jakobsson and Rosenberg, 2007) and graphical representations of cluster assignments were

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rendered with DISTRUCT (Rosenberg, 2004). ΔK (Evanno *et al*., 2005) was calculated to identify the appropriate number of clusters.

IBD (Isolation by distance) was estimated using the R package ecodist. Geographic distances were calculated as straight-line distances with the GeographicDistanceMatrix Generator version 1.2.3 (Ersts, Internet, http://biodiversityinformatics.amnh.org/open_source/gdmg/) and log transformed. Genetic distances were calculated as $F_{ST}/(1–F_{ST})$ (Rousset, 1997). A two-tailed Mantel test was carried out with 10^5 permutations.

Climate data analysis

Long-term high-resolution daily and monthly climate data sets were generated by the GIS unit of the International Center for Agricultural Research in Dry Areas (ICARDA), using ECMWF (European Centre for Medium-Range Weather Forecasts, ERA-40) with bias correction through use of the WRF (Weather Research and Forecasting) model. Spatial downscaling was done in ArcGIS to generate 1 km surfaces. These high-resolution 1 km time series gridded data sets were used to extract monthly precipitation and mean temperature values for all collecting sites for 1980–2013 for further analysis.

In our analysis, temperature and precipitation trends over the 34-year period from 1980 to 2013 were considered. Annual and monthly precipitation and mean temperatures were tabulated for all years. Precipitation of the driest and wettest month and quarter, and mean temperature of the hottest and coldest month and quarter were calculated. Temporal trends were analysed using least squares regression. These data were compared with precipitation and temperature data from Jordanian weather stations for the years 1978–2008.

Phenotypic analysis

All phenotypic measurements were tabulated and the 10 traits marked with * in Table 3 were used for phenotypic analysis. Given that the field trial was limited to a single season, and was carried out in a location outside the study area, our analysis was limited to relative changes in phenotypes and did not focus on single trait values. For this reason we summarized the individual phenotypes as a multivariate statistic through principal component analysis (PCA). We used the first principal component to describe these multi-trait phenotypes through a single value. We then used a two-way analysis of variance (ANOVA) to assess the influence of collecting year and site on the phenotype. The phenotype was described by the first principle component and treated as the dependant variable. The interaction effects between the independent variables site number and collecting year on phenotype were tested. Additionally, we used one-way ANOVAs to test the significance of phenotypic variation among sites within each collecting year. The first principle component describing phenotype was the dependant variable, and site was used as the independent variable for all phenotypes of the respective collecting year. PCA and ANOVAs were carried out in JMP® 12.0.1.

Correlation analyses

Correlations between climatic, phenotypic and genetic change, as well as correlations between geographical variables (latitude and elevation) and climatic, phenotypic and genetic change were tested.

Genetic change was measured using the metric F_{ST} and Δ allelic richness. F_{ST} estimated the differentiation within each site between the t_1 and t_2 population. Change in allelic richness was expressed as the difference in allelic richness estimated in each site for both collecting years. A composite measure of phenotypic change was generated through a PCA on average trait differences between collecting years. The average trait value for each of the 10 traits was calculated at each site and for each collecting year. The differences within each site between average trait values from both collecting years (t_2–t_1) were subjected to PCA and the first principal component was used for regression to climatic and genetic change measures.

Similarly, PCA was used to generate a composite measure of climatic change. Annual, quarterly and monthly precipitation and temperature differences between collecting years were calculated based on values averaged over the collecting year and its preceding year, i.e. values for 1980–1981 were used for t_1 and values for 2011–2012 for t_2. The differences between the five precipitation and five temperature variables within each site were subjected to PCA and the first principal component was used for regression to phenotypic and genetic change measures. Within each time point, we also tested whether single climate variables were correlated with genetic diversity or phenotypic traits, using appropriate Holm–Bonferroni correction (Holm, 1979) to avoid type I error inherent in multiple comparisons. All correlations were tested using least squares regression in JMP® 12.0.1.

