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ORIGINAL PAPER

The Horn of Africa as a centre of barley diversification and a potential domestication site

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Abstract According to a widely accepted theory on barley domestication, wild barley (*Hordeum vulgare* ssp. *spontaneum*) from the Fertile Crescent is the progenitor of all cultivated barley (*H. vulgare* ssp. *vulgare*). To determine whether barley has undergone one or more domestication events, barley accessions from three continents have been studied (a) using 38 nuclear SSR (nuSSRs) markers, (b) using five chloroplast SSR (cpSSR) markers yielding 5 polymorphic loci and (c) by detecting the differences in a 468 bp fragment from the non-coding region of chloroplast DNA. A clear separation was found between Eritrean/Ethiopian barley and barley from West Asia and North Africa (WANA) as well as from Europe. The data from chloroplast DNA clearly indicate that the wild barley (*H. vulgare* ssp. *spontaneum*) as it is found today in the ''Fertile Crescent'' might not be the progenitor of the barley cultivated in Eritrea (and Ethiopia). Consequently, an independent domestication might have taken place at the Horn of Africa.

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Introduction

The mode and the location of barley domestication has been the subject of vigorous scientific disputes (von Bothmer et al. 2003). Several hypotheses describing the domestication sites, the wild progenitor and the domestication of barley have been proposed (Molina-Cano et al. 1987; Badr et al. 2000; Molina-Cano et al. 2005). Currently the most widely accepted hypothesis is that wild barley, *Hordeum vulgare* subsp. *spontaneum* (abbreviated as *H. spontaneum* here), is the progenitor of cultivated barley. This is thought to have occurred in a single domestication event approximately 10,000 years ago in the Fertile Crescent, where *H. spontaneum* is indigenous and widely dispersed (Harlan and Zohary 1966). Alternative hypotheses propose that additional independent domestication events have occurred, citing the presence of wild six-rowed barley, *H. vulgare* subsp. *agriocrithon*, in Tibet (Åberg 1949) and the RFLP marker based diversity structure and presence of *H. spontaneum* in Morocco (Molina-Cano et al. 1999). An independent domestication was also proposed to have occurred in Ethiopia, based on the highly diverse phenotypic variation (Negassa 1985) and the presence of a unique flavonoid pattern (Fröst et al. 1975). For

all these hypotheses, contradictory results and theories have been published and discussed.

The high degree of polymorphism of molecular markers has made the detection of population structure simple and highly reliable. Nuclear Simple Sequence Repeats (nuSSR) markers are widely used and have become a powerful tool due to their locus-specific nature, high levels of polymorphism and to the co-dominant mode of inheritance. These characteristics make nuSSR markers particularly suitable for ecological, evolutionary and genetic diversity studies. They have also been applied to develop conservation strategies as well as core collections (Demissie et al. 1998; Nevo 1998; van Hintum 1999). Moreover, nuSSRs markers allow the analysis of population genetics and population structure studies at a high-degree of resolution (Powell et al. 1996; Provan et al. 1999; Matus and Hayes 2002; Woodhead et al. 2005).

However, in evolutionary studies, hyper-variable markers with high-mutation frequency, such as nuSSR, are not always the most appropriate tools. Chloroplast DNA (cpDNA) markers have several benefits compared to nuDNA-based markers for long-term evolutionary surveys. Chloroplast DNA is maternally inherited in the majority of angiosperms, including barley (Smith 1989), and mutates several times slower than genomic DNA (Holwerda et al. 1986). For these reasons, cpDNA provides ideal markers for tracing maternal ancestry and for taxonomic and evolutionary studies (Vanichanon et al. 2003). The discovery of highly polymorphic simple sequence repeats in the chloroplast genomes of plants (cpSSRs) has provided new opportunities for analysis in population studies (Powell et al. 1996; Provan et al. 1999). cpSSRs are more conservative in their evolution (mutation rate), with fewer alleles being reported at cpSSR loci compared to nuSSRs (Powell et al. 1996). Even in the non-coding region of the chloroplast, very low levels of variation have been found among closely related species (Small et al. 1998).

The objective of the present investigation was to study the diversity within and among different groups of barley. The barley lines that were investigated originated from Eritrea/Ethiopia, West Asia and North Africa (WANA) and Europe. Furthermore, the possibility of an independent centre of barley domestication in Eritrea/Ethiopia was investigated. The study was conducted using nuclear and chloroplastic microsatellite markers on barley lines collected from Eritrean farmer's fields, Ethiopia, the Fertile Crescent, North Africa and from Europe. Additionally, a non-coding region of the chloroplast genome was sequenced.

