Acta Agrobotanica

Article ID: 7343
DOI: 10.5586/aa.7343

Publication History
Received: 2020-05-08
Accepted: 2020-09-25
Published: 2020-12-28

Handling Editor
Joanna Kaczmarek; Institute of Plant Genetics, Polish Academy of Sciences, Poland; https://orcid.org/0000-0001-6437-5678

Authors' Contributions
SM: designed, performed, and conducted the experiments, analyzed the data, and wrote the manuscript; MK: participated in the writing of the article; MBY: supervised experiments and helped revise the manuscript

Funding
This study was supported by a research grant from the Korea–Africa Food & Agriculture Cooperation Initiative (KAFACI), Korea.

Competing Interests
No competing interests have been declared.

ORIGINAL RESEARCH PAPER in GENETICS

Assessment of Genetic Diversity and Population Structure of Tunisian Barley Accessions (*Hordeum vulgare* L.) Using SSR Markers

Salem Marzougui[1,2*], Mohamed Kharrat[2], Mongi ben Younes[1]

[1]Pôle Régional de Recherche Développement Agricoles du Nord-Ouest semi-aride à El Kef, Institution de la Recherche et de l'Enseignement Supérieur Agricoles (IRESA), Tunisia
[2]Field Crops Laboratory, National Institute of Agricultural Research of Tunisia (INRAT), University of Carthage, Tunisia

*To whom correspondence should be addressed. Email: marsalem79@gmail.com

Abstract

In barley breeding programs, information about genetic dissimilarity and population structure is very important for genetic diversity conservation and new cultivar development. This study aimed to evaluate the genetic variation in Tunisian barley accessions (*Hordeum vulgare* L.) based on simple sequence repeat (SSR). A total of 89 alleles were detected at 26 SSR loci. The allele number per locus ranged from two to five, with an average of 3.4 alleles per locus detected from 32 barley accessions, and the average value of polymorphic information content was 0.45. A cluster analysis based on genetic similarity was performed, and the 32 barley resources were classified into five groups. Principal coordinates (PCoA) explained 12.5% and 9.3% of the total variation, and the PCoA was largely consistent with the results of cluster separation of STRUCTURE software analysis. The analysis of genetic diversity in barley collection will facilitate cultivar development and effective use of genetic resources.

Keywords

barley; molecular screening; phylogenetic analysis; genetic resources

1. Introduction

Barley (*Hordeum vulgare* L.), one of the first and earliest crops domesticated by humans, is a major cereal grain grown in temperate climates globally. It is one of the oldest crops in the world and ranks fourth after wheat, rice, and maize (Poets et al., 2015). In Tunisia, barley is mainly cultivated in arid and semiarid climates in areas with annual rainfall of below 400 mm. In less developed Mediterranean countries such as Tunisia, barley plays a key role as its grain and straws are the principal feed for livestock. Small ruminants such as sheep and goats are the main livestock in Tunisia, representing a valuable dietary contribution in rural areas and a principal economic output (Medimagh et al., 2012). Genetic improvement to increase yield is underway in the Tunisian breeding program. Yield in barley is a complex trait governed by several genes and is a result of interactions between several components. The development of high yielding varieties adapted to local conditions depends on the understanding of the existing variability and genetic relation between barley accessions. Therefore, evaluating genetic diversity of barley lines using molecular markers is important in barley breeding for successful exploration, genetic stability, and effective conservation, because morphological characters are limited in number and unstable (Azartamar et al., 2015). Amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), and single nucleotide polymorphism

(SNP) have been used to study genetic diversity and structure in crops (Bwalya et al., 2020; Mwangi et al., 2019). Several studies have been performed on barley to assess their genetic diversity in different germplasm collections using molecular markers. However, most studies were based on either cultivar collections (Tondelli et al., 2013) or mixtures of cultivars and landraces (Elakhdar et al., 2016). Moreover, these studies have been conducted using SSRs (Yahiaoui et al., 2014), SNPs (Cronin et al., 2007), and DArT array (Ovesná et al., 2013). SSR markers have been broadly used in plant genetic research because they are available, highly informative, and distributed throughout the genome (Varshney et al., 2005).