Results

Genetic diversity

Population and collecting year-specific values for genetic diversity measures are summarized in Tables 1 and 2. Overall, genetic diversity was slightly but not significantly higher in 2012 than in 1981. The total number of alleles was 137 (t_1) and 149 (t_2). Sixteen alleles were unique to 1981 (with frequencies between 0.002 and 0.01) and 28 alleles were unique to 2012 (frequencies between 0.002 and

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0.031). In both collecting years these unique alleles were randomly distributed across the study area. Mean allelic richness per site increased from 1.66 (t_1) to 1.80 (t_2). Overall, the mean number of alleles per site increased significantly, from 63.4 (t_1) to 69.05 (t_2). Depending on site, the change in number of alleles ranged however from a reduction of 10 alleles to an increase of 25 alleles. In each site there were, on average, 52.2 alleles that were found in both collecting years, 11.2 alleles found only in 1981, and 16.85 found only in 2012. The average number of collecting sites in which a specific allele was found increased from 7.6 to 8.3.

The total number of ML genotypes increased from 164 (t_1) to 181 (t_2). The mean number of ML genotypes per population increased from 10.8 (t_1) to 12.1 (t_2). Of the 164 ML genotypes present in 1981, 118 (71.96%) were unique (i.e. found in one individual only), 25 were present in more than one individual within the same site, and 21 were shared across 2–14 sites (average 3.52). In 2012, 150 (82.87%) ML genotypes were present in only one individual, nine were present in more than one individual within the same site, and 22 were shared among 2–15 sites (average 3.82). Among both collecting years, a total of 317 ML genotypes were recorded. In total, 136 were found only in 1981, 153 only in 2012 and 28 were common to both collecting years. Of the 28 common genotypes, 10 were recorded in the same sites in both years (seven in only one site each, and one genotype in 5, 6 and 13 sites). Overall, all but four sites (7578, 7594, 7596 and 7635) had 1–3 ML genotypes found in both collecting years.

Population differentiation and structure

Population subdivision decreased as evidenced by a reduction in D and F_{ST}. D decreased from 0.157 (t_1) to 0.021 (t_2), F_{ST} from 0.486 (t_1) to 0.14 (t_2) (Table 1). D and F_{ST} values calculated at each site between t_1 and t_2 samples to estimate differentiation within a location varied from 0.0 to 0.54 (D) and 0.0 to 0.85 (F_{ST}) (Table 2). The F_{ST} values for sites 7594 and 7596 were particularly high (0.85 and 0.78), indicating much change, while the average F_{ST} of the remaining sites was as low as 0.07, indicating very little change. Pairwise genetic distances measured as F_{ST} within collecting years were significantly higher in 1981 than in 2012.

The high collecting year-specific F_{ST} and D values in 1981 were mainly caused by samples collected in sites 7594 and 7596. When these two sites were excluded from the estimates, the F_{ST} and D of the remaining 18 samples were reduced to 0.217 and 0.036 for 1981, while values for 2012 remained mostly unvaried with 0.147 and 0.021, still showing stronger subdivision in 1981.

The ΔK method (Evanno *et al.*, 2005) applied to InStruct results suggested subdivision into two clusters in 1981. Sites 7594 and 7596 constituted one small cluster, while all remaining 18 populations were assigned to the second cluster (Fig. 2). The average population assignment coefficient was >0.97 for both clusters. Fifteen individuals were genetically admixed, i.e. had a cluster assignment coefficient $q < 0.8$ and 14 of these were located in site 7578.

For comparison, we examined InStruct results for 2012 populations at the same $K = 2$. One cluster contained two sites, the other 18. The average population assignment coefficient to clusters was 0.82, significantly lower than in 1981 ($P < 0.0001$, Tukey–Kramer HSD (honestly significant difference)). Genetic admixture was much higher than in 1981, found in 135 individuals. Five populations were also physically admixed, i.e. where an individual assigned

Table 1. Genetic diversity measures by collecting year

Variable	1981	2012	Significance[a]
Total number of alleles	138	149	
Number of alleles unique to collecting year	16	28	
Mean allelic richness per population	1.658	1.799	ns
Mean number of alleles per population	63.4	69.05	$P = 0.0497$
D	0.157 CI (95%): 0.148–0.165	0.021 CI (95%): 0.017–0.026	
F_{ST}	0.486 CI (95%): 0.456–0.512	0.14 CI (95%): 0.113–0.168	
Total number of ML genotypes	164	181	
ML genotypes unique to collecting year	136	153	
ML genotypes recorded in one individual only	118	150	
ML genotypes repeated within a site	25	9	
ML genotypes repeated/shared across sites	21	22	
Mean number of ML genotypes per population	10.8	12.1	ns

ML, multi-locus.
[a]Tukey–Kramer HSD.

