Theor. Appl. Genet. 58, 27-31 (1980)

Linkage of the Hordein Loci *Hor1* and *Hor2* with the Powdery Mildew Resistance Loci *Ml-k* and *Ml-a* on Barley Chromosome 5

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Summary. The linkage relationship among the loci *Hor1*, *Hor2*, *Ml-k* and *Ml-a* on the short arm of chromosome 5 was studied by progeny testing the F_2 generation of two crosses. The loci *Hor1* and *Hor2* code for polypeptides of the storage protein hordein (prolamin) and the loci *Ml-k* and *Ml-a* determine the resistance reaction with some powdery mildew fungi cultures. The order of the loci is *Ml-k*, *Hor1*, *Ml-a*, and *Hor2*, the first named being nearest the centromere. The recombination percentage between *Hor1* and *Hor2* was determined in the F_1 and F_2 generations in both crosses, the combined estimate being 7.4 ± 0.9 per cent. The recombination percentage estimated between *Ml-k* and *Hor1* was 4.0 ± 1.3, between *Hor1* and *Ml-a*, 5.3 ± 1.1, and between *Ml-a* and *Hor2*, 6.1 ± 1.2. The estimates involving the *Ml-* loci were all probably a little too high.

Key words: Genetics – Loci – Powdery mildew – Prolamin – Recombination

Introduction

Nearly half of barley seed protein is the storage protein, hordein (Shewry et al. 1978a), with a low lysine content. Hordein production can be suppressed by a number of so-called high-lysine genes (Doll 1977). The discovery of the high-lysine genes has initiated studies on the chemistry, synthesis, and genetics of hordein (Brandt 1976; Doll 1977; Doll and Brown 1979; Holder and Ingversen 1978; Ingversen 1975; Kφie et al. 1976; Miflin and Shewry 1979; Shewry et al. 1977, 1978a, 1978b). Two chemically different groups of hordein, hordein-1 and hordein-2, previously designated C and B hordein, have been characterized (Kφie et al. 1976). Two probably complex loci designated *Hor1* and *Hor2* control the electrophoretic banding pattern of hordein-1 and hordein-2, respectively (Shewry et al. 1978b; Doll and Brown 1979).

The *Hor1* locus is apparently the same as *Pr-a* which was reported to be linked to a powdery mildew resistance gene at the *Ml-a* locus on chromosome 5 (Solari and Favret 1971) at a recombination percentage of 3.3. A powdery mildew resistance gene in the variety 'Sultan', (Ml-a(Ar)) (Torp et al. 1978), belonging to the *Ml-a* locus, was found to be linked to the *Hor2* locus at a 17.4 ± 2.8 recombination percentage (Oram et al. 1975). The recombination percentages between the *Hor1* and the *Hor2* loci were estimated to be 12.98 ± 2.94 and 4.58 ± 1.83, respectively, in male and female gametes by Shewry et al. (1978b), and to be 11 ± 2 by Doll and Brown (1979).

In 1978 Sozinov et al. (1979) reported five hordein loci *HrdA*, *–B*, *–C*, *–D*, and *–E*. Of these, *HrdA* and *HrdB* apparently correspond to *Hor1* and *Hor2*, respectively. They estimated the recombination percentages between *HrdA* and *HrdB* to be 6.87 ± 0.74 and 6.34 ± 0.90, respectively, in two materials. The order of the five loci was *HrdA*, *–B*, *–C*, *–D*, *–E*. The *HrdE* had only a 2.97 ± 0.45 per cent recombination with *HrdB*.

Netsvetaev (1978) found that the recombination percentages between the loci *HrdA* and *HrdB* were 13.44 ± 1.11 and 13.93 ± 1.13 in crosses involving the translocations T1-5f and T2-5a, respectively, and that the *Hrd* loci were on the short arm of chromosome 5 with *HrdA* nearest the centromere.

The present study elucidates the order and relative distances between the hordein loci *Hor1* and *Hor2* and the powdery mildew resistance loci *Ml-a* and *Ml-k* on chromosome 5.

