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SHORT PAPER

Chloroplast DNA diversity in wild and cultivated barley: implications for genetic conservation

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SUMMARY

Nine diverse lines of cultivated barley (*Hordeum vulgare*) and 11 lines of its wild progenitor (*H. spontaneum*) were assayed for variation in their chloroplast DNA by digestion with ten restriction endonucleases. The cultivated lines exhibited a single cpDNA polymorphism, whereas the wild material exhibited five. The significantly lower level of diversity among the cultivated lines was unexpected because both cultivated and wild lines had been selected for comparable levels of diversity for nuclear encoded isozyme loci. These results suggest that the level of cytoplasmic diversity was markedly restricted during the domestication of cultivated barley.

1. INTRODUCTION

Plant and animal breeding involves the bringing together of useful genes or gene combinations. The source of useful genes is the pool of genetic variation possessed by agriculturally important plant and animal species and by the wild relatives of these species. This pool of variation is being rapidly eroded by the cultural and technological changes associated with modern civilization. Germplasm conservation programs have the important role of preserving and documenting these vital resourses. Among the practical problems associated with this task are the choice of methods of preservation, the design of optimum sampling programmes and the documentation of the level and kinds of genetic diversity in economically important species.

The methods available for the assessment of genetic diversity have included a variety of biochemical and morphological measurements. Isozymes have provided a particularly important biochemical technique (Brown & Clegg, 1983). This technique permits the assay of genetic variation in many gene products (enzymes). Application of the isozyme technique to the study of genetic diversity in crop plants and their wild relatives has revealed a dramatic reduction in genetic diversity associated with cultivated forms (Rick & Fobes, 1975; Nevo *et al.* 1979; Brown & Munday, 1982). Studies of this kind are important because they validate the contention that a limited sample of extant variation is included in cultivated forms. Despite this, there are two major limitations to the use of the isozyme technique: first, the technique samples only those genes which are expressed as enzymic or structural proteins; secondly, the genetic loci included in isozyme surveys are encoded in the nuclear genome. This second limitation is particularly relevant to plant genetic resources because a number of photosynthetically important genes are chloroplast-encoded and are therefore cytoplasmically transmitted. Moreover, with

respect to the mitochondrial genome, the genetic characteristics of various cytoplasms may be of major economic significance as was the case in the recent corn leaf blight epidemic.

The analysis of plastid genome variation using type II restriction endonucleases has been successfully employed with maize and teosinte materials (Pring & Levings, 1978; Timothy *et al.* 1979; Weissinger *et al.* 1982). This approach has the advantage that it

Table 1. *Source of barley DNAs*

Sample	Working code	CPI no.*	Origin
H. vulgare			
Clipper	C	—	Australian cultivar
Atlas	A	77151	California cultivar
Himalaya	H	94435	Nepal cultivar
Murasaki-mochi	M	53285	Japanese cultivar, Ex USPI/95398†
Land race	L1	77168	Gonbad-e-qabus, Iran
Land race	L2	77169	Gorgan, Iran
Land race	L3	77158	Ilam-Chavar, Iran
Land race	L4	77170	Shiraz, Iran
Land race	L5	77155	Ilam, Iran
H. spontaneum			
Wild	W1	77133	Hermon-9, Israel
Wild	W2	77143	Tabigha-13, Israel
Wild	W3	77129	Atlit-37, Israel
Wild	W4	77129	Atlit-55, Israel
Wild	W5	77144	Talpiyyot-4, Israel
Wild	W6	77144	Talpiyyot-7, Israel
Wild	W7	77137	Mehola-7, Israel
Wild	W8	77135	Wadi Quilt-23, Israel
Wild	W9	77141	Sede Boker-21, Israel
Wild	W10	77154	Andimesk, Iran
Wild	W11	91870	Siroua Djebel, Morocco

All DNAs were prepared from single plant selections. Further descriptions of the Iranian material is given by Brown & Munday (1982), of the Israel *H. sponantaneum* collections by Nevo *et al.* (1979) and Doll & Brown (1979).