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Table 2. Genetic diversity measures by population

Site	IPK accession number 1981 samples[a]	IPK accession number 2012 samples	F_{ST} between collecting years	D between collecting years	Allelic richness 1981	Allelic richness 2012	ML genotypes 1981	ML genotypes 2012	ML genotypes present in both years	Alleles 1981	Alleles 2012	Alleles present in both collecting years
7574	HOR 22359	HOR 22643	0.0916	0.0052	1.70	1.60	11	12	1	65	61	55
7578	HOR 22363	HOR 22650	0.4701	0.1192	2.02	1.75	15	14	0	77	67	54
7582	HOR 22365	HOR 22653	0.0225	0.0012	1.31	1.72	8	8	2	50	66	45
7572	HOR 22358	HOR 22642	0.0511	0.0019	1.65	1.62	13	12	2	63	62	51
7566	HOR 22356	HOR 22633	−0.0097	2.00×10^{-04}	1.47	1.72	8	12	3	56	66	53
7567	HOR 22357	HOR 22634	0.0297	0.0039	1.70	2.09	11	12	3	65	82	55
7594	HOR 22369	HOR 22659	0.8466	0.5384	1.10	1.76	6	11	0	42	67	18
7596	HOR 22370	HOR 22662	0.7771	0.5119	1.43	1.92	3	12	0	55	73	33
7618	HOR 22377	HOR 22677	−0.0013	0.0018	1.88	2.22	5	16	3	72	86	63
7608	HOR 22374	HOR 22669	0.0943	0.0061	1.26	1.63	13	10	1	48	62	45
7611	HOR 22375	HOR 22671	0.0034	9.00×10^{-04}	1.57	1.67	12	11	2	60	64	56
7613	HOR 22376	HOR 22673	−0.0056	6.00×10^{-04}	1.65	1.83	10	13	2	63	70	61
7619	HOR 22378	HOR 22678	0.0047	0.0011	1.47	1.68	11	12	3	56	64	52
7620	HOR 22379	HOR 22679	0.0224	0.0018	1.70	1.60	12	10	2	65	61	55
7626	HOR 22382	HOR 22683	0.0358	0.0054	1.86	1.69	12	12	2	71	65	58
7622	HOR 22380	HOR 22680	0.0074	0.0016	1.96	2.00	14	16	1	76	77	62
7630	HOR 22383	HOR 22685	0.0976	0.0089	1.83	1.73	13	11	1	70	66	59
7633	HOR 22385	HOR 22686	0.0255	0.0027	1.70	1.97	12	15	1	65	76	55
7635	HOR 22386	HOR 22687	0.2261	0.0342	1.96	2.14	13	11	0	75	82	56
7639	HOR 22387	HOR 22688	0.0485	0.0032	1.93	1.67	13	12	2	74	64	58

ML, multi-locus.
[a]Seed samples obtained from NordGen were accessioned in IPK.