Material and Methods

A survey of the three parents used ('Iso1R', 'Iso4R' and 'Iso20R') is given in Table 1. They originate from crossing, respectively, the

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Table 1. The barley parent lines, the hordein and powdery mildew resistance genes studied, and the infection type produced by the two cultures of the powdery mildew fungus used

Designation of parent lines		Powdery mildew alleles		Infection type with powdery mildew culture		Hordein alleles	
Short name	CI nr.	Locus *Ml-a*	Locus *Ml-k*	201176-7	A6(290)	Locus *Hor1*	Locus *Hor2*
'Iso1R'	16137	*Ml-a*	*ml-k*	0	4	*Hor1Al*	*Hor2La*
'Iso4R'	16143	*ml-a*	*Ml-k*	4	1N	*Hor1Kw*	*Hor2Wk*
'Iso20R'	16151	*Ml-a6*	*ml-k*	4	0	*Hor1Fr*	*Hor2Rf*

varieties 'Algerian', 'Kwan', and 'Franger' with the variety 'Manchuria'. These crosses were then backcrossed three times to 'Manchuria' followed by selfing of plants heterozygous for, respectively, the powdery mildew resistance genes *Ml-a, Ml-k* and *Ml-a6* in 12-15 generations (Moseman 1972). Since then, plants homozygous for the powdery mildew resistance genes have been multiplied a number of generations by selfing. The three parents thus contain about 95% 'Manchuria' gene background and are quite homozygous. The powdery mildew resistance genes originate from the non-recurrent parents. 'Iso20R' had the *Hor* alleles from the non-recurrent parent. 'Iso1R' had the *Hor2* allele from the non-recurrent parent, whereas its hordein-1 pattern was different from that present in our seed samples of 'Algerian' and 'Manchuria'. The parents of 'Iso4R', 'Manchuria' and 'Kwan' have a similar electrophoretic banding pattern in both hordein-1 and hordein-2. The infection types of the three parents with the two powdery mildew cultures used, A6 (290), (Wiberg 1974a, 1974b) and 201176-7 (J.E. Hermansen, pers. comm.) are also shown in Table 1.

In the present study the electrophoretic banding pattern of hordein-1 and hordein-2 is considered to be controlled by alleles in the two loci *Hor1* and *Hor2*, respectively. As allele symbols at the *Hor1* locus, the two first letters of the name of the non-recurrent parent utilized to produce the parent line were used and at the *Hor2* locus the same two letters in reverse order were used (Table 1).

The two crosses utilized were 'Iso4R' × 'Iso1R' and 'Iso20R' × 'Iso1R'. The F_1 generation was grown in a growth chamber, and the F_2 generation was grown space planted in the field. The individual F_2 plants were machine threshed. About 25 F_3 seeds from each F_2 plant were sown in a greenhouse. Seven to ten days later the seedlings were inoculated with the first powdery mildew culture. Seven to ten days later the disease readings were made, classifying each F_2 plant as homozygous resistant, heterozygous, or homozygous susceptible. A similar test was made with the other powdery mildew culture on another sample of F_3 seeds. About half of the F_2 plants tested for disease reaction were tested for hordein genotype by analysing the electrophoretic banding pattern of the hordein of two F_3 seeds from each F_2 plant. Estimates of the recombination in the F_1 generation are based on segregation in the F_2 and are determined by tests in F_3. F_2 progenies (the two F_3 kernels) from double heterozygous genotypes thus provide data from which the recombination frequency in the F_2 generation is estimated.

The recombination frequencies were estimated as two-point tests by the method of maximum likelihood. The testing for hordein of only two F_3 kernels from each F_2 plant was accounted for in the estimation of the recombination frequency.

Protein and Hordein Extraction

Total protein was extracted from a single, crushed seed using 0.6 ml of stacking gel buffer containing 1% sodium dodecyl sulphate (SDS), 0.01% bromphenol blue, and 10% sucrose. After extraction at room temperature for about 2 hours, the sample was stirred briefly, centrifuged at 10^4 g for 10 min., and 0.2 ml supernatant was retained for electrophoresis. The protein was reduced by adding dithiothreitol to a concentration of 10 mmol one half hour before electrophoresis. Hordein was extracted from milled seed samples as described by Kφie and Nielsen (1977) after removal of lipids and salt-soluble proteins. The extracted hordein was prepared for electrophoresis as described above.