Materials and methods

Plant material

Four geographical/subspecies groups of samples adding up to a total of 549 accessions were analyzed (Table 1). The collection includes accessions from Eritrea/Ethiopia, WANA region, as well as from Northern Europe. The first group contains 240 Eritrean single-spike lines (10 spikes per field collected from 24 farmer's fields located in different agro-climate zones). This group also includes 23 lines from different agro-climate zones in Ethiopia, provided by the Gene Bank at the International Centre for the Agricultural Research in the Dry Areas (ICARDA), and two lines described as wild barley from Ethiopia, provided by the USDA-ARS National Small Grain Collection, Aberdeen, Idaho,

Table 1 Plant materials used in nuSSRs and cpSSRs profiling study

Group	Sources	Type	Country	No. of individuals
I	Own collection	*Hordeum vulgare* subsp. *vulgare*	Eritrea	240
	ICARDA[a], Syria	*H.v.* subsp. *vulgare*	Ethiopia	23
	USDA, USA[b]	*H.v.* subsp. *spontaneum*	Ethiopia	2
II	ICARDA[a], Syria	*H.v.* subsp. *vulgare*	Syria	10
	ICARDA[a], Syria	*H.v.* subsp. *vulgare*	Jordan	10
	ICARDA[a], Syria	*H.v.* subsp. *vulgare*	Turkey	10
	ICARDA[a], Syria	*H.v.* subsp. *vulgare*	Tunisia	10
	ICARDA[a], Syria	*H.v.* subsp. *vulgare*	Morocco	10
III	KVL, Denmark	*H.v.* subsp. *spontaneum*	Palestine/Israel	100
	ICARDA[a], Syria	*H.v.* subsp. *spontaneum*	Syria	10
	ICARDA[a], Syria	*H.v.* subsp. *spontaneum*	Jordan	10
	ICARDA[a], Syria	*H.v.* subsp. *spontaneum*	Turkey	9
	Spain[c]	*H.v.* subsp. *spontaneum*	Morocco	8
IV	KVL, Denmark	*H. vulgare* subsp. *vulgare*		96
	ICARDA[a], Syria	*H. marinum* subsp. *marinum*	Turkey	1
	Total			549

[a] The International Center for Agricultural Research in the Dry Areas, Syria

[b] The National Small Grains Collection USDA-ARS, Aberdeen, Idaho, USA

[c] Provided kindly by Dr. Molina Cano JL, Centre UdL-IRTA, Lleida, Spain

USA. The second group contains 50 landraces (*H. vulgare* subsp. *vulgare)* from WANA region (ten landraces from Syria, Turkey, Jordan, Tunisia and Morocco, respectively) provided by the ICARDA gene bank. The third group consists of 137 wild barley lines (*H. vulgare* subsp. *spontaneum*) of which 100 lines were collected from the Eastern Mediterranean region and maintained by the Department of Agricultural Sciences, The University of Copenhagen, Faculty of Life Sciences, Denmark. A further ten lines from Syria, ten lines from Jordan and nine lines from Turkey were provided by the ICARDA gene bank and eight lines of wild barley from Morocco were provided by Dr. Molina-Cano, Centre UdL-IRTA, Lleida, Spain. Group four consists of 96 Northern European varieties provided by the Danish Variety Releasing Authority, Tystofte, Denmark. One sample of sea barley, *H. marinum* subsp. *marinum* from Turkey, was provided by the ICARDA gene bank (Table 1).

Nuclear and chloroplast SSRs

DNA was extracted according to the CTAB-protocol (Saghai Maroof et al. 1984). All samples were tested with 38 microsatellite markers covering the entire barley genome and with five chloroplast microsatellite markers (hvcppsbA, hvcppsbK, hvcptrnS1, hvcptrnS2 and hvcptrnLF, Provan et al. 1999). PCR was performed as previously reported for each marker (Ramsay et al. 2000; Liu et al. 1996; Becker and Heun 1995). The forward primers were 5′-labeled with different fluorescent dyes (6-FAM, TET, HEX, NED or VIC). PCR products were analyzed with an ABI PRISM 377 DNA Sequencer (Applied Biosystem) and a MegaBace 1000 Sequencer (Molecular Dynamics). Data from the ABI PRISM was analyzed with GeneScan® and Genotyper® version 2.1 software and data from the MegaBace instrument was analyzed with Instrument Control Manager and Genetic profiler version 1.1.

Sequencing assay

A total of 105 samples representing different cpSSR haplotyles were selected and a non-coding region of chloroplast DNA was PCR amplified using trnT and trnE primers (Saltonstall 2001). PCR reactions were performed in a total reaction volume of 20 µl containing 100 ng genomic DNA, 1× PCR buffer provided with the enzyme, 1.5 mM $MgCl_2$, 0.1 units *Taq* polymerase, 250 µM dNTPs, and 1 µM of forward and reverse primers. PCR reactions were carried out with a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystem) using the following parameters: (1) denaturation at 94°C for 2 min; (2) 30 cycles of denaturing at 94°C for 1 min annealing at 56° for 1 min and extension at 72°C for 2 min; (3) final extension at 72°C for 5 min. PCR products were purified with Sephadex™ G-50 Fine (Amersham Biosciences) and freeze-dried. Purified PCR products were sequenced at MWG AG Biotech.

Data analysis

The analysis of genetic diversity was performed with the software NTSYSpc v.2.11Q (Exeter Software, Setauket, NY, USA) using the "SIMQUAL" module with "Simple Matching". Multi-dimensional scaling (MDS) was performed using the same software. For MDS, Principal Component Analysis was used as a starting point. Following MDS the result matrix was rotated to align the axes with the major axes of variation. The average genetic diversity was calculated using the software package 'Arlequin' (Excoffier et al. 2005). Nei's Standard Genetic Dissimilarity (Nei 1972) and the resulting Neighbour-joining tree were calculated using the software Power Marker v.3.23 (Liu and Muse 2005). Nei's Standard Genetic Dissimilarity was chosen because it assumes both genetic drift and mutation. The sequences were aligned with CLUSTALW and the phylogenetic tree was constructed with TREEFINDER using the assumption of a molecular clock (Jobb 2004).

