THE GENETIC ANALYSIS OF BARLEY STORAGE PROTEINS

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SUMMARY

Hordein polypeptide patterns in barley seeds are known to be controlled by structural genes at 2 loci, *Hor-1* and *Hor-2*, on chromosome 5. Two-dimensional and high resolution one-dimensional electrophoretic analyses of seeds of F_2 and doubled haploid progenies of four intervarietal crosses gave no evidence of recombination within these loci. Genetic analysis of the progenies showed that *Hor-1* is 0.161 ± 0.026 centimorgans from *Hor-2* and the mildew resistance locus, *Mla*, lies between them, 0.064 ± 0.020 centimorgans from *Hor-1* and 0.082 ± 0.024 centimorgans from *Hor-2*.

1. INTRODUCTION

THE storage protein fraction of barley (hordein) accounts for up to 50 per cent of the total grain N. When separated on the basis of molecular weight by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) the polypeptides can be classified into three groups "A", "B", and "C" (Koie, Ingversen, Andersen, Doll and Eggum, 1976; Miflin and Shewry, 1977a) of which only the "B" and "C" are thought to be true storage proteins (Miflin and Shewry, 1977b). Further separation into around 10-20 polypeptides (depending upon variety) can be achieved using two-dimensional (2-D) methods combining isoelectric focusing (IEF) in the first dimension with SDS-PAGE in the second (Shewry, Pratt, Faulks, Parmar and Miflin, 1979; Shewry, Ellis, Pratt and Miflin, 1978a).

The "C" hordeins differ from the "B" in having a greater content of glutamine, proline and phenylalanine and less lysine and sulphur amino acids, which results in differences in solubility and extraction behaviour (Shewry, Field, Kirkman, Faulks and Miflin, 1980; Miflin and Shewry, 1979). Oram, Doll and Koie (1975) showed that the "B" hordein pattern was determined by co-dominant alleles at a locus (later called *Hor-2*) linked to *Mla* on chromosome 5. It has since been shown that the "C" pattern is controlled by a second locus (called *Hor-1*) which is linked to *Hor-2* although the linkage of the *Hor-1* locus to *Mla* was not reported (Shewry, Pratt, Finch and Miflin, 1978c; Doll and Brown, 1979). Recently, Sozinov, Netsvetaev, Grigoryan and Obraztsov (1978) have reported that the hordein pattern in var. Steporoy is controlled by five linked loci (termed *Hrd A-E*) of which *Hrd A* and *Hrd B* appear to correspond to *Hor-1* and *Hor-2* respectively. The nature of the proteins coded by *Hrd C*, *D* and *E* is uncertain but they are present at low concentrations in the "C" hordein region of starch gels. These five loci, however, are based on the frequencies

of phenotypic classes in a homozygous F_{15} population and although the *Hrd A* and *Hrd B* loci have been confirmed by studying F_2 frequencies (Sozinov *et al.*, 1978) and crosses involving translocations (Netsvetaev, 1978), *Hrd C*, *D* and *E* remain to be confirmed.

The previous studies have failed to show recombination within either locus, although peptide mapping (Mifflin and Shewry, 1979; Mifflin, Matthews, Burgess, Faulks and Shewry, 1979) suggests that the individual polypeptides within the "B" group are the products of separate genes. This failure may have been due to the use of one-dimensional (1-D) analytical systems in which co-migration of polypeptides occurs (Shewry *et al.*, 1978a, 1979). We decided, therefore, to make a detailed study of the genetic control of hordein using 2-D and high-resolution 1-D analyses. We have used both F_2 material and, to eliminate the problems posed by scoring heterozygous triploid endosperms, doubled monoploid lines produced by the *Hordeum bulbosum* technique. Varieties with mildew resistance genes of the *Mla* complex on chromosome 5 were used so as to determine the position of this locus relative to both *Hor 1* and *Hor 2*.

2. MATERIALS AND METHODS

(i) Material

Table 1 shows the parental lines, their hordein patterns and their mildew resistance alleles. Previously known hordein patterns are presumed to be governed by single alleles at *Hor-1* (C-bands) and *Hor 2* (B-bands) named after the standard varieties containing them, *viz.* Proctor (Pr), Aramir (Ar),

TABLE 1

Parental lines and their hordein patterns and mildew resistance alleles. All loci are on chromosome 5 except Mlg on chromosome 4 and Mlv whose location is unknown

Line	Hordein pattern		Mildew resistance alleles		
	C-bands (<i>Hor-1</i>)	B-bands (<i>Hor-2</i>)	<i>Mla</i>	<i>MLv</i>	<i>Mlg</i>
Sultan	Pr	Pr			
Nigrinudum	Ni	Ni			
Hassan	Pr	Ar			
HJ69/41/224	Ar	Ze			
Goldspear	Ju	Ju	<i>Mla6</i>	<i>MLv</i>	
Athos	Ar	Ar	<i>Mlas</i>		<i>Mlg</i>
Porthos	Ar	Ar	<i>Mlas</i>		<i>Mlg</i>
Lud	Pr	Ze		<i>MLv</i>	<i>Mlg</i>

