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The distinctness and diversity of Ethiopian barleys

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Abstract The relative diversity and distinctness of Ethiopian barleys has been investigated using (1) morphology/isozyme/hordein polymorphisms and (2) RFLP markers. In the former a set of 51 landraces from over the whole of Ethiopia was compared with Iranian landraces based on data from previous studies and new hordein data. The two sets of landraces were found to have a comparable diversity. The Ethiopian ones are more diverse morphologically (5 traits), are similar in numbers of alleles per protein locus (17 loci) and in genetic differentiation, but are less diverse in average heterozygosity per locus and degree of polymorphism. However, on the basis of the hordein data the two sources of germplasm are very distinct. The correlation between morphological and protein diversity is very low. In the RFLP study 28 probes evenly distributed across the genome were used to analyse 43 Ethiopian landraces (represented by one single genotype) and to compare them with modern cultivars from North America, Europe and Japan, as well as 3 landraces from Iran, 1 from Nepal, and 1 accession of *H. spontaneum* from Afghanistan. The major finding was that the Ethiopian germplasm appears to be significantly less diverse than the modern germplasm but that it is genotypically very distinct. The apparent contradiction between a high diversity of useful genes coming from Ethiopia and an apparently low diversity at the DNA level is discussed.

Key words Genetic resources · Barley · Genetic markers · Landraces

Introduction

It was the pioneering expeditions of Vavilov and Harlan (see review in Qualset 1975) that first brought the diversity of Ethiopian barley germplasm to the attention of the scientific community. Based on the striking morphological diversities that he found, Vavilov suggested that Ethiopia was a centre of origin for barley. However, due to the absence of the wild progenitor, *Hordeum spontaneum*, this hypothesis had to be revised, and most people now consider Ethiopia to be a secondary centre of diversity for this crop. The value of the country's germplasm as a source of unique disease resistance genes, e.g., against barley yellow dwarf virus (BYDV, Qualset 1975) or powdery mildew (*ml-o*, Jørgensen 1993), is well known.

Still, comparisons with barleys from other regions are rather scanty. In their study on morphological variation Tolbert et al. (1979) found that Ethiopian barley landraces (LRs) were not more diverse than barleys from, for example, Eastern Europe. However, Cross (1994) showed a high degree of variation in agromorphological and biochemical traits, and the Ethiopian LRs were shown to be a very distinct group. As shown by Demissie and Bjørnstad (1996a), such comparisons are highly dependent on the traits used in the analysis.

Very few comparisons of protein or DNA polymorphisms have been reported. Zhang et al. (1992) compared the diversity at six isozyme loci in Tibetan and Ethiopian LRs. About equal levels of genetic diversity were detected at the single-locus level. However, the Tibetan diversity was significantly higher in terms of multilocus genotypes. As to DNA polymorphisms, Petersen et al. (1994) included three Ethiopian

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accessions in their diversity study of 26 genotypes from various parts of the world. They found *H. spontaneum* to be the most diverse, Nepalese and Ethiopian the least diverse, with European germplasm in an intermediate position. Obviously, results from different studies are rather divergent and difficult to compare.

In the study presented here we focused on the diversity and distinctness of Ethiopian barley germplasm by considering morphological, biochemical, and DNA (restriction fragment length polymorphisms, RFLP) markers. We compared the relative diversity and distinctness of Ethiopian LRs with respect to (1) Iranian LRs and (2) a wide sample of barleys from the world collection. The work is one of a series of papers on Ethiopian barley LRs (Demissie and Bjørnstad 1996a, b; Demissie et al. in prep; Lakew et al. 1997) in which its diversity is assessed and its conservation and utilization in the domestic crop improvement programmes is promoted.

Materials and methods

Plant materials

The genotypes included in the present investigation are listed in Table 1. For morphological and protein diversity 51 Ethiopian LR populations were used [see Demissie and Bjørnstad (1996a,b) for details]. Each sample consisted of 20 or 15 individuals per population (only 5 for hordeins), making a total sample of 943 genotypes. The 12 Iranian LRs were those used by Brown and Munday (1982). Sample size was 25 individuals per population (only 5 for hordeins), making a total sample of 300 genotypes. For certain comparisons, a random subset of 309 Ethiopian lines was generated.