Electrophoresis

SDS gel electrophoresis was carried out in 180 × 160 × 1 mm gels utilizing the discontinuous buffer system described by Chua and Bennoun (1975). The stacking and separation gels contained, respectively, 5 and 12% monomer consisting of acrylamide and N,N'-methylene-bisacrylamide in a ratio of 9:1 and 40:1, respectively. The gels were polymerized according to the principle described by Davis (1964). Electrophoresis was performed at a constant current of 10 mA in the stacking gel and then at 20 mA until the tracking dye had moved 14 cm into the separation gel. Gels were fixed/washed in a 150 ml solution containing 50% methanol and 10% acetic acid for 1.5 hours, washed in water for one half hour, and then stained overnight in 150 ml 0.0033% Coomassie blue R-250 in 15% trichloroacetic acid.

Results and Discussion

The electrophoretic banding pattern of the protein extract was used to classify each F_3 seed with respect to the *Hor1* and the *Hor2* loci. Direct extraction of the protein from the seed by SDS containing buffer was easier and it gave almost the same banding pattern as the hordein extract (Fig. 1). The minor additional bands obtained in the total protein extract in the hordein-2 region on the gel were ignored in reading the genotypes. Figure 1 shows the banding pattern of the hordeins of the variety 'Clipper', which is homozygous for the previously described alleles *Hor1Cl* and *Hor2Cl* (Doll and Brown 1979). Furthermore,

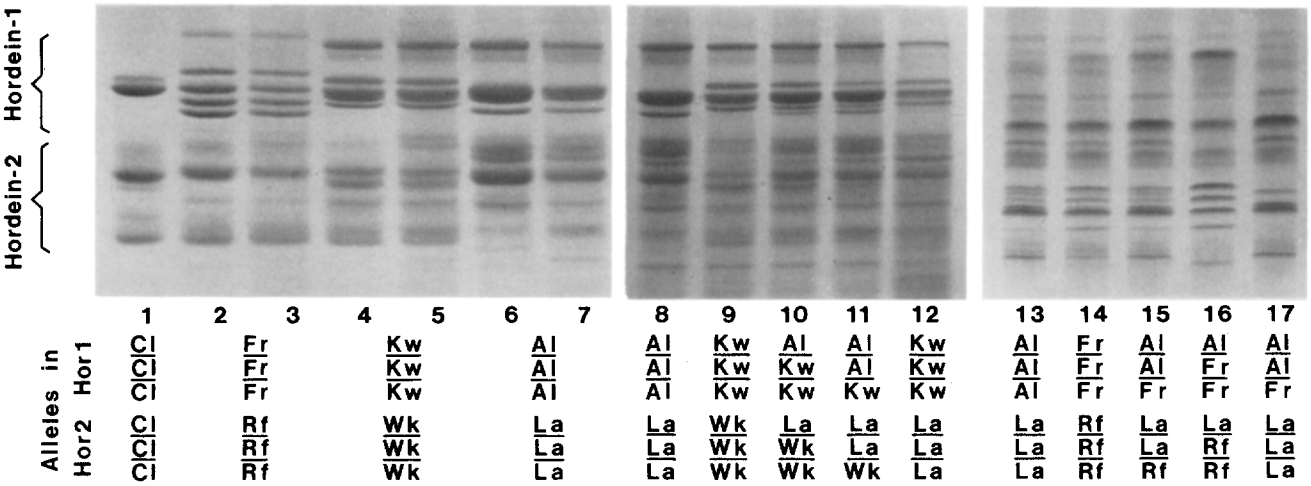

Fig. 1. SDS electrophoresis of hordein, tracks 1, 2, 4, and 6, and of total protein, tracks 3, 5, and 7-17, extracted from individual barley seeds. The banding patterns of the parent varieties 'Iso20R', 'Iso4R' and 'Iso1R' are shown in tracks 2-3, 4-5, and 6-7, respectively. The previously studied variety 'Clipper' is shown in track 1 for comparison. Selected F_3 seeds are shown in tracks 8-12 for the cross 'Iso4R' × 'Iso1R', and in tracks 13-17 for cross 'Iso20R' × 'Iso1R'. The patterns in track 12 and 17 derive from seeds containing recombinants between alleles in *Hor1* and *Hor2*