Zephyr (Ze) and Jupiter (Ju) (Shewry, Pratt and Mifflin, 1978b). Nigrinudum has a new pattern and its presumed *Hor-1* and *Hor-2* alleles are designated Ni. This variety is the only one used which has the dominant allele at the black lemma locus (*B*) on chromosome 5. Mildew resistance genes are named as in Wolfe and Schwarzbach (1978). For genetic analysis, F_2 seeds were used from cross 1 (Nigrinudum $\times$ Sultan) but doubled haploid lines were obtained from F_1 plants of crosses 2 (Hassan $\times$ HJ69/41/224), 3 (Goldspear $\times$ Athos) and 4 (Porthos $\times$ Lud) by methods similar to those described by Kasha and Kao (1970) and by Jensen (1976). The methods involved pollinating the intraspecific F_1 plants with *Hordeum bulbosum* pollen,

culturing the interspecific hybrid embryos so that the *H. bulbosum* genome was eliminated and diploidising the resultant barley haploids with colchicine. Such doubled haploids are homozygous and genetically equivalent to an unbiased sample (Kasha and Reinbergs, 1976) of diploidised eggs from the intraspecific F_1 . Doubled haploid lines were multiplied in the glasshouse and field. In cross 4, only lines resistant to mildew in a field trial were used for genetic analysis.

(ii) *Hordein pattern analysis*

Hordein fractions were extracted from half seed of cross 1 as described previously (Shewry *et al.*, 1978*b*). They were dissolved in 50 μ l of 8 M urea and 1 per cent (v/v) 2-mercaptoethanol, alkylated with 4-vinylpyridine (1.5 per cent v/v) and 25 μ l aliquots used for 2-D analysis. The remaining 25 μ l was dialysed against 1 per cent (v/v) SDS for 1-D analysis.

Five seeds of the Hassan $\times$ HJ69/41/224, Porthos $\times$ Lud and Goldspear $\times$ Athos lines were extracted by crushing with a hammer and stirring for 1 hour at 60° with 10 ml of 50 per cent (v/v) propan-1-ol 2 per cent 2-mercaptoethanol (Shewry *et al.*, 1978*a*). After centrifugation, the tissue was re-extracted by suspending in a sonic bath for 30 min with 5 ml of the same solvent. Hordein was precipitated from the bulked supernatants by the addition of 2 vols 0.75 M NaCl and standing overnight at 4°C. It was then reduced and either pyridylethylated or cyanoethylated (Shewry *et al.*, 1978*a*).

The fractions were separated by 1-D SDS-PAGE in 17.5 per cent acrylamide gels at pH 8.9, IEF in 5 per cent polyacrylamide in the pH range 5-9 or a 2-D procedure combining both systems (Shewry *et al.*, 1978*a*).

Cross 1 was analysed by 1-D SDS-PAGE and 2-D analysis (figs. 1 and 2). The "B" hordein was more completely resolved by the 2-D system (fig. 2) and was scored from these gels. Because of the diffuseness and poor staining reaction of the "C" polypeptides on the 2-D gels, this group was also scored from the 1-D separations (fig. 1). Four classes each of "B" and "C" patterns could be recognised in the F_2 progeny. These were the two homozygous parental patterns (figs. 1(*a*), (*b*); 2(*a*), (*b*)) and two types of heterozygote in which a weak pattern from one parent co-existed with a stronger pattern from the other (Figs. 1(*c*), (*d*); 2(*c*), (*d*)). These were interpreted as due to differences in the dosage of alleles from the male and female gametes.

Examination of the gels indicated that there was no recombination within either *Hor* locus. However, it was difficult to score for gene dosage in the heterozygotes, especially in the 2-D gels. In crosses 2-4, therefore, several seeds were used from each doubled haploid line for 1-D SDS-PAGE analysis and, in crosses 2 and 3, for 2-D analysis (fig. 3).

(iii) *Mildew tests*

In crosses 3 and 4, reactions of each doubled haploid line to isolates of powdery mildew (*Erysiphe graminis* DC f.sp. *hordei* Marchal) were scored on several seedlings in tests carried out in spore-proof conditions. Isolates AB3 (*V-a6*, *V-as*, *V-g*), AB14 (*V-a6*, *V-v*, *V-g*), CC1 (*V-as*) and CC15 (*V-a6*, *V-v*) were inoculated singly onto different subsamples of each line in cross 3 but only AB3 and AB14 were used in cross 4.