The main objective of this study was to analyze genetic diversity, which exist among the 32 Tunisian lines including four varieties, using 26 molecular markers. The study will facilitate cultivar development and effective use of genetic resources.

2. Material and Methods

2.1. Plant Materials and DNA Extraction

Thirty-two Tunisian barley lines; including four cultivars (Rihane, Manel, Lemsi, and Kounouz), one with uncertain improvement status, and 27 landraces; were used in this study. All accessions were obtained from the U.S. National Plant Germplasm System (NPGS) international database. According to the passport data, 28 accessions were collected or donated from Tunisia between 1922 and 1972 (Table 1). Eight seeds from each accession were germinated and leaves harvested at three-leaf stage after 15 days of planting. Genomic DNA was extracted using GRS Genomic DNA kit (Grisp, Portugal) according to the instruction of the manufacturer. DNA quality and quantity were determined using a UV-Vis spectrophotometer and visual comparison of 2% agarose gel electrophoresis.

2.2. PCR Amplification of SSR Markers

All accessions were typed using 25 SSR markers and one InDel marker (HvBM5-Intr) that were reported and obtained from GrainGenes marker report (https://wheat.pw.usda.gov/) (Table 2). PCR amplification was performed in a total volume of 10 µL, consisting of 6 µL of GRS Hotstart Taq Mastermix (Grisp, Portugal), 0.25 µL of each SSR marker (10 µM), and 1 µL of DNA (50 ng). The PCR product was analyzed on a 2% agarose gel, and DNA amplification performed in a FastGene Ultra Cycler (96-well) (Nippon Genetics, Germany). The PCR was then subjected to the following conditions: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C to 62 °C for 30 s, and final extension at 72 °C for 30 s.

2.3. Data Analysis of Genetic Diversity and Population Structure

The number of alleles, observed heterozygosity (Ho), expected heterozygosity (He), and loci polymorphic information content (PIC) were determined using CERVUS software version 3.0.7 (Kalinowski et al., 2007). Cluster analysis of relationships between accessions based on SSR marker data was performed with the method of ward using DARwin 6.0 (Perrier & Jaccquemond-Collet, 2014). SSR marker genotyping results were used to estimate the population structure of the 32 barley accessions using STRUCTURE software. The distribution of ΔK values was determined by evaluating the logarithmic likelihood [$L(K)$] (Evano et al., 2005). To determine the population structure of the studied accessions, genotyping data were processed with STRUCTURE software 2.3.4, which implements a model-based Bayesian cluster analysis (Pritchard et al., 2000). A putative number of subpopulations ranging from $K = 1$ to 10 was assessed using 50,000 burn-in iterations, followed by 50,000 recorded Markov chain iterations. To estimate the sampling variance of inferred population structure, 10 independent runs were carried out for each K. The actual number of subpopulations was determined using the logarithm of likelihood for each K; ln P(D) = $L(K)$, and the optimum value of ΔK was obtained by $\Delta K = [L''(K)]/SD$, according to the report of Evanno et al. (2005), to determine the most likely number of groups. Based on the subpopulations

Table 1 List of 32 Tunisian barley lines used for genotyping.