The RFLP study comprised 43 of the Ethiopian and 3 of the Iranian LRs (see Table 1), one *Hordeum spontaneum* line and 17 modern cultivars. All were represented by one single-seed progeny line. As to modern cultivars, the both parents of two mapping

studies were included ('Steptoe', 'Morex', 'Igri', 'Franka'). The 13 other genotypes were more or less randomly chosen as representatives of their respective geographic regions (grouped as North America, Europe, Japan, West Asia, and Nepal).

Morphological and biochemical data

The data on the Ethiopian and Iranian LRs were obtained from Demissie and Bjørnstad (1996a, Table 2) and Brown and Munday (1982, Table 3), respectively. They were reanalysed here for a comparative purpose.

The morphological characters of row type, rachilla, aleurone colour, lemma colour, and awn roughness were used to calculate the Shannon Weaver diversity index (H') from the formula $H' = \sum -p_i \ln p_i / n$, where p_i is the frequency of class i in each trait and n is the number of traits. The 17 isozyme and hordein loci studied are listed in Table 2. Brown and Munday (1982, Tables 5, 7 and 8) measured diversity at 25 isozyme loci. They did not, however, include the *Hor-1* and *Hor-2* loci. The supplementary data on hordein variation in Iranian LRs were obtained for this study as described by Demissie and Bjørnstad (1996b, which also gives data on isozyme protocols). Samples of Iranian LRs were grown in a greenhouse or analysed by isozymes to check that the morphological and isozyme characterization was comparable in the two studies. The parameters N_a (the number of alleles), P (% polymorphic loci, 95% criterion), H (expected heterozygosity) and G_{ST} (the genetic differentiation between populations) were calculated on a single-locus basis using the programme BIOSYS version 1.7 (Swofford and Selander 1989).

RFLP Analyses

The protocols for DNA extraction, blotting and hybridization used were as described by Kleinhofs et al. (1993). DNAs were digested by either *EcoRI* or *HindIII* enzymes. The probes used (Table 3) were selected to span all of chromosome arms evenly and preferably represent single locus polymorphisms, as inferred from knowledge obtained from known mapping populations, particularly 'Steptoe' \times 'Morex'. The 28 most polymorphic probe \times enzyme combinations were scored for presence/absence of bands using 'Morex' as a reference on each blot. These loci represent 2 loci on each chromosome arm, except for chromosome 4, which is represented by 1 locus on

Table 1 Genotypes analysed by RFLPs in the present study, listed by origin. For details on the Ethiopian accessions, see Demissie and Bjørnstad (1996a); for details on the Iranian accessions, see Brown and Munday (1982). Isozymes/hordeins were analysed only in the

Ethiopian and Iranian accessions. Unless indicated, all genotypes are spring barleys. The AUS numbers refer to accessions in the Australian Winter Cereals Collection

ETHIOPIA: BDI/E accession numbers 1753, 3965, 64210, 219142, 64068, 3235, 64300, 1628, 1837, 3291, 3409, 64077, 64121, 208816, 64097, 3352, 4261, 64193, 4300, 4229, 3267, 3664, 3939, 4522, 64049, 4941, 64166, 3244, 1621, 50, 1622, 1651, 1706, 3334, 1791, 3295, 3304, 3381, 3371, 3232, 3284, 3302, 3479. Hiproly.
Isozymes/hordeins only: 3966, 3267, 225775, 4393, 219910, 223185, 3631, 208733.

WEST-ASIA: Iran: AUS406101 (= "IranLR2": Ilam-Mehran), 406104 (= "IranLR3": Ilam), 406105, 406106 (= "IranLR1": Poledokhtar), 406107, 406110, 406111, 406112, 406113, 406116. RFLPs: only IranLR1-3.
Afghanistan: *Hordeum spontaneum* CI 211041

JAPAN: Amagi Nijo, Haruna Nijo

NEPAL: Himalaya

EUROPE: Germany: Alexis, Igri, Franka (Winter). United Kingdom: Halcyon (Winter). France: Express (Winter). Russia: Winer. Norway: Bode. Sweden: Agneta.