Results

The analysis with 38 nuSSR was successful for 540 out of 549 accessions from Eritrea, Ethiopia, WANA and Europe. Within the Eritrean population only two lines showed identical nuSSRs patterns (data not shown). Based on nuSSRs data of all populations, a distance matrix was calculated and a MDS analysis in two dimensions was carried out (Fig. 1). The distribution of the accessions in the two dimensions clearly reveals three groups. The first group on the left side of the plot consists of Eritrean and the Ethiopian barley lines. The second group on the upper part of the plot contains the European barley varieties and the last group on the right side of the plot includes all *H. spontaneum* as well as *H. vulgare* lines from WANA region. The last group is the most diverse group, exemplified by the greater spread in the MDS plot. Within this group, there is no apparent separation of the wild barley

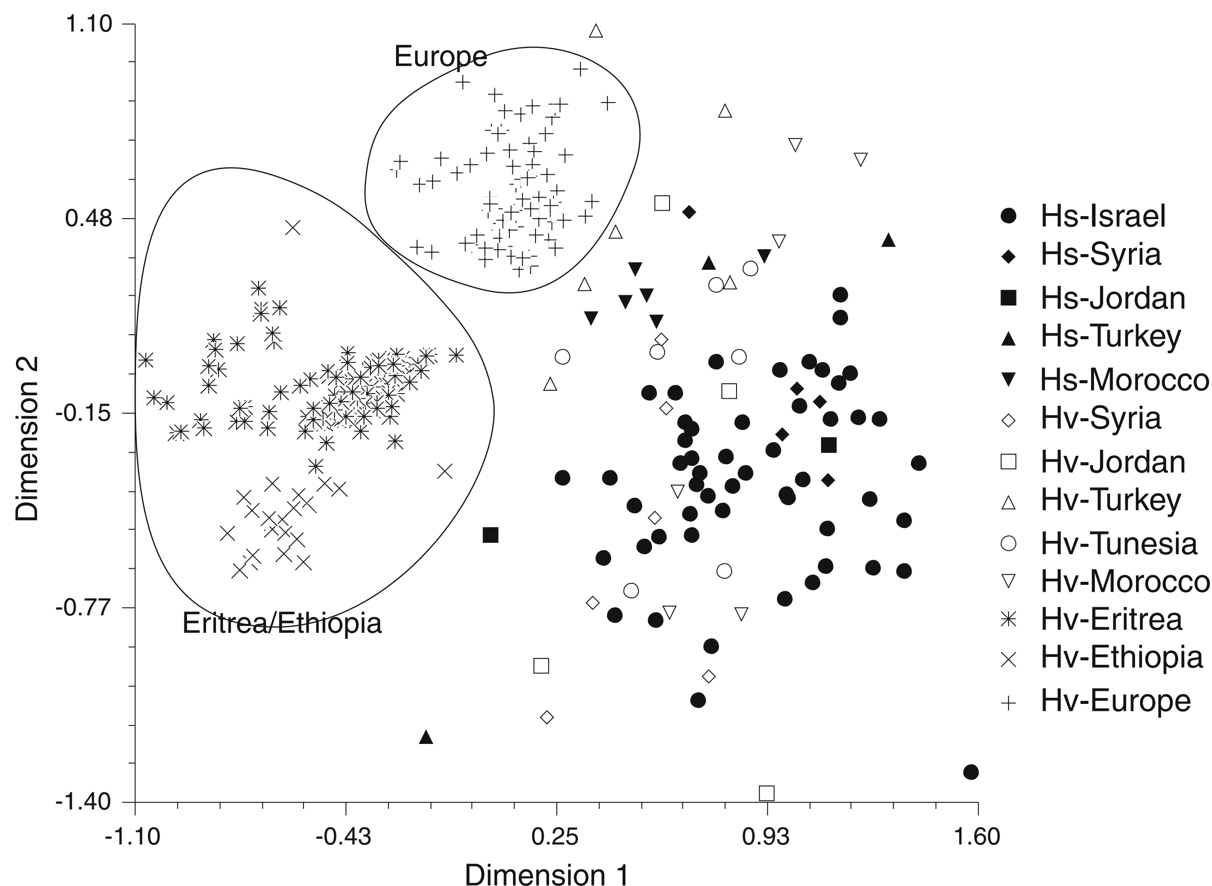

Fig. 1 MDS for all groups based on the nuclear SSRs distance. Encircled the groups with the European and the Eritrean/Ethiopian accessories, respectively

accessions from the landraces. Nor is there an apparent separation of barley accessions originating from different locations within the WANA region. The second group, containing the European cultivars, has dimensional axis scores more similar to the WANA barley than the Eritrean barley. The two lines described as wild barley from Ethiopia, did not show any phenotypic and genotypic differences from the Ethiopian cultivated lines, except their shattering. Therefore they were included in the group of Ethiopian *H. vulgare* lines.