Number	Accession name	Status	Origin
1	Rihane	Cultivar	Tunisia
2	Kounouz	Cultivar	Tunisia
3	Lemsi	Cultivar	Tunisia
4	Manel	Cultivar	Tunisia
5	175	Uncertain	Ariana
6	2528-23	Landrace	Siliana
7	3124-8	Landrace	Siliana
8	djebali	Landrace	Manouba
9	djebali	Landrace	Manouba
10	djebali	Landrace	Manouba
11	djebali	Landrace	Manouba
12	frigui	Landrace	Kebili
13	frigui	Landrace	Kebili
14	djebali	Landrace	Kebili
15	1110-30	Landrace	Kebili
16	jebali	Landrace	Kebili
17	jebali	Landrace	Kebili
18	djebali	Landrace	Kebili
19	hmira	Landrace	Kebili
20	djebali	Landrace	Kebili
21	jebali	Landrace	Kebili
22	djebali	Landrace	Kebili
23	frigui	Landrace	Kebili
24	jebali	Landrace	Kebili
25	jebali	Landrace	Kebili
26	djebali	Landrace	Kebili
27	jebali	Landrace	Bizerte
28	tounsi	Landrace	Tozeur
29	safra	Landrace	Tozeur
30	commune A	Landrace	Unknown
31	cowra	Landrace	Unknown
32	cowra	Landrace	Unknown

inferred by structural analysis, we carried out analysis of molecular variance (AMOVA) to assess the population differentiation using GenAlEx version 6.5 (Peakall & Smouse, 2012) with 999 times boost-strapping.

3. Results

3.1. Allelic Diversity of SSR Markers

In this study, we used 32 Tunisian barley lines, including four cultivars developed by the Tunisian breeding program. Twenty-six molecular markers, distributed across the seven chromosomes of barley, were used to genotype the selected lines. The number of polymorphic alleles ranged from two to five in the studied barley accessions. A total of 89 alleles were detected, with an average of 3.4 alleles per locus (Table 2). EBmac0701 and Bmag0496 SSR markers recorded the highest number of alleles. The value of polymorphic content (PIC) ranged from 0.088 (EBmag0793) to 0.703 (Bmac0134), with an average of 0.45. The average value of PIC for all the 26 polymorphic primers was 0.45. The mean of Ho was 0.23 ranging from 0 to 0.750, whereas the mean of He ranged from 0.094 to 0.731, with a mean value of 0.51.

Table 2 Information of 26 molecular primers used for analysis of 32 genotypes in this study.