AMERICA: USA: Steptoe, Morex, Glacier, Pirolina. Canada: Harrington, Betzes

Table 2 A comparison of the diversity statistics^a of the protein loci (number of alleles in Ethiopian and Iranian landraces of barley. Ethiopian isozyme data are based on 51 populations/939 individuals (Demissie and Bjørnstad 1996b); the N_a values for Ethiopia in parentheses are based on a random subsample of 309 individuals (16 populations); those from Iran (Brown and Munday 1982, excluding *Hor-1* and *Hor-2*), on 12 populations/300 individuals. The hordein data are from the same samples, with 254/79/46 analysed individuals, respectively

Locus	N_a		H		G_{ST}	
	Ethiopia	Iran	Ethiopia	Iran	Ethiopia	Iran
<i>Est-1</i>	4 (2)	3	0.28	0.44	0.47	0.65
<i>Est-2</i>	4 (3)	3	0.09	0.38	0.58	0.72
<i>Est-4</i>	4 (2)	3	0.26	0.45	0.49	0.73
<i>Est-5</i>	6 (4)	2	0.29	0.01	0.45	0.03
<i>Est-6</i>	1 (1)	2	0.00	0.09	–	0.53
<i>Aco-1</i>	3 (3)	3	0.02	0.32	0.45	0.55
<i>Aco-2</i>	2 (2)	2	0.03	0.01	0.06	0.03
<i>6-Pgd-1</i>	2 (2)	1	0.01	0.00	0.06	0.03
<i>6-Pgd-2</i>	3 (3)	3	0.03	0.21	0.09	0.09
<i>Idh-1</i>	2 (1)	1	0.00	0.00	0.10	–
<i>Idh-2</i>	2 (2)	4	0.05	0.48	0.28	0.33
<i>Aat-1</i>	2 (1)	1	0.01	0.00	0.16	–
<i>Aat-2</i>	2 (2)	3	0.03	0.02	0.15	0.04
<i>Aat-3</i>	2 (2)	–	0.03	–	0.15	–
<i>Acp-3</i>	5 (5)	4	0.38	0.57	0.39	0.34
<i>Hor-1</i>	11 (9)	10	0.37	0.60	0.54	0.34
<i>Hor-2</i>	8 (7)	11	0.41	0.61	0.53	0.33
Mean/locus	3.9 ± 1.4 (3.0 ± 1.1)	3.5 ± 1.3	0.13 ± 0.16	0.26 ± 0.24	0.31 ± 0.19	0.34 ± 0.27
Mean/LR	1.5	1.4	0.10	0.08	–	–

^a N_a , Number of alleles; H, heterozygosity; G_{ST} , genetic differentiation

Table 3 Chromosomes/arms, probe designations and enzyme used, type/function and sources of the RFLP probes used in the study. For details on sources, see Kleinhofs et al. (1993)

Chromosome arm ^a	Designation ^b restriction enzyme	Type/function	Source	Designation/ restriction enzyme	Type/function	Source
1P	MWG851/H	Genomic	A. Graner	KFP127/H	Cold regulated	Cattivelli and Bartels
1M	KFP41/E	Peroxidase1A	S. Rasmussen		Crg 3B	
2P	KFP112/E	Catalase3	R. W. Skadsen	Risø1c10/E	Genomic	H. Giese
	KFP148/H	Aleurone specific	R. Aalen/ O.-A. Olsen	KFP13/H	β -amylase 2	M. Kreis
2M	KFP84/H	Gln 2	B. G. Forde	MWG503/E	Genomic	A. Graner
3P	KFP127/H	Cold regulated	Cattivelli and Bartels	MWG798/H	Genomic	A. Graner
		Crg 3B				
3M	MWG902/H	Genomic	A. Graner	ABC174/E	cDNA	NABGMP
4P	MWG634/E	Genomic	A. Graner			
4M	MWG058/H	Genomic	A. Graner	KFP14/H	β -amylase 1	M. Kreis
	ABG472/H	Genomic	NABGMP			
5P	KFP108/H	Actin	R. W. Skadsen	MWG036/E	Genomic	A. Graner
5M	MWG912/E	Genomic	A. Graner	MWG706/E	Genomic	A. Graner
6P	KFP37a/E	Nitrate reductase	A. Kleinhofs	MWG652a/E	Genomic	A. Graner
	Risø37d7/E	Rrn1	H. Giese			
6M	ABG001/H	genomic	NABGMP	KFP11/E	Amylase 1	J. Rogers
7P	KFP21/E	Dehydrin 2	T. J. Close	Risø37d7/E	Rrn2	H. Giese
7M	KFP002/H	Aleurain	J. Rogers	ABC309/E	cDNA	NABGMP