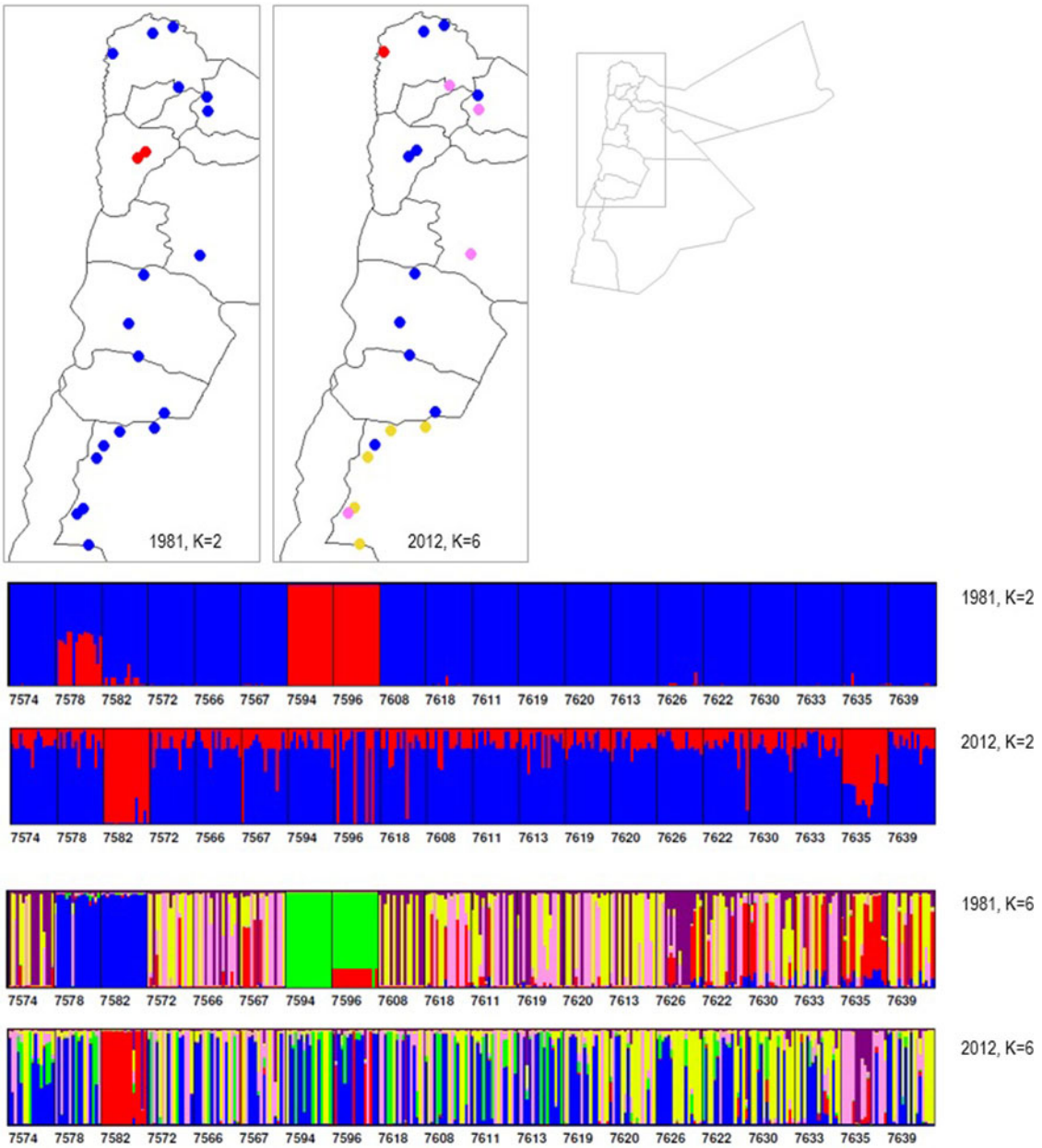

Fig. 2. Assignment of barley landrace individuals and populations to clusters identified by InStruct for $K = 2$ and $K = 6$.

to one cluster resides in a site dominated by individuals belonging to another cluster.

The ΔK method applied to InStruct results for the samples collected in 2012 suggested a subdivision into six clusters (Fig. 2). All 20 populations were physically admixed containing individuals assigned to 2–5 different clusters. When we applied $K = 6$ to the 1981 populations, 13 populations were physically admixed with individuals assigned to two or three populations. The population assignment coefficient was not significantly higher in 1981 (0.54) compared with 2012 (0.47).

Phenotypic variation

Growth habit, leaf hairiness, stem pigmentation, awn type, awn barbs, lemma colour, kernel covering, spike density and row number showed no or little variation between populations and collecting years. Quantitative traits varied within sites and across the study area in both collecting years (Table 3 and online Supplementary Table S4). Days from emergence to heading, plant height at harvest, number of tillers, spike length, total seed harvested per plant, showed comparatively larger differences between

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collecting years than other traits. Plants from 2012 tended to take more days from emergence to heading, to have more and longer tillers, and to produce more seed per plant (Table 3).

The first principal component represented 31.1% of phenotypic variation. ANOVA showed that multi-trait phenotype, measured as the first principal coordinate, differed among sites in 1981 but not in 2012 (Table 4). Site by collecting year effect was not significant, indicating that differences among collecting years were similar in direction and magnitude within sites. Relative change in phenotype among collecting years was statistically significant (Table 4).

Climate changes in Jordan from 1980 to 2013

Values for all five temperature variables (annual mean temperature, mean temperature of coldest and hottest month and quarter) increased significantly over the 34-year period in the 20 sites. Precipitation values measured as annual precipitation, precipitation of wettest and driest month and quarter showed a negative trend over the 34-year period in all 20 sites. The decreases over time in precipitation of driest month and quarter were statistically significant in most sites. The results of the regression analyses on temporal trends are summarized in online Supplementary Table S3. These trends confirm that the precipitation and temperature values of the two collecting years are part of a broader trend and do not represent anomalous values.