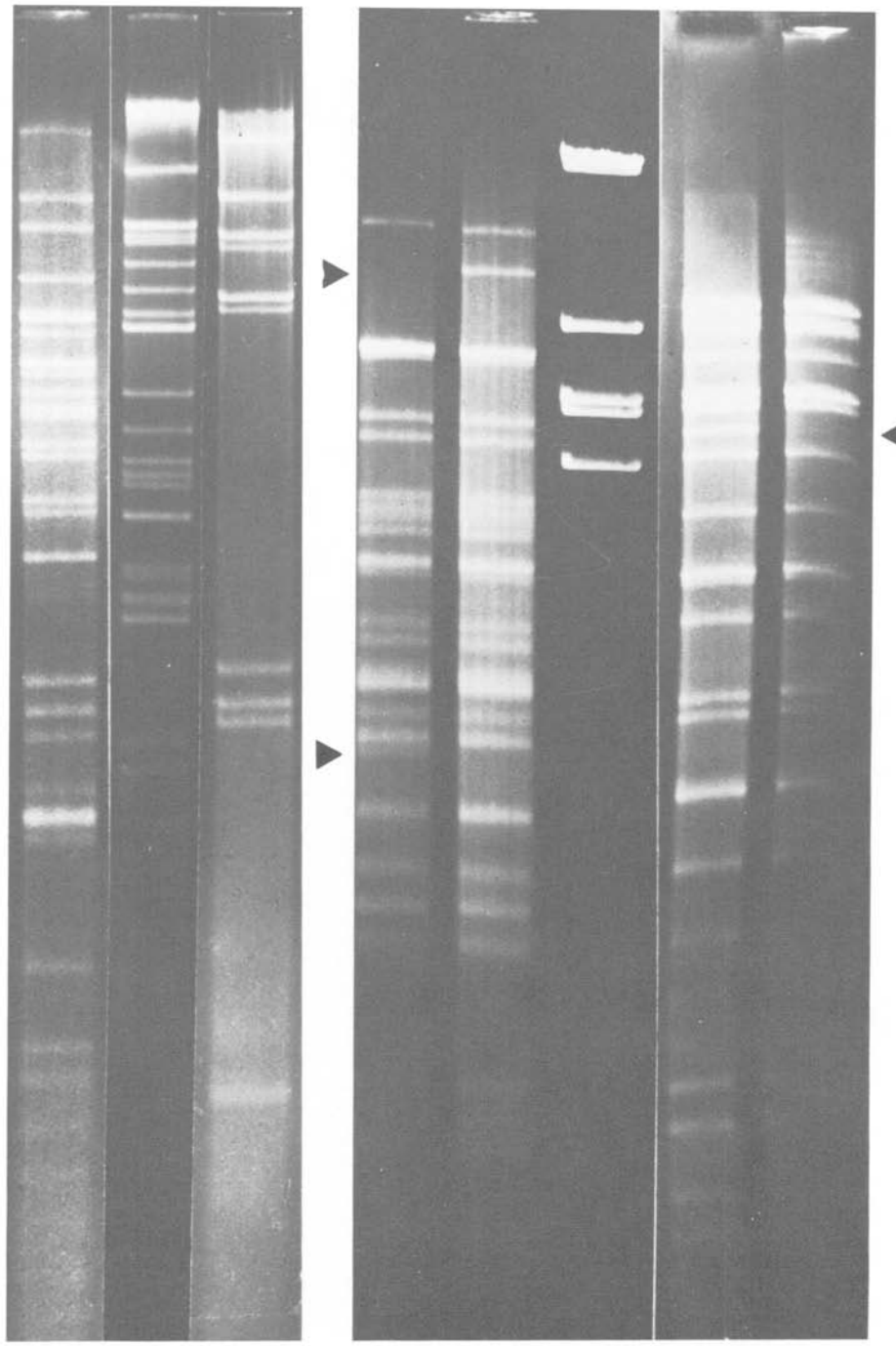
* CPI refers to Commonwealth Plant Introduction.