^a P, Plus arm; M, minus arm (Kleinhofs et al. 1993.)

^b KFP, Known-function probe; E, *EcoRI*; H, *HindIII*

the Plus (or long) arm and 3 on the Minus (or short) arm. Independent scorings were done by two observers, and bands not reliably scored on all blots were deleted. The genotypes 'Golden Promise' and 'TR306' were discarded from the analyses for this reason.

The data were analysed for genetic similarity (\hat{F} in Nei 1987) and genetic distance (\hat{d} in Nei 1987) according to the method described by Nei (1987, eqs 5.53–5.55). The computer programme RESTSITE (Miller 1991) was used for this purpose. Theoretically, genetic distance is measured from the degree of nucleotide substitutions between two "operational taxonomic units" (OTUs, such as genotypes,

regional groups, etc.) since their putative common ancestor. For fragment data this can only be indirectly estimated as the proportion of RFLP fragments [or morphs (m)] that are common to the two OTUs:

$$\text{Genetic similarity} = \hat{F} = 2m(xy)/m(x) + m(y)$$

F is then used to estimate genetic distance, \hat{d} . The \hat{d} estimates between two OTUs were derived as "corrected" values by subtracting the average within-OTUs distances from that between the OTUs (Miller 1991).

The \hat{F} and \hat{d} matrices for individual genotypes as well as regions (Ethiopia, West Asia, East Asia, Europe, North America) were obtained. \hat{F} values within each region were used as a within-region diversity measure. Standard errors for \hat{F} and \hat{d} were obtained by the jack-knife method. Differences in \hat{F} estimates within regions were tested by *t*-tests. Cluster analyses using the UPGMA algorithm was performed using the \hat{d} estimates.

Results

The relative diversity and distinctness of Ethiopian and Iranian LRs (morphology, isozymes, hordeins)

Morphological diversity

Phenotypic diversity (Shannon-Weaver index, H') was found to be slightly higher in Ethiopia ($H' = 0.52$), than in Iran ($H' = 0.45$). There are, however, marked differences in individual characters which confirm their *distinctness*. The Ethiopian germplasm is more diverse in row type characteristics (irregulars missing altogether from Iran) and has a higher frequency of black lemma (0.24 vs. 0.09). On the other hand, Iranian LRs have a higher frequency of smooth awns, whereas rachilla and blue aleurone show only minor differences.

Isozyme and hordein diversity

In Table 2 measures of relative diversity are summarized. As to the number of alleles per locus (N_a) there is no major difference between the two sets of data when all loci are considered. Also, the N_a per LR population is also similar in Ethiopia and Iran. When the Ethiopian data based on 51 populations are considered, the N_a is slightly higher than in Iran. If both samples are of similar size, the Ethiopian LRs are less diverse, and the apparent excesses in *Est-1* to *-4* vanish (Table 2, left). However, individual loci differ markedly. *Idh-2* is clearly more variable in Iran than in Ethiopia, *Est-5* is the other way around. Also, the higher numbers of hordein alleles from Iran in spite of a smaller sample size for these loci may point to a somewhat higher diversity.