Precipitation of wettest month was significantly lower and all temperature values were significantly higher in t_2 compared with t_1. Precipitation and temperature values for t_1 and t_2 are summarized in online Supplementary Figs S1 and S2. Changes in precipitation and temperature values within single sites are visualized in online Supplementary Figs S3 and S4. These latter show that increases in warmest quarter and warmest month temperatures as well as decrease in annual and wettest quarter precipitation were more pronounced in the southern sites.

Table 3. Phenotypic trait values by collecting year, providing percentages for categorical variables and means followed by standard deviation for quantitative variables

Trait	1981	2012
Days to emergence[a]	9 days: 70% 10 days: 30%	9 days: 100%
Growth habit[a]	Erect	Erect
Leaf hairiness	Present	Present
Stem pigmentation	Green: 29.47% purple (basal only): 70.53%	Green: 35.63% Purple (basal only): 64.38%
Awn type	Awnleted: 5.46% Awned: 94.53%	Awnleted: 4.76 Awned: 95.24
Awn barbs	Smooth: 17.42% Intermediate: 68.39% Rough: 14.19%	Smooth: 0.0% Intermediate: 71.75% Rough: 0.95%
Lemma colour	Amber	Amber
Days from emergence to heading*	61.82 (10.17)	65.51 (9.61)
Plant height*	65.87 (14.86)	76.57 (15.31)
Number of tillers*	9.08 (8.41)	12.53 (9.81)
Spike length*	6.4 (1.85)	7.3 (1.59)
Spike density	Lax: 70.0% Intermediate: 30.0%	Lax: 66.56% Intermediate: 33.44%
Kernel number per ear*	14.36 (5.95)	15.76 (4.44)
Kernel row number	2	2
Kernel covering	Semi-covered	Semi-covered
TSW*	52.15 (7.64)	52.97 (7.06)
Seed area*	32.9 (2.44)	32.26 (2.13)
Seed width*	3.46 (0.17)	3.46 (0.17)
Seed length*	11.52 (0.76)	11.29 (0.61)
Number of seeds harvested per plant*	71.76 (64.32)	103.00 (80.83)

[a]Traits 'days to emergence' and 'growth habit' were recorded at population level, all other traits at individual level.

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Table 4. One way ANOVA on multi-trait phenotypes for each collecting year to test effect of site within year (type of ANOVA = 1) and two way ANOVA on multi-trait phenotypes of both collecting years to test effect of year, site and their interaction (type of ANOVA = 2)

Type of ANOVA	Source of variation	Number of parameters	Degrees of freedom	Sum of squares	*F* Ratio	Prob > *F*
1	Site in 1981	19	19	121.63	2.13	0.0048
1	Site in 2012	19	19	50.21	0.8	0.7088
2	Collecting year	1	1	68.16	21.55	<0.0001
2	Site	19	19	97.25	1.62	0.0475
2	Collecting year × site	19	19	74.14	1.23	0.2246

Temperature and precipitation data at 16 weather stations in Jordan showed the same trends as the interpolated data at the collecting sites. Annual mean temperature measured at the weather stations trended significantly upwards, while annual precipitation fell during the period 1978–2008 (data not shown).

Correlation analyses

Overall climate change, expressed as the first principal component (64% of variation) was strongly correlated with latitude ($R^2 = 0.85$, $P < 0.0001$) and altitude ($R^2 = 0.66$, $P < 0.0001$). No other significant correlation between climate, phenotypic and genetic change was found. Neither was phenotypic or genetic change correlated with latitude or elevation. We found no correlations in any time point between phenotypic traits and genetic or climatic variables.

Discussion

In the present study we used resurrection of old seed samples and re-collection (Davis *et al.*, 2005; Franks *et al.*, 2008) to assess genotypic and phenotypic changes over time. We compared landrace barley from Jordan, collected in the same locations 31 years apart. We observed a slight increase in genetic diversity between 1981 and 2012. Samples collected in 2012 were more admixed than in 1981 and differentiation among samples was lower in 2012. There was a relative change in phenotype among collecting years, and phenotypes were found to be more homogeneous among sites in 2012. In two sites, we observed complete replacement of the old material. Climate became hotter and dryer, but we did not identify any correlation with the observed genetic and phenotypic variations.