Marker name	Linkage group	Forward primer	Reverse primer	Na	Ho	He	PIC
Bmac0213	1H	ATGGATGCAAGACCAAAC	CTATGAGAGGTAGAGCAGCC	4	0.563	0.71	0.647
GBM1461	1H	AAACCATGCATTCTTCAGAGA	TTTAGACCGACCCGATGAAG	3	0.258	0.525	0.458
scssr07759	2H	GCAACTCCTCATCATCTCAGG	CAACAGCCAGAAGGTCTACG	4	0.219	0.5	0.439
GBM1214	2H	ATGCTACAGCAAGCATGCAC	TGGTGAGGATGTCGGAGAAC	4	0.219	0.377	0.349
Bmag0125	2H	AATTAGCGAGAACAAAATCAC	AGATAACGATGCACCACC	4	0.129	0.647	0.568
EBmag0793	2H	ATATATCAGCTCGGTCTCTCA	AACATAGTAGAGGCGTAGGTG	2	0.031	0.091	0.085
HVM0054	2H	AACCCAGTAACACCGTCCTG	AGTTCCCTGACCCGATGTC	3	0.219	0.601	0.503
Bmag0138.3H	3H	ACCAGGAGGAATGAGAGAG	AATAAACCTTGAGACGATGG	2	0.097	0.094	0.088
EBmac0708	3H	AATTTTTGTTTCCCATGC	AGCCCTATTGTCACAGTTTT	3	0.344	0.335	0.285
Bmag603	3H	ATACCATGATACATCACATCG	GGGGGTATGTACGACTAACTA	2	0.3	0.413	0.324
Bmac209	3H	CTAGCAACTTCCCAACCGAC	ATGCCTGTGTGTGGACCAT	4	0.063	0.229	0.211
HVOle	4H	GATGGATGTCAGTCGGTC	ATGAGCAGTAGTACAACTCTAAGC	4	0.219	0.648	0.574
EBmac0906	4H	CAAATCAATCAAGAGGCC	TTTGAAGTGAGACATTTCCA	3	0.25	0.54	0.468
EBmac0775	4H	GCTTCCTTCATAGACCCAT	ATATCATGCCAATGGTGTC	4	0.125	0.688	0.616
Bmac0134	4H	CCAACTGAGTCGATCTCG	CTTCGTTGCTTCTCTACCTT	4	0.733	0.67	0.703
EBmac0701	4H	ATGATGAGAACTCTTCACCC	TGGCACTAAAGCAAAAGAC	5	0.161	0.731	0.673
Bmag0138.4H	4H	ACCAGGAGGAATGAGAGAG	AATAAACCTTGAGACGATGG	2	0.031	0.448	0.344
Bmag0751	5H	CACTGCAAATATTAAAATGGA	GATCTACTGGTCCATAGTTGC	4	0.125	0.282	0.262
scssr15334	5H	GGGAGCCGTAAGTAAGAACC	CGACCTCTGAATCTCAAATCC	4	0.467	0.593	0.54
scssr03907	5H	CTCCCATCACACCATCTGTC	GACATGGTTCCCTTCTTCTTC	4	0.094	0.634	0.561
HvBM5-Intr	5H	CTTGCATGTGTTGTCGGTCT	GCTGGGACAAGACTCTACGG	3	0	0.478	0.416
Bmac0316	6H	ATGGTAGAGGTCCCAACTG	ATCACTGCTGTGCCTAGC	4	0.194	0.704	0.636
Bmag0500	6H	GGGAACTTGCTAATGAAGAG	AATGTAAGGGAGTGTCCATAG	3	0.12	0.569	0.496
Bmag0496	6H	AGTATAACCAACAGCCGTCTA	CTATAGCACGCCTTTGAGA	5	0.281	0.724	0.671
Bmag120	7H	ATTTCATCCCAAAGGAGAC	GTCACATAGACAGTTGTCTTCC	3	0	0.666	0.582
GBMS183	7H	TAATGGTGATGGTCTTGAGGC	AAGACTCGCGTGCCTTTTAA	2	0.750	0.476	0.359
Total				89	5.992	13.373	11.858
Mean				3.42	0.23	0.51	0.45

PIC – polymorphic information content; Na – the number of observed alleles; Ho – observed heterozygosity; He – expected heterozygosity.