The total number of alleles may, however, sometimes be misleading, since in the Ethiopian data many loci contain null alleles. These were mostly "locally rare" types (Marshall and Brown, 1975) that tended to occur once or twice in the entire sample (e.g. *Aco-1*, *6-Pgd-1*, *6-Pgd-2*, *Aat-2*, *Aat-3*). However, in other cases they were locally common in LRs (e.g. *Est-2* and *Aco-2*). The former case is in accord with the findings of Bekele (1983). The frequency of null alleles in Iran is not indicated by Brown and Munday.

The presence of very rare alleles may also explain why the *average heterozygosity per locus*, H , is somewhat lower in Ethiopia (the only major exception is *Est-5*). This is also reflected in the estimates of %

polymorphic loci, P (not in Table 2). While the average P per LR population is closely similar in the two countries (32.0% and 32.1%), the overall P is higher in Iran (74.0% vs. 40.0%). The G_{ST} values are, however, on average similar in the two regions, although they tend to be higher in Iran in loci with high diversity estimates (Table 2). Hordeins are the exception, but this may be due to small sample size.

Thus, although some measures tend to show somewhat more diversity and even more allelic frequencies in Iran, the two sets of LRs tend to show basic similarities in their landrace biology, in spite of the absence of *H. spontaneum* introgression in Ethiopia. This analysis does not, however, reveal the *distinctness* of these two LR sources. The allelic identities at each cannot be directly compared. However, samples from all Iranian populations have been analysed for all isozyme loci, and basically the same alleles occur, albeit at different frequencies (results not shown). The alleles at the *Est-1* to *-5* and *Acp-3* loci tend to be well-known alleles (Linde-Laursen et al. 1987).

Conversely, the *Hor-1* and *Hor-2* loci displayed striking differences. Although similar in diversity (Table 2), only 3 alleles at each locus were common to the Iranian and Ethiopian LRs. At *Hor-1*, the most common allele in both sets was the same as that in the check 'Havila' (frequencies 0.44 and 0.28, respectively). At *Hor-2*, the most common Ethiopian allele ($P = 0.25$) was rare in Iran ($P = 0.02$). Only, 3 alleles at each locus reached frequencies higher than 0.10, the others being rare. One particular LR from Tigray (Acc. no. 223185) was the unique occurrence in Ethiopia of a *Hor-1* allele that appeared widespread in Iran. It is often assumed that barley has been introduced through Yemen to Eritrea and Tigray (Northern Ethiopia), and this piece of data may reflect such a process.

The sample sizes are obviously too small to draw firm conclusions. Overall, however, the hordein data point to Ethiopia and Iran as two genetically very distinct sources of LRs. This conclusion is also supported by the RFLP analyses (below).

The relative RFLP diversity of Ethiopian LRs

Diversity within geographical regions

Table 4 (diagonal) summarizes estimates of genetic similarity (\hat{F}) within the geographical regions. The estimate in Ethiopia is significantly ($P < 0.001$) higher than in Europe and North America, indicating a lower genetic diversity in the Ethiopian germplasm. Obviously, the different sample sizes play a role, but this should be expected to favour the largest sample, the Ethiopian one. The difference between Ethiopia and West Asia is significant, but just significant ($P < 0.05$), which may support the tendency observed in the protein data but not the morphological data. However, the very distinct

Table 4 Genetic similarity (\hat{F} lower half) and genetic distance (\hat{d} , upper half) between barley accessions from different geographical areas. The within-regional \hat{F} estimates are given along the diagonal (bold face)

Origin	Ethiopia	North America	Europe	Japan	Nepal	West Asia
Ethiopia	0.863 \pm 0.030	0.011 \pm 0.003	0.015 \pm 0.004	0.020 \pm 0.005	0.020 \pm 0.005	0.023 \pm 0.005
North America	0.831 \pm 0.042	0.603 \pm 0.041	0.001 \pm 0.001	0.006 \pm 0.002	0.016 \pm 0.004	0.015 \pm 0.004
Europe	0.778 \pm 0.058	0.972 \pm 0.029	0.635 \pm 0.041	0.008 \pm 0.002	0.020 \pm 0.005	0.015 \pm 0.004
Japan	0.707 \pm 0.062	0.897 \pm 0.030	0.877 \pm 0.033	0.825 \pm 0.065	0.023 \pm 0.006	0.016 \pm 0.005
Nepal	0.710 \pm 0.059	0.756 \pm 0.047	0.707 \pm 0.063	0.671 \pm 0.063	—	0.024 \pm 0.006
West Asia	0.677 \pm 0.054	0.781 \pm 0.061	0.780 \pm 0.060	0.763 \pm 0.070	0.668 \pm 0.068	0.693 \pm 0.048