Figure 1 shows the banding pattern of the parent varieties 'Iso20R', 'Iso4R' and 'Iso1R', which are homozygous for the *Hor1* alleles designated *Fr, Kw* and *Al*, and the *Hor2* alleles designated *Rf, Wk* and *La*, respectively. Figure 1 also shows the banding pattern of selected genotypes from the crosses 'Iso4R' × 'Iso1R' and 'Iso20R' × 'Iso1R', respectively. The *Hor1* and the *Hor2* alleles are codominant and show dose effects upon electrophoresis of both the total protein and the hordein extracts from the triploid endosperm, which has two alleles from the female and one from the male gamete. A distinction of the two heterozygotes was sometimes difficult and has not been utilized in the present study.

In F_2 progeny testing of cross 'Iso4R' × 'Iso1R' with powdery mildew culture 201176-7, a reduced germination of the F_3 kernels was apparently correlated with susceptibility. It was noticed that progenies with all susceptible seedlings were particularly poor germinators but in segregating progenies there were also apparently too few susceptible seedlings. Statistical tests for genotype specific reduction in germination were significant. In estimating the recombination frequency, the reduced germination was taken into account by simultaneously estimating the germination reduction.

In the cross 'Iso20R' × 'Iso1R' the F_2 progenies were tested with both powdery mildew cultures 201176-7 and A6(290) for detection of possible recombinants between the *Ml-a* and the *Ml-a6* resistance genes. The indications of allelism between the two genes *Ml-a* and *Ml-a6* are based on rather limited materials (Hiura 1960; Karimi 1965; Moseman et al. 1965; Starling et al. 1963; Wiberg 1974a). Therefore, it is still possible that the genes may recombine with a low frequency. As no recombinants were obtained between the *Ml-a* and the *Ml-a6* gene in this study, they are treated as alleles.

In the cross 'Iso4R' × 'Iso1R', 355 F_2 progenies were tested for the two *Ml* loci but only about half were tested for the two *Hor* loci. In cross 'Iso20R' × 'Iso1R', 168 F_2 progenies were all tested for the two *Hor* loci and for the two *Ml-a* alleles.

The recombination percentages obtained in the two crosses are shown in Table 2. No heterogeneity was detected between the recombination percentages determined in different crosses or in different generations, and a combined estimate is given. The segregation ratios observed did not deviate from those expected taking the estimates of recombination and reduced germination into consideration.

Based on the recombination percentages obtained it is obvious that the order of the loci must be *Ml-k, Hor1, Ml-a, Hor2* (Fig. 2). The recombination estimated between *Ml-k* and *Hor2* and between *Hor1* and *Hor2* is considerably smaller than the percentages obtained by adding the recombination percentages of the subdistances. We have indications that a few F_3 kernels were occasionally transferred from one F_2 progeny to another, probably during the machine threshing. It can be shown that such a mixing in the present test-procedure is expected to cause too many plants to be classified as recombinants either between the two *Ml* loci or between one of the *Ml* loci and one of the *Hor* loci. In accordance with this the recombination percentage between *Ml-k* and *Ml-a* of 7.5 per cent on the current linkage map (Jensen 1979) is lower than the 4.0 + 5.3 = 9.3 per cent on the map in Figure 2.

Table 2. Recombination percentages and their standard deviation in the two crosses studied

Loci	Generation	'Iso4R' × 'Iso1R'	'Iso20R' × 'Iso1R'	('Iso4R', 'Iso20R')[a] × 'Iso1R'
Ml-k × *Hor1*	F_1	*4.0 ± 1.3*[b]		
Ml-k × *Hor2*	F_1	11.3 ± 2.0		
Ml-k × *Ml-a*	F_1	10.6 ± 1.4		
Ml-a × *Hor1*	F_1	5.8 ± 1.8	4.9 ± 1.5	*5.3 ± 1.1*[b]
Ml-a × *Hor2*	F_1	4.3 ± 1.7	7.5 ± 1.8	*6.1 ± 1.2*[b]
Hor1 × *Hor2*	F_1	6.9 ± 1.7	7.2 ± 1.6	7.0 ± 1.2
Hor1 × *Hor2*	F_2	9.3 ± 2.1	6.5 ± 1.7	*7.9 ± 1.3*[b]
Hor1 × *Hor2*	(F_1, F_2)[a]	7.9 ± 1.2	6.9 ± 1.1	7.4 ± 0.9