Study system and sampling

Genebanks maintain living germplasm and associated provenance information from collections of agriculturally important species. Well-documented and conserved collections can be used to reveal past diversity and serve as a starting point for temporal studies in genetic diversity through re-collecting contemporary samples for comparison (Maxted and Guarino, 2006; Franks *et al.*, 2008; Deu *et al.*, 2010; van de Wouw *et al.*, 2010; Thormann *et al.*, 2015). Several studies have used re-collecting to investigate temporal changes in landrace diversity, for example in sorghum and pearl millet in Niger (Bezançon *et al.*, 2009) and rice in Guinea (Barry *et al.*, 2008). Others have compared barley and wheat samples collected from the same areas several decades apart (Khlestkina *et al.*, 2004, 2006).

Differences in sampling protocols between historical and contemporary collections have been suggested as possible reasons for observed temporal genetic changes (del Rio *et al.*, 1997; Barry *et al.*, 2008). Up to 200 spikes per sample were collected in 1981, trying to maximize variability expressed in phenotypes. Thus, rare types could likely have been oversampled compared with common types. Samples of up to 100 spikes were randomly collected from each site in 2012. These sample sizes are considered sufficient to adequately represent the diversity of a population in the sample (Brown and Marshall, 1995; Hoban and Schlarbaum, 2014). The difference in sampling method between 1981, where phenotypic variability was maximized along a transect, and 2012, where sampling was random, does not explain our results as higher genetic diversity might have been expected in the 1981 samples, but this was not observed, in fact the diversity was slightly higher in 2012.

Genetic diversity in *ex situ* collections can be affected by regeneration and/or other management practices, including the sample sizes (Rao *et al.*, 2006; Dulloo *et al.*, 2008). Gomez *et al.* (2005) observed lower genetic diversity in *ex situ* conserved bean samples compared with re-collected *in situ* samples due to regeneration processes. The 1981 seeds were from the original collected samples, which had not been regenerated and been conserved under standard long-term storage conditions. This excludes the possibility that genetic diversity has been affected by

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inappropriate regeneration and/or other management practices.

Another concern related to seed storage is the potential selective loss of genotypes during long-term conservation. Barley has an orthodox, desiccation tolerant seed storage behaviour under long-term storage conditions (van Hintum and Menting, 2003; Nagel *et al.*, 2009) and all samples used in the present study germinated normally. There is no indication to suspect that major genotypic changes have affected seed samples from 1981. In a similar study on pearl millet landraces, no impact of seed storage over time was found (Vigouroux *et al.*, 2011). To the best of our knowledge, no study has yet demonstrated conclusively that selective mortality occurs during storage.

Our study has furthermore included a relatively large number of sites covering a range of agro-ecological conditions to provide a good estimate of the overall distribution of diversity across Jordan. We used 38 SSRs to describe genetic diversity, while 20 are considered sufficient to provide resolution and representation of diversity for genetic erosion studies (Hoban *et al.*, 2014). We therefore assume that the seed samples used in this study provide comparable snapshots of diversity existing at the respective collecting times, and that observed differences are not significantly affected by sampling bias or *ex situ* management practices.

Changes in genetic diversity and population structure

Landraces are typically genetically heterogeneous (Brown, 1999; Camacho Villa *et al.*, 2005). This has been shown for barley in Jordan and Syria (Jana and Pietrzak, 1988; Russell *et al.*, 2003, 2011), Ethiopia (Bekele, 1983), Iran (Brown and Munday, 1982), Nepal (Pandey *et al.*, 2006) and Spain (Yahiaoui *et al.*, 2008). Landraces of predominantly self-fertilizing species like barley (Wagner and Allard, 1991; Parzies *et al.*, 2000) are usually composed of mixtures of many, mostly homozygous, ML genotypes (Pérez de la Vega and Garcia, 1997). It should also be noted that the barley landraces collected in Jordan were predominantly used as animal feed and thus, most likely very limited selection might have taken place by farmers other than for straw quality and seed yield.