H. spontaneum accession is included in the West Asian group, which may bias the results in favour of West Asia. While the North America versus Japan comparison was also statistically significant, biologically it was not since the two Japanese cultivars are related (Seko 1987) and may not be representative.

Distinctness among geographical regions

Table 4 also gives \hat{F} and \hat{d} estimates between regions. The \hat{d} data are displayed in the cluster diagram of Fig. 1. The two major forks demonstrate the marked distinctness of the West Asian cluster in relation to the other regions, possibly reflecting *H. spontaneum* introgression. The single *H. spontaneum* line is the genotype that has the highest d value in relation to other genotypes. The other major fork is cleaved into one comprising Nepal and Japan (with a relatively large d between them) and one comprising Ethiopia, Europe, and North America. The close similarity between European and North American germplasm is apparent, and the high within-region diversity is reflected in the unusually high SE of the \hat{d} estimate. This probably reflects a common historical origin as well as frequent introgressions by breeders in the two regions.

In Fig. 2a and b two autoradiographs with different patterns of polymorphisms are shown.

Distinctness among individual accessions

The matrix of genetic distances between individual accessions is too unwieldy to be included. However, some features will be briefly mentioned.

It is apparent that many of the Ethiopian accessions seem to be closely related, with shorter average \hat{d} s than those found among, for example, European accessions. The minimum and maximum genetic distances ranged from 0.0006 to 0.0207. (For comparison, the maximum distance observed (between the Swedish 'Agneta' and the *H. spontaneum* line D was 0.051.)

For comparison, the small sample of 3 Iranian LRs spanned the full range of individual accession distances present in the Ethiopian set. They are all from Western Iran, close to Iraq. The IranLR1 (from Poledokhtar) is

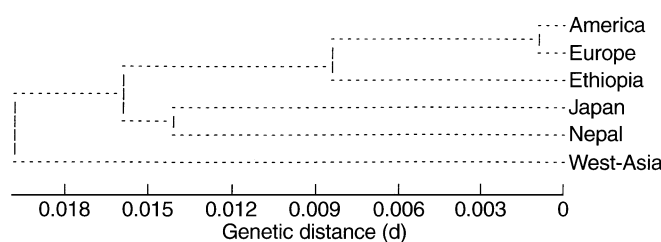


Fig. 1 Dendrogram displaying differentiation in RFLP patterns between the major geographical regions studied. This dendrogram is based on genetic distance (\hat{d}) estimates according to Nei and Li (1979) and the UPGMA clustering procedure in RESTSITE (Miller 1991)

geographically distant from the other 2 that come from the same area (Ilam), and the low genetic distances may reflect this (data not shown).

The "modern" cultivars (North American, European, and Japanese genotypes) present a somewhat confusing pattern. Consistent with the high degree of genetic similarity between American and European germplasm, there was no clear cut grouping of the individual lines. Some subgroups, such as winter barleys, were, however, discernible.

Discussion

A critical premise for using markers to assess genetic diversity is the number of loci studied and their adequacy in representing the whole genome. From this study, we have tried to meet the latter requirement in selecting the RFLP probes (see Table 3). However, for unambiguous fingerprinting the number of probes is probably too low. This can be inferred from studies that try to correlate genetic distance based on neutral markers like RFLPs with pedigree data, measured by the coefficient of coancestry, f . In a RFLP study of European barleys (Graner et al. 1994) the correlation was found to be low, with R^2 values of 0.06 and 0.14 in winter and spring barleys, respectively. Still, in this study, 136 probe \times enzyme combinations were measured (48 single-copy probes \times three enzymes). In another study of European wheats Siedler et al. (1994) found good agreement between RFLP-based genetic