[a] Combined values

[b] These four estimates are nearley independent and they contain nearly all the obtained information concerning the mapping of the four loci

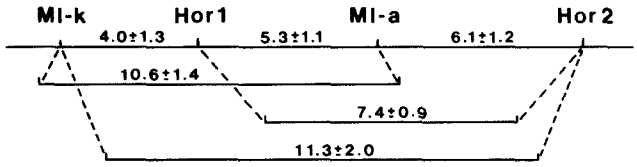

Fig. 2. The linear order of and the recombination percentage between the two powdery mildew resistance loci *Ml-k* and *Ml-a*, and the two hordein loci, *Hor1* and *Hor2*

The recombination percentage estimated between the two *Hor* loci of 7.4 is in agreement with the two values 6.87 ± 0.74 and 6.34 ± 0.90 estimated by Sozinov et al. (1979). The somewhat higher estimates obtained by Shewry et al. (1978b) and by Doll and Brown (1979) have a higher standard deviation and are not significantly different from the present results. On the other hand, the two estimates obtained by Netsvetaev (1978), 13.93 ± 1.13 and 13.44 ± 1.11, are significantly higher. This may be due to the simultaneous segregation of translocations linked with the *Hor* loci in that study. However, Netsvetaev's data indicate that the *Hor2* locus was more distal than *Hor1* on the short arm of chromosome 5 in accordance with the results of the present study. Recently, P.R. Shewry and colleagues (pers. comm.) found that the *Ml-a* locus is between *Hor1* and *Hor2* in accordance with our finding. However, they identified a considerably higher recombination percentage between *Hor* loci than we have estimated in the present study.

The complexity of the *Hor* loci is indicated by the formation of independent messenger RNA molecules for individual hordein-2 polypeptides (Brandt 1979). Each *Hor* locus is likely to contain a number of structural genes for hordein polypeptides and offers opportunities for fine structural mapping.

Literature

Brandt, A. (1976): Endosperm protein formation during kernel development of wild type and a high-lysine barley mutant. Cereal Chem. **53**, 890-901

Brandt, A. (1979): Cloning of double stranded DNA coding for hordein polypeptides. Carlsberg Res. Commun. **44**, 255-267

Chua, N.-H.; Bennoun, P. (1975): Thylakoid membrane polypeptides of *Chlamydomones reinhardtii* wild type and mutant strains deficient in photosystem II reaction center. Proc. Nat. Acad. Sci. (Wash.) **72**, 2175-2179

Davis, B.J. (1964): Disc electrophoresis II. Methods and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**, 404-427

Doll, H. (1977): Storage proteins in cereals. In: Genetic diversity in plants (eds.: Muhammed, A.; Aksel, R.; von Borstel, R.C.), pp. 337-347. New York, London: Plenum Publ.

Doll, H.; Brown, A.H.D. (1979): Hordein variation in wild *(Hordeum spontaneum)* and cultivated (*H. vulgare*) barley. Can. J. Genet. Cytol. **21**, 391-404

Hiura, U. (1960): Studies on the disease resistance in barley. 4. Genetics of the resistance to powdery mildew. Ber. Ohara Inst. Landwirtsch. Biol. Okayama Univ. **11**, 235-300

Holder, A.A.; Ingversen, J. (1978): Peptide mapping of the major components of in vitro synthesized barley hordein: Evidence of structural homology. Carlsberg Res. Comm. **43**, 177-184

Ingversen, J. (1975): Structure and composition of protein bodies from wild-type and high-lysine barley endosperm. Hereditas **81**, 69-76

Jensen, J. (1979): Coordinator's report. Chromosome 5. Barley Genet. Newsl. **9**, 118-120. Colorado State Univ.