Genetic diversity was maintained between 1981 and 2012. Most of the alleles were found in both time points. Only 12 and 18% of alleles in 1981 and 2012 were unique to the respective time point, and these alleles were found at very low frequencies, between 0.002 and 0.031. Given that most alleles were found in both time points and ML genotypes were maintained in collecting sites, no replacement by exotic or uniform material seems to have taken place. Also the very low D and F_{ST} values between time points within most collecting sites indicate limited genetic change between time points within sites. Ceccarelli and Grando (1999, 2009) reported that conventional breeding and high-yielding varieties have had rather limited success in the Fertile Crescent. However, two sites, 7594 and 7596, present an exception. Their very high site-specific D and F_{ST} values indicate that the samples collected there in 2012 are quite different from those collected earlier. Samples from 1981 collected in these two sites were genetically and phenotypically distinct from all other samples, as they formed a separate genetic cluster and were significantly smaller in height and less productive. In 2012, samples collected in these two sites were no longer distinct. The barley landraces grown in these two sites in 1981 appear therefore to have been replaced with material similar to that re-collected in other sites.

We observed important changes in population structure. The collection in 2012 showed less genetic differentiation among sites and smaller average genetic distances between samples. The clustering at $K = 6$, increased genetic admixture, and the presence of physical admixture in all populations in 2012, indicate that population structure has decreased. Furthermore, the reduction in D and F_{ST} values shows that barley samples have become more homogeneous across the territory compared with 1981. This homogenization might be the result of increased seed flow within the country and increased use of common seed sources. Accordingly the number of ML genotypes found in 2012 was higher and the number of ML genotypes unique to the collecting time point increased from 72 to 83%.

In the 1980s, the barley grown in Jordan was primarily landrace material, from farmers who had been using their own seeds for generations (Weltzien, 1982, 1988; Jana and Pietrzak, 1988; Brush, 2004; Russell *et al.*, 2011). The three decades between 1981 and 2012 were characterized by increased urbanization and infrastructure development due to rapid population growth in Jordan, conversion of rangelands into cultivated land, and a generalized intensification of agricultural activities (Khresat *et al.*, 1998; Al-Bakri *et al.*, 2001, 2008; FAO, 2006; NCARTT, 2007). Jordan operated a conventional national barley-breeding programme, which had released a first variety in 1966. Further varieties were released in the late 1980s and 2004 (Friedt *et al.*, 2011). Since 2000, the breeding programme has been decentralized by adopting and implementing the participatory plant breeding (PPB) approach (ICARDA, 2003; Ceccarelli and Grando, 2007) involving farmers in different ecogeographical regions. About 30 PPB varieties were distributed to a number of regions in Jordan (Al-Yassin, 2012). The Jordan Cooperative Corporation (JCC) started to coordinate seed production and distribution throughout Jordan in 1982 (Al-Yassin, 2012). JCC has become the primary seed source for the majority of barley farmers (N. Al-Hajaj, pers. commun. NCARE; ICARDA, 2003). The increased availability of varieties released by the breeding programme

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and the operation of the seed cooperative have likely contributed to decreasing differentiation and reduction in population structure, because seed exchange and genetic population structure in landraces are found to be interdependent (Parzies *et al.*, 2004; Allinne *et al.*, 2008; Thomas *et al.*, 2011; Samberg *et al.*, 2013).

Similar observations of weak and unstructured clustering between barley landrace samples were made in Ethiopia (Abebe and Leon, 2013; Abebe *et al.*, 2013) and Tunisia (Ould Med Mahmoud and Hamza, 2009). These observations were attributed to seed-mediated gene flow among farmers. Farmer seed management can have a stronger influence on population structure than any landscape structure (Samberg *et al.*, 2013), and can explain why correlations between genetic structure and ecogeographic variables are often weak or non-existent (Ould Med Mahmoud and Hamza; 2009; Abebe and Leon, 2013). Traditional practices and cultural factors are known to influence population structure and genetic diversity in landraces of other crops and regions (Guo *et al.*, 2012; Westengen *et al.*, 2014). To explain population structure and spatial distribution of diversity in landraces and to design conservation strategies, it appears therefore necessary to complement the knowledge about the species biology and the study of the ecogeographical landscape with the farmers' social landscape and their seed management and exchange practices.

Phenotypic variation

High phenotypic variation was found in both collecting periods in our study in Jordan, consistent with the findings of other studies. Barley landraces collected from the Fertile Crescent (Weltzien, 1982, 1988; Ceccarelli *et al.*, 1987; Jaradat 1989a, b) and other countries (Damania *et al.*, 1985; Demissie and Bjornstad, 1996; Lasa *et al.*, 2001; Assefa and Labuschagne, 2004; Haseneyer *et al.*, 2010) are reported to be morphologically very variable. These observations can also be confirmed for Ethiopia during the mid-1980s from collecting over 100 barley landraces across the country (Engels 2016, unpublished data).