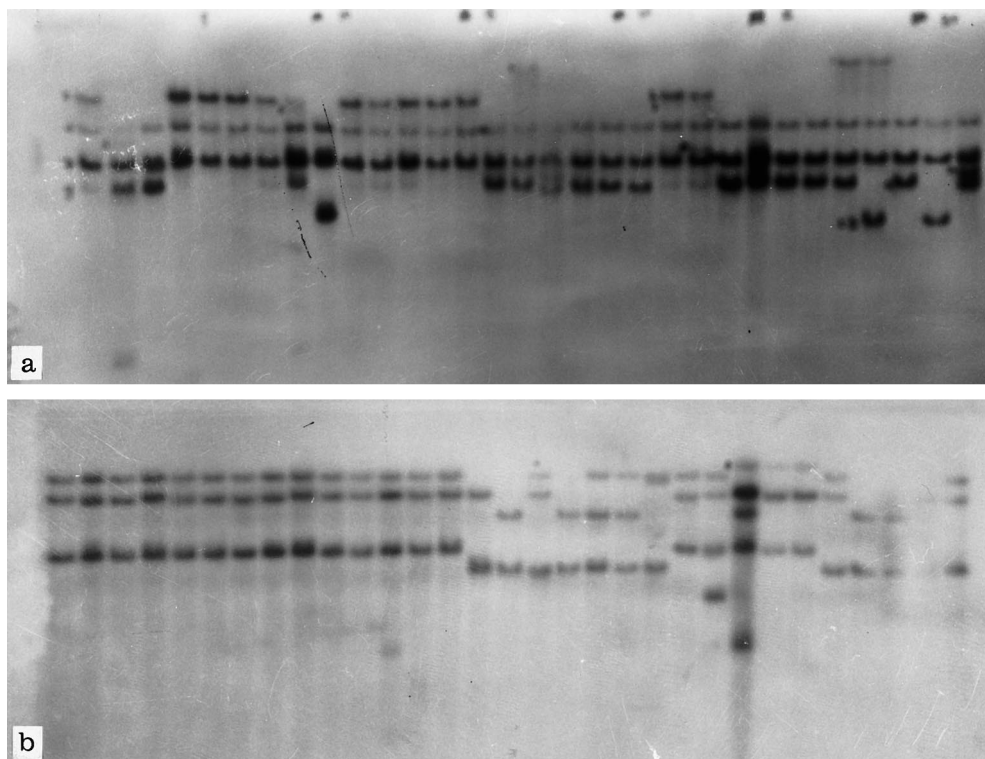


Fig. 2a, b Different patterns of RFLP polymorphisms. **a** The probe MWG036, one of the most variable probes, (chromosome 5 M, situated close to the *Ml-a* locus and between *Hor-1* and *Hor-2*) shows ample polymorphism in Ethiopia as well as in other regions. Variability within and between Ethiopian LRs is evident. **b** For the probe ABG001 (chromosome 6M) the Ethiopian genotypes are invariant, but polymorphism is manifest elsewhere. In only 1 case out of 28 (KFP148, chromosome 2P) was there polymorphism in the Ethiopian set, whereas the other regions seemed to be monomorphic (not shown). The genotypes are, from left to right Lanes 1–4 Acc. 3284 (4 lines), 5–8 Acc. 1621 (4 lines); 9–12 Acc. 1622 (4 lines); Acc. 4941, Acc. 64300; ‘Betzes’, ‘Alexis’, ‘TR306’, ‘Harrington’, ‘Pirolina’, ‘Glacier’, ‘Himalaya’, ‘Hiprolly’, *H. spontaneum* CI211041, IranLR1, IranLR2, IranLR3, ‘Haruna Nijo’, ‘Amagi Nijo’, ‘Halcyon’, ‘Express’, ‘Morex’