Seedlings were scored as either resistant or susceptible based on the mildew recording scale 0-4 used by Jones and Pickering (1978). Plants with reaction type 0-2 were classified as resistant and with 3 or 4 as susceptible.

(iv) *Genetic analysis*

When linkage between two loci was detected, it was estimated by methods described by Bailey (1961). In populations of n plants where a and d were the frequencies of parental phenotypes and b and c the frequencies of recombinant phenotypes, the recombination fraction, p was calculated as $(b+c)/n$, or when allelic ratios at both loci differed significantly from 1 : 1, as $\sqrt{bc}/(\sqrt{bc} + \sqrt{ad})$. The standard error of p was calculated as $\sqrt{((p-p^2)/n)}$ or as $1/2(p-p^2)\sqrt{(1/b+1/c+1/a+1/d)}$, respectively. Map distances were calculated as $-1/2 \log_n(1-2p)$ with standard error (s.e. of $p)/(1-2p)$.

3. RESULTS

Table 2 gives phenotype frequencies in progenies of crosses 1-4. There was no sign of recombination within a *C*-band pattern or a *B*-band pattern and so *Hor-1* and *Hor-2* were treated as simple loci with multiple alleles.

TABLE 2
Frequencies in progeny of crosses 1-4 of phenotypes governed by 8 pairwise combinations of the loci, Hor-1, Hor-2, Mla, Mlv and B.

Governing locus 1	Governing locus 2	Cross	Phenotype frequency			
			Parental types: maternal allele expressed at		Recombinant types: maternal allele expressed at	
			both loci	neither loci	locus 1	locus 2
<i>Hor-1</i>	<i>Hor-2</i>	1. (♀)	28	30	7	3
		1. (♂)	30	30	3	5
		2.	11	12	2	3
		3.	45	33	9	2
		4.	53	27	4	10
	<i>Mla</i>	3.	51	34	3	1
		4.	56	31	1	6
	<i>MLv</i>	3.	30	13	24	22
		4.	12	30	45	7
	<i>B</i>	1. (♀)	13	9	6	16
		1. (♂)	16	9	6	13
<i>Hor-2</i>	<i>Mla</i>	3.	46	36	1	6
		4.	59	28	4	3
	<i>MLv</i>	3.	26	16	21	26
		4.	13	25	50	6
	<i>B</i>	1. (♀)	15	7	9	18
		1. (♂)	18	9	7	15
	<i>Mla</i>	3.	28	13	24	24
		4.	14	27	48	5

Plate I

FIG. 1.—SDS-PAGE of pyridylethylated hordeins from F_2 half-seeds of cross 1 ((a)-(d)) and single doubled haploid seeds of cross 4 ((e)-(i)). B-band patterns are not scorable in (a)-(d). (a) *Hor-1* Ni,Ni,Ni; (b) *Hor-1* Pr,Pr,Pr; (c) *Hor-1* Ni,Ni,Pr; (d) Ni,Pr,Pr; (e), (f), (h) *Hor-1* Ar,Ar,Ar/*Hor-2* Ar,Ar,Ar; (g) *Hor-1* Pr,Pr,Pr/*Hor-2* Ar,Ar,Ar; (i) *Hor-1* Pr,Pr,Pr/*Hor-2* Ze,Ze,Ze.

Plate II

FIG. 2.—Two-dimensional analysis of pyridylethylated hordeins from F_2 half-seeds of cross 1. *Hor-1* alleles are not scorable; *Hor-2* alleles are (a) Pr,Pr,Pr; (b) Ni,Ni,Ni; (c) Ni,Pr,Pr; (d) Ni,Ni,Pr.

FIG. 3.—Two-dimensional analysis of cyanoethylated hordeins from milled grains of parents and recombinant doubled monoloids of crosses 2 ((a)-(c)) and 3 ((d)-(f)). (a) HJ69/41/224 (*Hor-1* Ar,Ar,Ar/*Hor-2* Ze,Ze,Ze); (b) recombinant (*Hor-1* Pr,Pr,Pr/*Hor-2* Ze,Ze,Ze); (c) Hassan (*Hor-1* Pr,Pr,Pr/*Hor-2* Ar,Ar,Ar); (d) Athos (*Hor-1* Ar,Ar,Ar/*Hor-2* Ar,Ar,Ar); (e) recombinant (*Hor-1* Ju,Ju,Ju/*Hor-2* Ar,Ar,Ar); (f) Goldspear (*Hor-1* u,Ju,Ju/*Hor-2* Ju,Ju,Ju).