The diversity index $\hat{H}$, and the number of alleles per locus were calculated for all populations based on the nuSSRs data (Table 2). In order to account for the unequal sample sizes, the same calculation was performed with a maximum of 50 lines per group that were randomly selected from the three large groups. After 50 repetitions the mean was calculated. Only marginal differences for $\hat{H}$ were found compared with the calculation with the full samples (data not shown). Wild barley had a significantly higher diversity than all other groups. Landraces from WANA region were less diverse than the wild barley, but significantly more diverse than the barley landraces from Eritrea, Ethiopia and the Northern European varieties. The average number of alleles per locus within the groups had a similar pattern. The very high average number of alleles per locus in the wild barley and the relatively high average number of alleles per locus in the Eritrean landraces are most likely caused by the higher number of individuals. The average number of alleles per locus from 50 randomly selected individuals for the wild

Table 2 Sample size, the average number of alleles per locus, diversity index $\hat{H}$ and its standard deviation (SD) based on *nuclear* SSRs for all populations

Population	Sample size	Loci	No. of alleles	$\hat{H}$	SD $\hat{H}$
H.sp. WANA	138	35	17.51	0.8258	0.0262
H.v. WANA	50	35	10.89	0.7624	0.0295
H.v. Eritrea	240	35	9.37	0.5729	0.0410
H.v. Ethiopia	23	35	6.06	0.6203	0.0418
H.v. Europe	96	35	6.57	0.5934	0.0293

SD standard deviation

barley was 13.14 and 6.16 for the Eritrean landraces, respectively (data not shown).

Five cpSSRs markers in non-coding plastome regions resulted in eight distinct bands. Two of the additional bands for hvcptrnS1 consistently followed the pattern described by Provan et al (1999) and Molina-Cano et al (2005), and therefore contributed no additional information and were therefore removed from the analysis. For hvcppsbA, we observed a previously unreported 130 bp polymorphic band. As we could not exclude an amplification of non-chloroplast-DNA, we decided to omit this band from our analysis. In total, 471 accessions were included in the analysis. Lines with complete marker information were grouped according to their chloroplast microsatellite pattern (haplotypes). Table 3 shows the diversity index $\hat{H}$ and the average number of alleles per locus for the chloroplast microsatellites. Again, a resampling strategy was used to obtain a diversity index approximately the same as the full samples. Generally, the diversity indices for chloroplast microsatellites were smaller than the diversity indices for nuclear microsatellites. The highest diversity indices were found for the wild barley from WANA region, followed by Eritrean and Ethiopian barley. Again, the European cultivars had the lowest diversity indices. The ranking of the groups shows differences compared with the respective data from the nuSSR, especially for the landraces from Eritrea and Ethiopia. For cpSSR, the Eritrean landraces have a slightly lower diversity index than the wild barley lines, while the landraces collected all over WANA region show an even lower $\hat{H}$-value than the landraces collected in Eritrea over a small area.

While all but two of the 540 lines analyzed with the nuSSR showed individual banding patterns, the five cpSSR-loci resulted in 107 different haplotypes. Table 4 shows the number of lines in each population, the resulting number of haplotypes and the average number of accessions per haplotype. The last column of Table 4 shows the average genetic distance between

Table 3 Sample size, the average number of alleles per locus, diversity index $\hat{H}$ and its standard deviation (SD) based on *chloroplast* SSRs for all populations

Population	Sample size	Loci	No. of alleles	$\hat{H}$	SD $\hat{H}$
H.sp. WANA	118	5	5.00	0.3990	0.0785
H.v. WANA	41	5	2.20	0.2060	0.0875
H.v. Eritrea	214	5	4.60	0.3481	0.0980
H.v. Ethiopia	22	5	2.20	0.2532	0.0726
H.v. Europe	92	5	2.60	0.1774	0.0726

SD standard deviation

Table 4 Sample size, number of pattern groups and the average distance of lines within a population

Population	Sample size	Groups	Accessions/ group	Average gen. dist
H. sp. WANA	117	48	2.4	0.40
H v. WANA	39	5	7.8	0.21
H.v. Erit/Eth.	219	56	3.9	0.34
H.v. Europe	91	8	11.4	0.18
H.v. Europe[a]	86	6	14.3	0.12

[a] European varieties without two groups having the ''Eritrean-like'' pattern

haplotypes within each population based on differences between repeat numbers. The different populations did not contribute equally to the number of haplotypes. The 117 wild barley lines showed 48 different haplotypes, while only 6 different haplotypes were found within the 86 European barley varieties. This is also reflected by the number of accessions per group. The number of accessions per haplotype for the landraces from Eritrea/Ethiopia is second lowest, with 3.9, compared to 2.4 for the wild barley. This is due to the fact that 98 of 219 Eritrean/Ethiopian accessions showed only 3 different haplotypes, while the remaining 121 accessions are distributed in 53 different haplotypes. The highest average genetic distance was obtained for wild barley from WANA with a value of 0.68. Within the European lines, 5 lines have very different haplotype patterns compared to the remaining 86 lines. These 5 European lines show the same haplotype patterns as lines from Ethiopia/Eritrea. The analysis of their pedigree showed ancestors originating from Ethiopia. Thus, the calculations in Table 4 are shown both for all European varieties (*H.v.* Europe) as well as for European varieties without the 'Eritrean/Ethiopian-like haplotypes' (*H.v.* Europe). The exclusion of these 5 lines resulted in the reduction, from 0.18 to 0.12, of the average genetic distance.