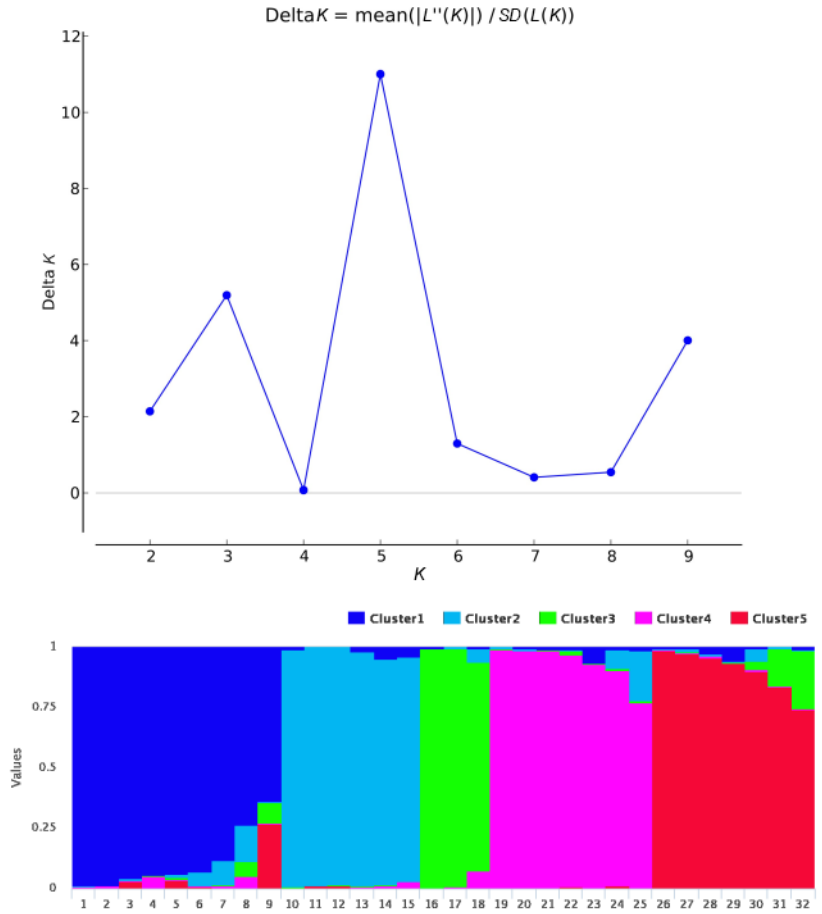

Figure 1 Population structure of 32 barley accessions genotyped using 26 markers. Means of log likelihoods and their standard deviations computed with STRUCTURE software over 10 runs and for a number (K) of expected clusters ranging from 1 to 10 and ΔK values as a function of K (Figure 2A). As indicated in this figure, K value for 5 was optimal. This indicates that accessions could be divided into 5 clusters. Each cluster was represented by different color (Figure 2B).

3.2. Clustering and Population Structure

Estimated likelihood [ln P(D)] was found to be greatest when $K = 5$, suggesting that the population used in this study can be divided into five clusters (Figure 1). The modern cultivars, Rihane and Lemsi, were found in Cluster 1, whereas Kounouz and Manel were distributed in Cluster 2.

The average distance (expected heterozygosity) between accessions in each cluster was 0.46. The highest value of 0.53 was observed in Cluster 5, indicating greater genetic diversity within the clusters; however, Cluster 3 showed the lowest value of 0.42. Genetic differentiation (F_{ST}) ranged from 0.21 in Cluster 5 to 0.46 in Cluster 3, with a mean of 0.34.

AMOVA test was applied to the codominant data matrix to obtain information on the variation within and among populations using GenAlEx software. The results of the AMOVA indicated that most genetic variation was among individuals (47%) (Table 3).

3.3. Phylogenetic Analysis

Unweighted neighbor-joining dendogram was constructed based on Nei's similarity coefficient of 32 genotypic data and revealed the genetic relationship among the accessions. The tree showed four groups of accessions (Figure 2). All accessions collected from Kébili (south of the country) were found in Groups 3 and 4. Cultivars

Table 3 Analysis of molecular variance (AMOVA) using 26 molecular markers of the genetic variation of the 32 barley lines.

Source	*df*	*SS*	*MS*	Estimated variation	%
Among Pops	4	87.324	21.831	0.986	14
Among Indiv	27	256.520	9.501	3.360	47
Within Indiv	32	89.000	2.781	2.781	39
Total	63	432.844		7.127	100

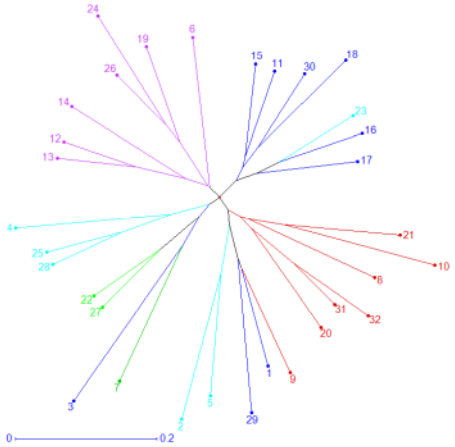

Figure 2 Unweighted neighbor-joining dendrogram showing genetic relationship among the 32 barley accessions based on the genetic dissimilarity matrix data of SSR markers alleles. All the accessions were divided into four groups. The colors of branches indicate accessions corresponding to the clusters (Cluster 1 to 5) from population structure analysis as in Figure 1. Numbers indicate accessions mentioned in Table 1.