Karimi, H. (1965): Untersuchungen zur Vererbung von Mehltauresistenz der Braugerste als Beitrag wirksamer Krankheitsbekämpfung durch Resistenzzüchtung. Z. Pflanzenzücht. **53**, 205-225

Kφie, B.; Ingversen, J.; Andersen, A.J.; Doll, H.; Eggum, B.O. (1976): Composition and nutritional quality of barley protein. In: Evaluation of seed protein alterations by mutation breeding, p. 55-61. Vienna: Intern. Atomic Energy Agency

Kφie, B.; Nielsen, G. (1977): Extraction and separation of hordeins. In: Techniques for the separation of barley and maize seed protein (eds.: Miflin, B.J.; Shewry, P.R.), pp. 25-35. Luxembourg: CEC Eur. 5687e

Miflin, B.J.; Shewry, P.R. (1979): The biology and biochemistry of cereal seed prolamins. In: Seed protein improvement in

cereals and grain legumes. Vol. 1., pp. 137-157. Vienna: Intern. Atomic Energy Agency

Moseman, J.G. (1972): Isogenic barley lines for reaction to *Erysiphe graminis* f. sp *Hordei*. Crop. Sci. **12**, 681-682

Moseman, J.G.; Macer, R.C.F.; Greeley, L.W. (1965): Genetic studies with cultures of *Erysiphe graminis* f. sp. *hordei* virulent on *Hordeum spontaneum*. Trans. Br. Mycol. Soc. **48**, 479-489

Netsvetaev, V.P. (1978): Mapping of loci *Hrd* in chromosome 5 of barley with the help of reciprocal translocations. Biologicheskie Osmovy Ratseonalnogo Ispolzovaniya i Jivotnogo Rastitelnogo Myra, pp. 145-146. Riga (USSR): Zinatne (in Russian, translated by V.P. Netsvetaev)

Oram, R.N.; Doll, H.; Kφie, B. (1975): Genetics of two storage protein variants in barley. Hereditas **80**, 53-58

Shewry, P.R.; Pratt, H.M.; Charlton, M.J.; Miflin, B.J. (1977): Two-dimensional separation of the prolamin of normal and high-lysine barley (*Hordeum vulgare* L.) J. Exp. Bot. **28**, 597-606

Shewry, P.R.; Hill, J.M.; Pratt, H.M.; Leggatt, M.M.; Miflin, B.J. (1978a): An evaluation of techniques for the extraction of hordein and glutelin from barley seed and a comparison of the protein composition of Bomi and Risφ 1508. J. Exp. Bot. **29**, 677-692

Shewry, P.R.; Pratt, H.M.; Finch, R.A.; Miflin, B.J. (1978b): Genetic analysis of hordein polypeptides from single seeds of barley. Heredity **40**, 463-466

Solari, R.M.; Favret, E.A. (1971): Polymorphism in endosperm proteins of barley and its genetic control. In: Barley genetics II (ed.: Nilan R.), pp. 23-31. Washington State Univ. Press

Sozinov, A.A.; Netsvetaev, V.P.; Grigoryan, E.M.; Obraztsov, I.S. (1979): Mapping of the *Hrd* loci in barley (*Hordeum vulgare*). Soviet Genetics **14**, 1137-1147 (Translation of Genetica **14**, (1978) 1610-1619)

Starling, T.M.; Pinda, C.R.; Chen, K.; Moseman, J.G. (1963): Loci of genes conditioning resistance in several barley varieties to race 9 of *Erysiphe graminis* f. sp. *hordei*. Crop Sci. **3**, 151-154

Torp, J.; Jensen, H.P.; Jφrgensen, J.H. (1978): Powdery mildew resistance genes in 106 northwest European spring barley varieties. In: Royal Veterinary and Agricultural University, Yearbook, pp. 75-102. Copenhagen

Wiberg, A. (1974a): Genetical studies of spontaneous sources of resistance to powdery mildew in barley. Hereditas **77**, 89-148

Wiberg, A. (1974b): Sources of resistance to powdery mildew in barley. Hereditas **78**, 1-40

Received February 2, 1980
Communicated by D. von Weltstein

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