For the 107 haplotypes, the genetic distances based on number of repeats were calculated and further analyzed by MDS on two dimensions (Fig. 2). Two groups can be clearly distinguished. One group on the left side of the plot contains the majority of the *H. spontaneum* lines, all *H. vulgare* lines from WANA region and most of the European cultivars, but none of the Eritrean or Ethiopian lines. No apparent subgrouping is observed within this group neither concerning the subspecies (*vulgare* or *spontaneum*) nor the geographical origin. The second group on the right side of the plot includes all *H. vulgare* lines from Eritrea and Ethiopia, but only five haplotypes containing *H. spontaneum* lines from Eastern Mediterranean region, Jordan and Syria (mixed with Eritrean barley lines),

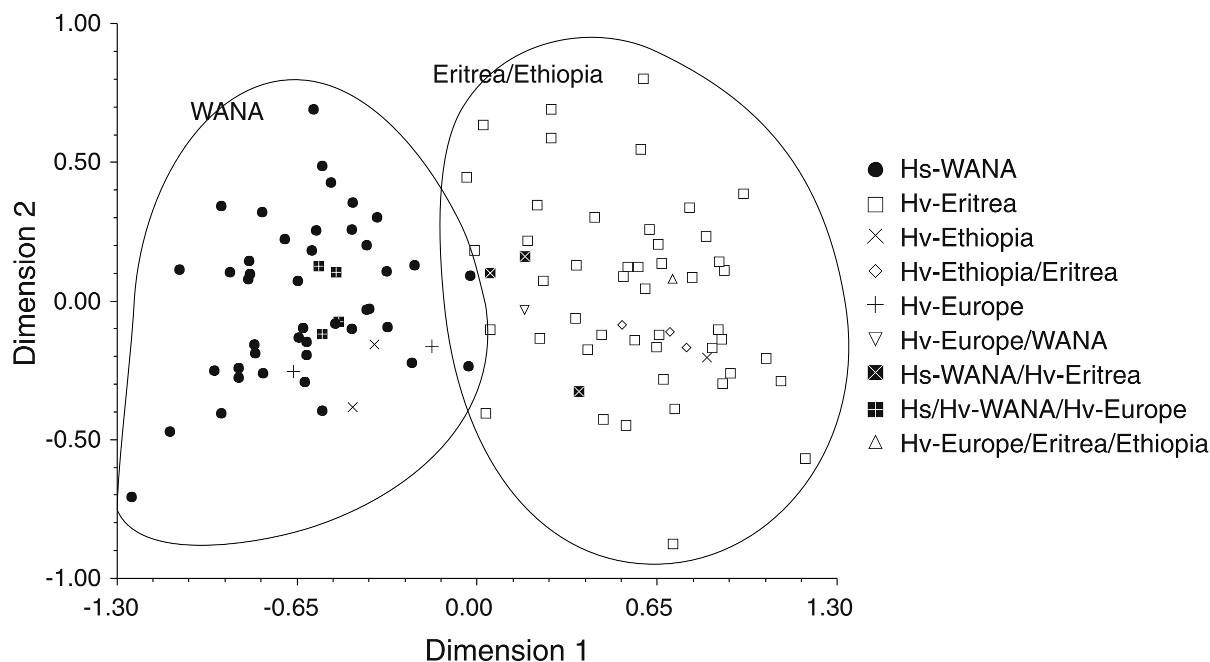

Fig. 2 MDS based on five cpSSRs repeat distance. Encircled the lines with the Eritrean/Ethiopian and the WANA pattern (the latter also including most of the European lines)

one haplotype containing European varieties and a line from the WANA region. The two lines described as wild barley from Ethiopia clustered together with the cultivated Ethiopian lines in the case of nuSSR-MDS, as described before. In the analysis of the cpSSR, they were not distinguishable from cultivated Ethiopian/Eritrean barley by haplotype.

In order to quantify the differentiation between the different populations in our study, Nei's Standard Genetic Dissimilarity was calculated among all populations based on both nuSSR and cpSSR data (Table 5). This statistic can have values higher than one. Figure 3a and b show Neighbour-joining trees based on these similarities. Generally, the dissimilarity values between the groups are higher for the nuSSR than for the cpSSR. The highest dissimilarity values were found between the barley lines from Eritrea/Ethiopia and the other three groups for both microsatellite types. Furthermore, the dissimilarity value between barley lines from Eritrea and from Ethiopia is very low from both microsatellite types. The main difference between the dissimilarity tables based on the two marker types is the dissimilarity value of the European lines compared to the *H. vulgare* lines from WANA region. The dissimilarity value is low (0.0123) for the cpSSR data, while it is much higher (0.6381) in the nuSSR analysis. This results in the differentiation of two groups with cpSSR and three groups with nuSSR, as suggested by the MDS analysis (Fig. 1).