Most alleles segregated in the expected Mendelian ratios except mildew resistance alleles in cross 4 where selection for resistance had been practised. Other cases where allelic ratios deviated from those expected were still included in linkage estimates since the linkage data were homogenous. *Hor-1*, *Hor-2* and *Mla* were linked with one another but *B* and *Mlv* showed no linkage. Table 3 gives recombination fractions and map distance estimates between the linked loci, *Hor-1*, *Hor-2* and *Mla*. Pooled data showed that *Mla* lies between *Hor-1* and *Hor-2* at distances of 0.064 ± 0.020 cM and 0.082 ± 0.024 cM from each, respectively. The sum of these separate estimates (0.146) agrees well with the pooled estimate (0.161 ± 0.026) of the largest distance, *Hor-1* to *Hor-2*. In cross 4 genes for yellow rust resistance

TABLE 3

Recombination fractions and map distances between Hor-1, Hor-2 and Mla in crosses 1-4.

Locus 1	Locus 2	Cross	Recombination fraction	Map distance in cM
<i>Hor-1</i>	<i>Hor-2</i>	1. (♀)*	0.147 ± 0.043	0.174 ± 0.061
		1. (♂)	0.118 ± 0.039	0.134 ± 0.051
		1. (♀ + ♂)	0.132 ± 0.029	0.154 ± 0.040
		2.	0.179 ± 0.072	0.222 ± 0.112
		3.	0.124 ± 0.035	0.142 ± 0.046
		4.	0.149 ± 0.037	0.177 ± 0.052
		1. + 2. + 3. + 4.	0.138 ± 0.019	0.161 ± 0.026
	<i>Mla</i>	3.	0.045 ± 0.022	0.047 ± 0.024
		4.	0.075 ± 0.027	0.081 ± 0.032
		3. + 4.	0.060 ± 0.018	0.064 ± 0.020
<i>Hor-2</i>	<i>Mla</i>	3.	0.079 ± 0.029	0.086 ± 0.034
		4.	0.079 ± 0.029	0.086 ± 0.034
		3. + 4.	0.076 ± 0.020	0.082 ± 0.024

* The ♀ and ♂ refer to the gametic genotypes deduced as previously (Shewry *et al.*, 1978c).

were also present, the *Yr4* gene, which is located on chromosome 5, in Lud and an unlocated gene derived from Arabische in Porthos. It was not possible to distinguish between the presence of these two genes in many of the lines from this cross. However, of the 28 lines thought to have the *Yr4* gene 22 were *Hor 1 Pr*, *Hor 2 Ze*, four were *Hor 1 Pr*, *Hor 2 Ar* and one each were *Hor 1 Ar*, *Hor 2 Ze* and *Hor 1 Ar*, *Hor 2 Ar*. This indicates that the *Hor 1* locus is located more closely to the *Yr 4* locus than the *Hor 2* locus.

4. DISCUSSION

The study confirms the previously reported linkage between the *Hor 1* and *Hor 2* loci. Our pooled estimate of 0.138 ± 0.019 for the recombination fraction agrees well with the estimates of Shewry *et al.* (1978c), Doll and Brown (1979) and Netsvetaev (1978), but is about twice that of Sozinov *et al.* (1978). We found no evidence for additional hordein loci as described by Sozinov *et al.* (1978). Although we have examined the equivalent of a total of 347 gametes (253 by 2-D analysis), we have found no evidence of recombination within either the *Hor 1* or *Hor 2* loci. However, with this number of analyses it is unlikely (5 per cent probability) that recombination

occurring within a map distance of about 0.010 cM would have been detected. These results are thus not inconsistent with previous proposals that these are complex loci.

The mildew resistance data show that *Hor 1* and *Hor 2* are located on either side of the *Mla* locus on chromosome 5. The results from cross 4 with the *Yr4* gene tentatively suggest that *Hor 1* is on the centromere side of *Mla*. Recent work by J. Jensen and colleagues (pers. commun.) using *Mla* and *MLK* provides much stronger evidence for this conclusion.

The close linkage of *Hor 1* and *Hor 2* with *Mla* (and *MLK* Jensen pers. commun.) results in the association of certain types of mildew resistance with specific hordein alleles. In some cases the hordein alleles are derived from exotic varieties which have been introduced into breeding programmes as a source of disease resistance. For example, the *Hor 1 Ar* alleles are associated with the *Mlas* gene and are derived from the exotic variety Arabische. Of 15 varieties which have the *Mlas* gene, 11 were found to have the *Hor 1 Ar* and *Hor 2 Ar* alleles while two more had the *Hor 2 Ar* allele associated with the *Hor 1 Pr* allele (Shewry et al., 1979).

The linkage with *Mla* also means that hordein analysis can be used as an initial screen for disease resistance and vice versa in breeding programmes. The analyses can be made on half of either the F_1 or F_2 seed, and the embryo half retained to be germinated. Because the hordein alleles are readily scored in the seed, they are also of potential value as markers for the fine structural mapping of chromosome 5.

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