† USPI is United States Plant Introduction.

provides a direct means to characterize cytoplasmically transmitted genomes, and it detects DNA sequence variants throughout the plastid genome without regard to gene expression. We have undertaken a survey of wild (*Hordeum spontaneum*) and cultivated barleys (*H. vulgare*) to (*a*) evaluate the level and distribution of chloroplast DNA (cpDNA) diversity and (*b*) to compare the distribution of genetic diversity among wild and cultivated materials for cpDNA and nuclear encoded genes (allozymes).

2. MATERIALS AND METHODS

The barley materials selected for study were chosen from several sources to give a diverse coverage of wild and cultivated types (Table 1). The criterion used for selection was to include entries spanning the known isozyme diversity of barley. The sample of *H. vulgare* collections consisted of four cultivars and five primitive land races from Iran. Eleven lines of *H. spontaneum*, including nine from Israel and one each from Iran and Morocco, comprised the sample of wild materials.



Restriction fragments of barley chloroplast DNA. Lane 1: line W9 digested with *Bgl* II; 2, line W9 with *Cla* I; 3, line M with *Hpa* I; 4, 5, lines W9, W3 with *Bcl* I; 7, 8, lines W5, W4 with *Hind* III. Lane 6 is from an *Eco*RI digestion of phage λ DNA into 21·4, 7·4, 5·8, 5·4, 4·7 kb fragments. Arrowheads mark fragment differences for the two examples of variable digests (*Bcl* I, *Hind* III).

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Chloroplasts were prepared by a non-aqueous procedure and the DNA purified by phenol extraction (Bowman & Dyer, 1982). Chloroplast DNAs were digested with each of ten hexanucleotide recognizing restriction endonucleases (listed in Table 2). The digested cpDNA samples (1 μg) were run on 1% agarose slab gels, stained with with ethidium bromide and photographed under UV light. A standard cpDNA was included on every gel for each digest to facilitate the comparison of fragment patterns among samples.

Eighteen enzyme systems representing 36 genetic loci comprise the isozyme data. Twenty of these loci were polymorphic in the collection of 20 barley lines. Assay conditions are as previously described (Brown & Munday, 1982).

Table 2. *Distribution of cultivars and land-races of* Hordeum vulgare, *and of wild* H. spontaneum *lines (W's) among chloroplast DNA types*

cpDNA type	*BamH*I	*Bcl* I	*Eco*RI	*Hind* III	Lines
A	—	—	—	—	C, A, H, M, L3, L4, L5, W2 W10, W11
B	I	—	—	—	L1, L2
C	I	I	—	—	W4, W7, W8, W9
D	I	—	I	I	W1, W5, W6
E	I	—	I	I+II	W3

The '—' denotes the standard pattern shown by cpDNA type A. The symbol 'I' indicates the first subset of altered fragments observed for a digest. Similarly 'II' denotes the second subset of altered fragments observed for a particular enzyme. As noted in the text, all variant patterns except for those produced by *Hind* III are consistent with the loss or gain of a single site. Restriction endonucleases yielding invariant fragmentation patterns across digests were *Bgl* II, *Bst* EII, *Cla* I, *Hpa* I, *Pvu* II and *Xba* I. Enzymes were purchased from Biolab and Boehringer Mannheim and used as recommended by the supplier. Slab gels were run in 40 mM Tris acetate, 20 mM sodium acetate, 2 mM EDTA (pH 7·5) with 0·5 mg ethidium bromide/ml for 18 h at 35 V, 20 mA.

3. RESULTS AND DISCUSSION

Five different cpDNA fragment patterns were resolved. Differences among barley lines were observed upon digestion with *BamH*I, *Bcl* I, *Eco*RI, and *Hind* III. Each of the remaining six restriction endonucleases produced a single fragmentation pattern for all barley lines. Table 2 summarizes the interline comparisons for the subset of variable digests. Text-fig. 1 shows some of the cpDNA fragment patterns observed, for both uniform and variable digests. The average number of fragments observed per digest was 25, although the *Hpa* I, *Pvu* II and *BstE* II digests formed a distinct subset averaging only about ten fragments (Plate 1). Differences among the lines for the variable digests could be explained by the gain or loss of a single site except for the *Hind* III digests, for which one wild barley line (W3) differed by at least two events from all the cultivated and seven of the wild lines. In general, the barley DNAs are quite similar, in agreement with other studies of cpDNA variation (Pring & Levings, 1978; Timothy *et al.* 1979; Palmer & Zamir, 1982; Clegg, Rawson & Thomas, 1984). It is, however, noteworthy that plants W3 (type E) and W4 (type C) come from the same population yet differ for at least four sites, indicating that marked levels of cpDNA polymorphism can exist within single populations.