Further evidence for the relationships found in the chloroplast microsatellite analysis should be revealed by the analysis of the intergenic-spacer region of chloroplast DNA from the 105 accessions representing different cpSSR-haplotypes that were selected. In these lines a 468 bp fragment from an intergenic-spacer of chloroplast DNA was sequenced (trnE-trnT, Saltonstall 2001) and yielded one hundred useful sequences of which 17 were unique. A phylogenetic tree

Table 5 Nei's standard genetic dissimilarity between groups of limes based on nuclear SSR (lower left) and chloroplast SSR (upper right)

	H.s. WANA	*H.v.* WANA	*H.v.* Eritrea	*H.v.* Ethiopia	*H.v.* Europe
H.s. WANA		0.1385	0.4126	0.4756	0.1992
H.v. WANA	0.2633		0.6788	0.6571	0.0123
H.v. Eritrea	0.7211	0.5892		0.0359	0.6910
H.v Ethiopia	0.8577	0.7093	0.2722		0.6134
H.v. Europe	0.6419	0.6381	0.9398	1.0399	

H.s.: *Hordeum vulgare* ssp. *spontaneum*, *H.v.*: *Hordeum vulgare* ssp. *vulgare*

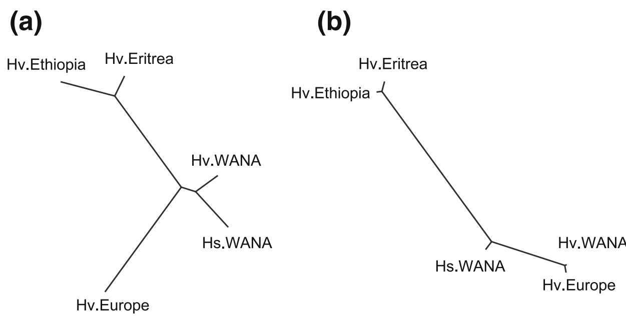

Fig. 3 Neighbour-joining tree based on Nei-dissimilarity from nuclear SSRs (**a**) and chloroplast SSR (**b**) as presented in Table 5. *Hv*: *Hordeum vulgare*, *Hs*: *Hordeum spontaneum*

was constructed by Maximum Likelihood (Fig. 4) including one line of sea barley *H. marinum* subsp. *marinum* that belongs to the tertiary gene pool of *Hordeum* to root the tree. Besides the rooting branch containing *H. marinum*, two distinct branches were identified. One branch includes all *H. vulgare* lines from Eritrea (''Hv.Eritr.''), while the other includes lines from WANA and most of the lines from Europe. ''Mixed.Grp.1'' includes 39 Eritrean lines, 5 *H. spontaneum* lines from West Asia and one landrace from Syria as well as the European variety 'Ceylon'. The 'non-Eritrean' lines grouped by sequencing to the

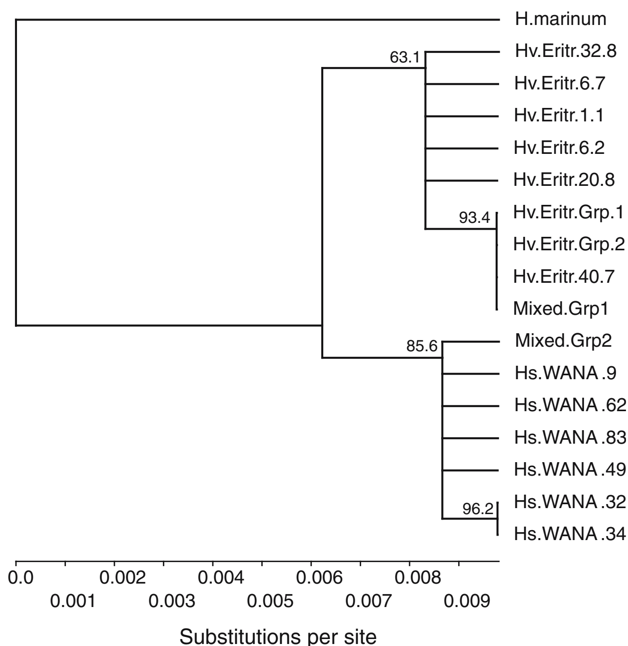

Fig. 4 Phylogenetic tree for 105 *H. vulgare* and *H. spontaneum* lines and varieties from WANA, Europe and Eritrea based on sequence differences in a chloroplast intergenic spacer (trnE-trnE). The numbers near the branches are likelihoods from a Local rearrangement Paired-sites test ranging from 0 to 100

''Mixed.Grp.1'' showed also in the cpSSRs 'Eritrean-like' patterns. ''Mixed.Grp.2'' belonging to second clade includes 8 European varieties, 2 landraces from Jordan and 26 *H. spontaneum* lines from WANA. Interestingly, none of the Eritrean/Ethiopian lines were clustered with WANA barley group, while some of the lines from WANA and one European cultivar show Eritrea-like patterns.