We observed a significant change in multi-variate phenotype, where plants from 2012 were on average taller, had longer spikes and more tillers. While there was significant heterogeneity between sites in 1981, this was not found in 2012 anymore. Because our data do not include multi-year and multi-location data, the observed differences in phenotype could be attributable to maternal effects and environmental plasticity (Kirkpatrick and Lande, 1989; Mousseau and Fox, 1998). The year of harvest and the age of seeds – the 1981 seeds being 31 years older – could have led to differences in the fitness of the seeds and played a role in development of the plants. On the other hand, farmers in Jordan are known to prefer taller plants, which are easier to harvest from stony fields, and plant height has been an important selection criterion in PPB activities (ICARDA, 2003). To determine whether observed phenotypic differences between collecting years might be influenced by farmer selection, seed fitness or G × E effects, would require a complex set of additional common garden experiments in different environments.

Responses to environmental variation

The analysis of the climate data demonstrated that climate has been changing, in particular the already dry months have become drier and temperatures have been increasing. This presents an increasing challenge to agriculture. These changes were not found to have affected genotypic diversity, however. Two studies have made similar findings. Significant changes in phenotypic traits were observed by Cui *et al.* (2016) in a temporal study (1980–2007) on rice landraces in China, as a decrease in plant height and an increase in number of grains per panicle. This was attributed to on-farm selection. Vigouroux *et al.* (2011) observed earlier flowering and a reduction in plant and spike size as an adaptation to drought in pearl millet in a similar diachronic study (1976–2003) conducted in Niger. Analogous to our results, in both cases, genetic diversity was maintained in landraces on-farm despite environmental change.

Genetic erosion in landraces and on-farm conservation

Climate change, replacement with improved varieties, environmental degradation, changing agricultural systems and needs, legislation and policy, civil strife and war are reported as main causes for past genetic erosion and threats to the survival of existing diversity (FAO, 2010, 2012). The picture that so far has emerged from research about the extent of genetic erosion in landraces, however, appears very complex and no coherent body of data are available because methods of assessment are not uniform (Thormann and Engels, 2015).

While many studies and reports document genetic erosion (FAO, 2010; Thormann and Engels, 2015), other studies show that in some cases introduction of improved varieties has not caused loss in landrace diversity or landrace diversity has been maintained on-farm. Steele *et al.* (2009) monitored the adoption of modern rice varieties in a high-altitude region in Nepal. They found that partial adoption of a modern variety can increase the overall genetic diversity within the agricultural system, if at least 35% of the cultivated area continues to be planted to traditional varieties. Landrace diversity of pearl millet and sorghum in Niger (Bezançon *et al.*, 2009), sorghum in eastern

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Ethiopia (Mekbib, 2008) or rice in Yunnan, China (Cui *et al*., 2016) and Guinea (Barry *et al*., 2008) was maintained over time by farmers. No loss of genetic diversity was found in wheat collected over an interval of 40–50 years in Albania, Austria, India and Nepal (Khlestkina *et al*., 2004).

The presence and extent of erosion varies for the same crop, by geography, national policy environment and agricultural system. While Khlestkina *et al*. (2006) observed maintenance in diversity in barley collected from Austria and India over an interval of 40–50 years, loss of barley landrace diversity has been reported from Serbia (Petrovic and Dimitrijevic, 2012) and Ethiopia (Megersa, 2014).

Our study has for the first time analysed temporal variation in barley landrace diversity in Jordan. We revealed a localized loss of genetically and phenotypically distinct populations in two nearby sites. While genetic diversity in terms of allelic richness and ML genotypes has been maintained – and even slightly increased, we observed a decrease in differentiation between populations, which have become less locally distinctive. Our results underline the importance of seed management practices on shaping diversity in landraces maintained by farmers, which have likely led to reduced distinctiveness and potential for local adaptation of landrace populations. The potential for evolution and adaptation to changing environmental conditions is one of the major advantages of active on farm conservation of landrace diversity. The assessment of changes in landrace diversity over time, combined with analysis of seed management practices, can provide useful input to devise concrete interventions for conservation of landrace diversity.

Supplementary Material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262117000028

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