and accessions collected from the north and north west of the country were located in Groups 1 and 2. When the unrooted phylogenetic tree was compared with the clusters obtained from the STRUCTURE analysis, the phylogenic tree matched well with the cluster separation in the STRUCTURE analysis. Accessions in Cluster C4 belonged to Group A3, accessions in Clusters C3 belonged to group A2, and accessions in Cluster C5 belonged to Group A1. Accessions in Clusters C1 and C2 belonged to Groups A1, A2, and A4.

3.4. Principal Coordinate Analysis

Principal coordinate analysis (PCoA) was conducted to further assess the population structure identified using SRUCTURE. The principal coordinates explained 12.5% and 9.3% of the total variation. The PCoA was largely consistent with the results of STRUCTURE. The first principal coordinate (PCo1) clearly separated 32 barley accessions into 5 groups (Figure 3).

4. Discussion

Estimating the genetic diversity of plant genetic resources is one of the important prebreeding activities in crop breeding. Assessing genetic diversities is important in identifying genotypes that underlie important phenotypic and genetic shifts during domestication (Vigouroux et al., 2008) and distinct genetic groups for retention of germplasm (Agrama & Eizenga, 2008). Identification of barley cultivars, lines, and accessions of Tunisian genetic resources have been based on phenotypic traits and agromorphological data. Such methods cannot provide reliable information for calculation of genetic distance and validation of pedigree (Stanton et al., 1994).

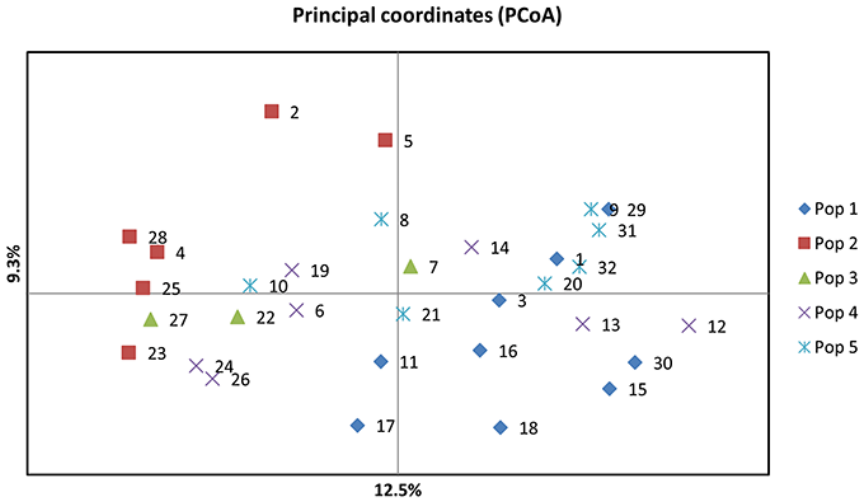

Figure 3 Principal coordinates analysis (PCoA) of 32 barley accessions. Coordinate 1 (12.5%) and Coordinate 2 (9.3%) refer to the first and second principal component, respectively.

In this study, we used 32 Tunisian barley lines (*Hordeum vulgare* L.), including four cultivars developed by the Tunisian breeding program. Twenty-six molecular markers, distributed across the seven chromosomes of barley, were used to genotype the selected lines. The average PIC value was 0.45 and is similar to the values reported by Hamza et al. (2004) (0.45) and Zhang et al. (2014) (0.46) for 96 barley accessions generated from 69 loci but less than those reported by Jilel et al. (2008) (0.78) and Pasam et al. (2014) (0.54). The average PIC value obtained in this study is higher than the average PIC (0.36) reported by Elakhdar et al. (2016). In general, a PIC value higher than 0.5 is useful in genetic studies because it can distinguish the polymorphism of a marker (DeWoody et al., 1994). He values demonstrate the diversity level of markers, and the values obtained in this study are high; the diversity of markers reported by Pompanon et al. (2005) is also high. He values ranged from 0.094 to 0.731, with a mean value of 0.51, suggesting that there is an extensive genetic variation within the 32 barley accessions genotyped in this study.