distances and coancestry using 58 probes but concluded that this was the minimum number required for distinguishing well between wheat genotypes. In a study of maize inbreds Messmer et al. (1993) recommended a minimum of 100 probe \times enzyme combinations. It appears not unexpected, then, that the 28 probe \times enzyme combinations used by us may be insufficient for distinguishing between individual barley genotypes, be they Ethiopian, European, or American. However, the main purpose of our study—determination of the relative diversity and distinctness of Ethiopian barleys as a group—they appear to be adequate. Genetic similarity and distance estimates are, however, quite sensitive to the number of loci included, their polymorphism, as well as to missing values. If, in addition, genotypes are largely similar, like in the Ethiopian LRs, a low number of probes may bias the results. Thus, the \hat{F}/\hat{d} between individual Ethiopian accessions varies

somewhat with the number of loci included in the analyses (Demissie et al. in prep).

On the other hand, the mean similarity estimate for Europe ($\hat{F} = 0.645$) obtained by us is markedly smaller than those reported by Melchinger et al. (1994). In spring barleys they found a mean similarity of 0.840, in winter barleys 0.853, but a significantly smaller value (0.757) was obtained in the combined group. This may be explained from the fact that Graner et al. (1994) used 48 probe \times three enzyme combinations, without regard to polymorphism, whereas in our study the 28 most polymorphic combinations were chosen. This will tend to increase the differences.

The clear distinctness, but relatively low RFLP variation, in the Ethiopian germplasm confirms the results of Petersen et al. (1994), who used 21 probes (one enzyme only) and 26 barley genotypes. The patterns of distinctness between Nepal, Ethiopia, Europe, and *H. spontaneum* resembles that found by us. Also, they found *H. spontaneum* to be the most diverse, Nepalese and Ethiopian the least, with European germplasm in an intermediate position. Obviously this latter group was overrepresented (13 against 5 Nepalese, 3 Ethiopian, and 5 *H. spontaneum*). It may also be noted that the typical genetic similarity among Ethiopian accessions is of the same magnitude as that between European spring wheats and spelt (Siedler et al. (1994) give a $\hat{F} \approx 0.86$). Similarly, \hat{d} estimates among Ethiopian accessions of the order 0.01 or less are comparable to those found between 13 cultivars from a world collection of *Capsicum annuum* L. (Lefebvre et al. 1993).

There is an apparent paradox, then, between the high isozyme and morphological variation in Ethiopia and the low RFLP variation. This may be explained in two ways:

1) The morphological or isozyme diversity does not reflect a random and chromosomally balanced sample of the genetic variation. The lack of correlation between morphological and protein diversity in Iran and Ethiopia has been noted (Brown and Munday 1982; Demissie and Bjørnstad 1996b). Also, the correlations between morphological, protein, and RFLP diversity in Ethiopian barleys were found to be low (Demissie et al., in prep). Hence a high morphological diversity in Ethiopia (Table 2) need not reflect a higher average genetic diversity at other levels. Also, in *Capsicum annum* L. the RFLP variation was "much lower than that suggested by the observation of morphological variations" (Lefebvre et al. 1993).

2) The probes have been selected on the basis of high polymorphism in European/American mapping studies. Thus, they may bias the diversity estimates in that direction. This is not supported, however, by Zhang et al. (1993), who found similar estimates of N_a and H in *H. spontaneum* when using seven allozyme and 17 RFLP loci derived from the 'Steptoe' × 'Morex' map. The two marker classes, however, differed strongly in population differentiation estimates. Thus, different marker types tend to reflect different areas of the genome as well as a difference between expressed genes and RFLPs.

The Ethiopian germplasm is well known as a source of important genes for barley breeders worldwide. Recently, its utility to Ethiopian barley breeders as the primary source of indigenous barley improvement was demonstrated (Lakew et al. 1996). However, the variation in quantitative characters, morphology, or allozymes may not reflect the overall genetic diversity of Ethiopian barleys. Since its introduction into the country the well-documented evolution of diversity in particular adaptive traits may have occurred at a limited number of loci. In 15 of the LRs included in the present study, data on quantitative characters are available (B. Lakew et al., personal communication). The relationships between adaptive/agronomic traits and RFLPs will be analysed in a later paper.

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