Discussion

Comparing the two types of markers used in the present analysis, namely microsatellites derived from nuclear DNA and microsatellites derived from chloroplast DNA, significant differences were found for the diversity index ($\hat{H}$) in the different populations (Tables 2 and 3). nuSSR show higher $\hat{H}$ values ranging from 0.593 to 0.826 compared with the cpSSR ranging from 0.177 to 0.399. This is also reflected by the differences in the average number of alleles per SSR locus ranging from 6.6 to 17.5 for the nuSSR and from 2.6 to 5.0 for the cpSSR. This may be caused by the lower number of loci for the cpSSR. However it supports the results of Provan et al. (1999), which reported that the mutation rate of cpSSR, and therefore the number of different alleles, is considerably lower for cpSSR than for nuSSR. Nevertheless, the analyses based on these two marker types agree that the *H. spontaneum* lines from WANA region have very high diversity indices, while the modern varieties from Europe have the lowest diversity indices. Assuming that the wild barley from WANA region is the progenitors of European cultivated barley, and accepting the postulation that domestication is a severe genetic bottleneck and subsequent breeding efforts caused genetic erosion in European cultivars (Russell et al. 2000), our results are not surprising. However, contradictory evidence was obtained for the ranking of the *H. vulgare* lines from Eritrea and Ethiopia using the SSR derived from different DNA types (nuclear and chloroplast). While the diversity indices for the nuSSR were as low as those found in modern European varieties, the $\hat{H}$ values for the cpSSR were nearly as high as the values found for the *H. spontaneum* lines from WANA region. While the first finding might be explained by the relatively small collection area, of approximately 100 km in diameter, for the Eritrean accessions, the large diversity for the cpSSR was unexpected. The differences in diversity indices between the populations using the two SSR types are also shown by the representation of the diversity data in the MDS (Figs. 1, 2). The distribution of the accessions from Eritrea and Ethiopia in the two

dimensions on the MDS plot is much larger for the cpSSR than for the nuSSR. The divergent results must be caused by the different properties of cpSSR and nuSSR. SSR derived from chloroplasts have, as mentioned above, a lower mutation rate. Furthermore, chloroplast genomes are effectively haploid, non-recombinant and, in angiosperms, maternally inherited (Provan et al. 2001). Additionally and partly caused by high conservation of the cpDNA and the lack of recombination in cpDNA, a lower or no selection pressure can be expected on cpSSR. Consequently, cpSSRs are an ideal tool to track seed flow and the development of a species not affected by panmixia and pollen flow within and between subspecies and even species. In order to explain our observations, we assume that in Eritrea/Ethiopia, an ancient wild barley population was subjected to a domestication process on a broad base without a strong bottleneck effect on the level of cpSSR diversity, as little or no selection occurred at the chloroplast level. This would explain the unexpected variation at the level of cpDNA. As this population is subjected to recombination through pollination and subsequent phenotypic selection, genetic erosion might be expected on the nuclear level. The case is completely different for the European varieties, where a strong bottleneck obviously occurred during the domestication at the level of cpSSR, most likely the result of a narrow ‘starter population’ (Tanksley and McCouch 1997; Provan et al. 1999; Piffanelli et al. 2004). Similar results were obtained by Neale et al. (1988) for American barley cultivars compared with *H. spontaneum* genotypes using RFLP marker for cpDNA.

This leads to the issue of the genetic and phylogenetic relationship of the different populations analyzed in this study. The comparison of the genetic dissimilarity values of nuSSR and cpSSR (Table 5, Fig. 3) reveals similar patterns for nuSSR compared with cpSSR. The lowest dissimilarity index for nuSSR and cpSSR can be found between *H. vulgare* and *H. spontaneum* from WANA region and between the Eritrean and Ethiopean lines. The Eritrean and Ethiopean lines share the same geographic region and might therefore also share the same domestication process. The main differences between the results obtained with the two marker types are: (1) The European varieties are much more separated from the two WANA groups in the case of the nuSSR than in the case of cpSSR (Fig. 3). The same fact can be seen in the results from the MDS analyses (Figs. 1, 2), where the European varieties show a clear grouping in the results based on nuSSR, but not on cpSSR. This might be due to the fact that these two gene pools have been subjected to different influences and selection pressures. While the WANA *H. vulgare* is still under genetic influence of *H. spontaneum* and selected for improved yield stability and adaptability to harsh climatic condition (Ceccarelli et al. 1987), modern European varieties are often improved for disease resistance by exotic material from geographically distant regions (Fischbeck 1992; Jahoor and Fischbeck 1993; Garvin et al. 1997) and are selected for high yielding potential under optimized conditions. (2) The relatively clear separation between the Eritrean/Ethiopian lines and all other barley lines is also revealed by the cpSSR, but is not as clear from the nuSSR. This can be explained by both pollen flow and higher selection pressure on nuSSR (by linkage with advantageous or disadvantageous alleles).

Nevertheless, for both datasets (cpSSR and nuSSR), the largest dissimilarity values were observed between the Eritrean/Ethiopian accessions and all other populations. This finding is further supported by the MDS analyses for the two types of microsatellites (Figs. 1, 2). The cluster of landraces from Eritrea and Ethiopia is clearly separated from the rest of the data points. Only very few exceptions can be found and are easily explained by single migration events, such as the Ethiopian line (‘‘Abyssinian’’) used as a donor for powdery mildew resistance in European barley breeding (Fischbeck 1992). The grouping of barley lines from Eritrea/Ethiopia against all other accessions analyzed is even more emphasized by the phylogenetic tree of the sequences presented in Fig. 4.