Unrooted phylogenetic tree was compared with the clusters obtained from STRUCTURE analysis using SSR markers. The phylogenic tree matched well with the cluster separation in STRUCTURE analysis. The phylogenetic tree clearly differentiated groups according to their geographic origin.

The estimation of genetic diversity and population structure of 32 Tunisian barley lines using molecular markers may provide more accurate information to barley breeders than the classical pedigree method. The 26 primer pairs used in this study may also be of potential value for further research on genetic mapping, segregation analysis, and phylogenetic status analysis of newly introduced germplasm.

References

Agrama, H. A., & Eizenga, G. C. (2008). Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of and its wild relatives. *Euphytica*, *160*(3), 339–355. https://doi.org/10.1007/s10681-007-9535-y

Azartamar, F. S., Darvishzadeh, R., Ghadimzadeh, M., Azizi, H., & Aboulghasemi, Z. (2015). Identification of SSR loci related to some important agro morphological traits in different oily sunflower (*Helianthus annuus* L.) lines using association mapping. *Crop Biotechnology*, *5*(10), 73–87.

Bwalya, E. C., Marzougui, S., Mwangi, E., Wooseon, C., & Lee, M. C. (2020). Genetic diversity analysis and population structure of some African and Asian finger millet (*Eleusine coracana* L.) accessions using expressed sequence tags-simple sequence repeat (EST-SSR) markers. *International Journal of Environment, Agriculture and Biotechnology*, *5*(3), 643–646. https://doi.org/10.22161/ijeab.53.17

Cronin, J. K., Bundock, P. C., Henry, R. J., & Nevo, E. (2007). Adaptive climatic molecular evolution in wild barley at the *Isa* defense locus. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 2773–2778. https://doi.org/10.1073/pnas.0611226104

DeWoody, J. A., Honeycutt, L., & Skow, L. C. (1994). Microsatellite markers in white tailed deer. *Journal of Heredity*, *86*(4), 317–319. https://doi.org/10.1093/oxfordjournals.jhered.a111593

Elakhdar, A., Abd EL-Sattar, M., Amer, K., Rady, A., & Kumamaru, T. (2016). Population structure and marker-trait association of salt tolerance in barley (*Hordeum vulgare* L.). *Comptes Rendus Biologies*, *339*(11), 454–461. https://doi.org/10.1016/j.crvi.2016.06.006

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Hamza, S., Hamida, W. B., Rebaï, A., & Harrabi, M. (2004). SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. *Euphytica*, *135*, 107–118. https://doi.org/10.1023/B:EUPH.0000009547.65808.bf

Jilal, A., Grando, S., Henry, R. J., Lee, L. S., Rice, N., Hill, H., Baum, M., & Ceccarelli, S. (2008). Genetic diversity of ICARDA's worldwide barley landrace. *Genetic Resources and Crop Evolution*, *55*(8), 1221–1230. https://doi.org/10.1007/s10722-008-9322-1

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Medimagh, S., Felah, M. E., & Gazzah, M. E. (2012). Barley breeding for quality improvement in Tunisia. *African Journal of Biotechnology*, *11*(89), 15516–15522. https://doi.org/10.5897/AJB11.2786

Mwangi, E. W., Marzougui, S., Sung, J. S., Bwalya, E. C., Choi, Y. M., & Lee, M. C. (2019). Assessment of genetic diversity and population structure on Kenyan sunflower (*Helianthus annus* L.) breeding lines by SSR markers. *Korean Journal of Plant Resources*, *32*(3), 244–253. https://doi.org/10.7732/kjpr.2019.32.3.244