From Table 2 it can also be seen that there are just two cpDNA patterns among cultivated barleys; there is a single *BamH*I fragment difference separating to land-race or primitive barleys from the remaining seven cultivated barleys. These patterns will be denoted B and A, respectively. It is interesting to note that three wild barleys also exhibit

pattern A. The remaining eight wild barleys show three distinct fragment patterns, denoted C, D and E.

To test whether the fragment pattern distributions differ statistically among wild and cultivated types, we take as a null hypothesis that the cpDNA differences are independent of the origin of the samples (i.e. independent of whether the samples were wild or cultivated). First, consider the *Bcl* I distribution. If cpDNA is independent of species origin, the probability that all four 'rare' variant lines are *H. spontaneum* is

Table 3. *Analysis of variation among lines of barley for chloroplast DNA and for allozymes*

	cpDNA (per variable site)		Allozymes (per variable locus)	
	Diversity*	No. of differences†	Diversity	No. of alleles
Within *H. vulgare*	0·08	1·20	0·28	1·90
Within *H. spontaneum*	0·43	2·00	0·33	2·50
Between H.s. and H.v.	0·39	0·80	0·03	1·60
Total *Hordeum*	0·33	2·00	0·33	3·00

* Diversity is computed as the average probability per variable site (or isozyme locus) that two lines will differ.

† Calculated as the average number of site differences per variable site, in analogy to the average number of alleles per locus. Thus within *H. vulgare*, two site differences are observed at Bam HI and one for each of the remaining enzymes. There are thus $\frac{6}{5} = 1{\cdot}2$ site differences.

$(11 \times 10 \times 9 \times 8)/(20 \times 19 \times 18 \times 17) = 0{\cdot}068$. The same figure applies for the *Eco* RI and one of the *Hind* III variant patterns. The probability for the other *Hind* III variant is 0·55. Therefore, the probability that variation at these four sites is confined to *H. spontaneum* under the null hypothesis is $< 0{\cdot}0002$. If the *BamHI* result is included as a polymorphism shared between the two species, and the question modified to asking what is the probability under independence of at least four confined polymorphisms, the result is still $< 0{\cdot}0002$. We therefore conclude that the two distributions differ and that the wild materials are more diverse than the cultivated barleys.

The relationship among fragment patterns can be inferred by assuming each fragment difference represents at least one event (Avise *et al.* 1979). Thus, for example, type A differs from B by one event while A differs from D by three events. Note that the common cultivated pattern (A) still exists among wild barleys and in fact is geographically widespread, because the three wild lines with pattern A originated from Iran, Israel and Morocco. On the other hand, it is also apparent that the cultivated barleys represent a very limited sample of the cpDNA diversity extant in the wild materials.

The sample of cultivated barleys was selected to include the full range of isozyme diversity found in cultivated materials. Table 3 summarizes the allozyme and cpDNA diversity within and among the cultivated and wild materials. If allozyme diversity and cpDNA diversity are correlated, then the selection of a sample of high nuclear gene (allozyme) diversity should yield a sample of high cpDNA diversity. Such a correlation would be desirable in the documentation of genetic resources because a single class of variation could serve as a useful indicator for other classes of variation. From Table 3 it is evident that cpDNA diversity is poorly correlated with nuclear encoded allozyme diversity. The level of cpDNA diversity within the cultivated barleys is sixfold less than within the wild barleys, despite approximately equal levels of allozyme diversity.

The present data suggest that the process of domestication has greatly restricted the level of chloroplast DNA diversity in barley. There are moderate levels of cpDNA

diversity in the wild progenitor of cultivated barley, and in at least one case, cpDNA polymorphism within populations. Nevertheless, the cultivated barleys are depauperate in cpDNA variation and probably trace from a restricted base of cytoplasm types.

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