Finally, the chloroplast sequence analysis provides further support, besides a few exceptions, that Eritrean/Ethiopian barley accessions form a distinct group only distantly related to the other *H. spontaneum* and *H. vulgare* genotypes analyzed. Therefore, *H. spontaneum* as it is found in WANA, and especially in the Eastern Mediterranean region might not be the wild ancestor of Eritrean/Ethiopian barley.

In contrast to our conclusions, the predominant theory for the domestication of cultivated barley claims a single domestication event in the WANA region (von Bothmer et al. 2003). This theory was proposed by Harlan and Zohary (1966), based on the occurrence of wild barley in this region. Recently, Badr et al. (2000) concluded based on results from AFLP marker and the sequence of *Bkn-3* that cultivated barley is of monophyletic origin. However, their analysis was restricted to nuclear DNA and their sampling was strongly skewed, by including only 24 landraces and old varieties from Mediterranean, Balkan, and African locations in the 374 accessions they analyzed. They also included 21 landraces from the Himalayan-Indian

region, as this area has been proposed as the origin of six-rowed barley (Åberg 1949). Based on their marker and sequence results, Badr et al. (2000) concluded that the Himalayan region is a centre of diversification and not a centre of origin for barley.

The hypothesis that Morocco is a centre of origin of barley was proposed by Molina-Cano et al. (1987), based on agromorphological traits and protein patterns. This hypothesis was later supported by an analysis of 35 *H. spontaneum* accessions with different geographical origin, using nuclear RFLP markers (Molina-Cano et al. 1999). Recently, Molina-Cano et al. (2005) used the same cpSSR as applied in this study, to analyze 186 barley lines, including 8 *H. spontaneum* and 37 *H. vulgare* lines from Morocco as well as 39 *H. vulgare* lines from Ethiopia. Significantly, the 32 Ethiopian accessions included in their analysis clustered separately, similar to our results for all 263 lines from Eritrea and Ethiopia. Additionally, three of the 37 *H. vulgare* lines from Morocco and two old Spanish cultivars showed the ''Ethiopian-like'' pattern. None of the *H. spontaneum* lines from Morocco show these patterns and consequently the authors discuss a very early migration of wild barley into Northwest Africa and from there to Spain. However, none of the 8 *H. spontaneum* accessions from Morocco we included showed an ''Eritrean-like'' pattern. Nevertheless, as we restricted the number of this specific group of accessions to eight, we neither can nor want to contradict those results that might reveal an interesting additional aspect to the cultivation of barley.

The theory that the region covered today by Ethiopia and Eritrea, is a second centre of domestication of cultivated barley has been stated before. It was first based on the large phenotypic variation represented by 485 landraces collected in 12 Ethiopian provinces (Negassa 1985). Additionally, a flavonoid pattern nearly exclusively occurring in Ethiopian lines added evidence to the independent domestication theory (Fröst et al. 1975). The opponents of the hypothesis of a second centre of origin of barley at the Horn of Africa mainly use two arguments. The first argument is the relatively low genetic diversity determined by molecular markers (Bjørnstad et al. 1997). Our experiments came to the same result, but only for markers based on *nuclear* DNA and we discussed the reasons for finding low nuSSR-based diversity in Eritrean/Ethiopian barley. The second argument is that there is no wild progenitor of barley present in Eritrea and Ethiopia except the two wild barley lines maintained at USDA. However, in our analysis, these two barley lines did not show a distinct marker pattern and no phenotypic differences, besides shattering, were found in comparison with the cultivated barley lines from Eritrea and Ethiopia. Distinguishing the wild form from the cultivated form by a single gene, for example in this case the shattering gene might not be enough (von Bothmer et al. 2003). Here, a back-mutation could have occurred in brittle rachis loci as discussed by Abbo et al. (2001). Nevertheless, the clear separation between the Eritrean/Ethiopian germplasm and the WANA/European germplasm showed that *H. spontaneum*, as it is found today in the Fertile Crescent, could not be the progenitor of Eritrean/Ethiopian barley. We found that the genetic dissimilarity between the Eritrean/Ethiopian barley and the *H. spontaneum* accessions, as well as the *H. vulgare* lines from WANA and Europe, at both the level of cpSSR and nuSSR, indicate an independent domestication of the Eritrean/Ethiopian barley. We found a high diversity at the cpSSR level, resulting in a very distinct and dissimilar population for the Eritrean/Ethiopian barley in the MDS. Finally, we obtained clear indications for a separate group from the sequence information of a chloroplast intergenic spacer. Therefore, we are looking for alternative hypotheses. There might have been an independent domestication of barley in East Africa. The significant cpSSR diversity suggests that the domestication event might have occurred on a very broad base, without forming genetic bottlenecks during domestication, because many different genotypes might have been taken into cultivation by many ancient farmers. During this broad domestication process in East Africa, the wild progenitor might have been superseded in its habitat, and/or it vanished subsequently due to intensive grazing of goats and other domestic animals. Summarizing all our arguments, we conclude that the Horn of Africa, namely Eritrea and Ethiopia, is at least a center of diversification of barley; alternatively we suggest a new independent domestication site of barley in this geographical area.

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