Ovesná, J., Kučera, L., Vaculová, K., Milotová, J., Snape, J., Wenzl, P., Huttner, E., Kilian, A., Martelli, G., & Milella, L. (2013). Analysis of the genetic structure of a barley collection using DNA diversity array technology (DArT). *Plant Molecular Biology Reporter*, *31*, 280–288. https://doi.org/10.1007/s11105-012-0491-x

Pasam, R. K., Sharma, R., Walther, A., Özkan, H., Graner, A., & Kilian, B. (2014). Genetic diversity and population structure in a legacy collection of spring barley landraces adapted to a wide range of climates. *PLoS ONE*, *9*, Article e116164. https://doi.org/10.1371/journal.pone.0116164

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic Analysis in Excel. Population genetic software for teaching and research – An update. *Bioinformatics*, *28*, 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Perrier, X., & Jacquemoud-Collet, J. P. (2014). *DARwin* [Computer software]. http://darwin.cirad.fr/

Poets, A. M., Fang, Z., Clegg, M. T., & Morrell, P. L. (2015). Barley landraces are characterized by geographically heterogeneous genomic origins. *Genome Biology*, *16*, Article 173. https://doi.org/10.1186/s13059-015-0712-3

Pompanon, F., Bonin, A., Bellemain, E., & Taberlet, P. (2005). Genotyping errors: Causes, consequences and solutions. *Nature Review Genetics*, *6*, 846–847. https://doi.org/10.1038/nrg1707

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multi locus genotype data. *Genetics*, *155*, 945–959. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Stanton, M. A., Stewart, J. M., Percival, A. E., & Wandel, J. F. (1994). Morphological diversity and relationships in A-genome cottons, *Gossypium arboreum* and *Gossypium herbaceum*. *Crop Science*, *34*, 519–527. https://doi.org/fqqfcr

Tondelli, A., Xu, X., Moragues, M., Sharma, R., Schnaithmann, F., Ingvardsen, C., Manninen, O., Comadran, J., Russell, J., Waugh, R., Schulman, A. H., Pillen, K., Rasmussen, S. K., Kilian, B., Cattivelli, L., Thomas, W. T. B., & Flavell, A. J. (2013). Structural and temporal variation in genetic diversity of European spring two-row barley cultivars and association mapping of quantitative traits. *The Plant Genome*, *6*, 1–14. https://doi.org/10.3835/plantgenome2013.03.0007

Varshney, R. K., Graner, A., & Sorrells, M. E. (2005). Genic microsatellite markers in plants: Features and applications. *Trends in Biotechnology*, *23*, 48–55. https://doi.org/10.1016/j.tibtech.2004.11.005

Vigouroux, Y., Glaubitz, J. C., Matsuoka, Y., Goodman, M. M., Sanchez G., J., & Doebley, J. (2008). Population structure and genetic diversity of New World maize races assessed by DNA microsatellites. *American Journal of Botany*, *95*, 1240–1253. https://doi.org/10.3732/ajb.0800097

Yahiaoui, S., Cuesta-Marcos, A., Gracia, M. P., Medina, B., Lasa, J. M., Casas, A. M., Ciudad, F. J., Montoya, J. L., Moralejo, M., Molina-Cano, J. L., & Igartua, E. (2014). Spanish barley landraces outperform modern cultivars at low-productivity sites. *Plant Breeding*, *133*, 218–226. https://doi.org/10.1111/pbr.12148

Zhang, M., Mao, W., Zhang, G., & Wu, F. (2014). Development and characterization of polymorphic EST-SSR and genomic SSR markers for Tibetan annual wild barley. *PLoS ONE*, *9*, Article e94881. https://doi.org/10.1371/journal.pone.0094881