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Resistance to barley scald (*Rhynchosporium secalis*) in the Ethiopian donor lines 'Steudelli' and 'Jet', analyzed by partial least squares regression and interval mapping

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The resistance of barley (*Hordeum vulgare* L.) to *Rhynchosporium secalis* (scald) has been investigated in two crosses between the susceptible cv. 'Ingrid' and two resistant Ethiopian landraces, 'Steudelli' and 'Jet'. Doubled haploids were inoculated in replicated tests using two isolates of *R. secalis*, '4004' and 'WRS1872'. Expression of resistance differed widely between replicated tests. AFLP, SSR and RFLP markers were used to develop chromosome maps.

Results have been analysed using partial least squares regression (PLSR) and interval mapping. In PLSR the major covariance structures or 'latent variables' between X (markers) and Y (isolates, tests) are modelled as principal components and their optimal number determined by cross-validation. In 'Steudelli' two QTL were detected, one on each of chromosomes 3H and 7H, in 4 out of 5 tests, while in 'Jet' only one (different) allele at the 3H locus was found. The validated R^2 varied between 11.0% and 64.9% in the replicated tests with '4004'.With isolate 'WRS1872' the 7H locus and another 3H locus were detected. By interval mapping the QTL detected were less stable and generally gave lower R^2 values than PLSR. PLSR does not depend on maps, but interval mapping based on values predicted by PLSR had R^2 around 90%. It is suggested that PLSR may be a useful tool in QTL analysis.

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Rhynchosporium secalis (Oudem.) J.J. Davis causes a serious foliar disease (leaf scald) on barley (*Hordeum vulgare* L.). and occurs worldwide wherever barley is grown. The disease can be controlled most effectively by growing resistant cultivars; however, the genetic variability in virulence and in DNA markers is very high in *R. secalis* populations (HANSEN and MAGNUS 1973; MCDONALD et al. 1989; SALAMATI et al. 2000). At least 15 resistance genes or loci have been designated in different cultivars or lines and mapped on barley chromosomes 3H, 4H, 6H and 7H (DYCK and SCHALLER 1961; HABGOOD and HAYES 1971; BOCKELMAN et al. 1977; ABBOTT et al. 1992; SCHWEIZER et al. 1995; see BJØRNSTAD et al. 2002, for a revised nomenclature leaving basically 7 loci designated by an *Rrs* prefix).

The loci mapped to chromosome 4H, however, remain rather elusive and GRØNNERØD et al. (2002) were unable to trace the '*Rh9*' locus previously reported to this chromosome in 'Abyssinian'. In the present paper, we attempt to map the '*rh6*' locus reported on this chromosome , by BAKER and LARTER (1963), who described two temperature-sensitive, complementary recessive resistance genes, designated '*rh6*' and '*rh7*', in the two Ethiopian accessions 'Jet' and 'Steudelli'. Later HABGOOD and HAYES (1971) concluded that '*rh7*' was an allele at the *Rrs1 (=Rh)* complex on chromosome 3H and re-designated it as 'rh^5'. BOCKELMAN et al. (1977) assigned '*rh6*' to chromosome 4H and '*rh7*'/'rh^5' on chromosome 3H. No reports on closer localisation of '*rh6*' on chromosome 4H have been published. However, ABBOTT et al. (1992) identified linkage of scald resistance in several BC_3-lines from *H. spontaneum* spp. *spontaneum* to the isozyme locus *Acp2*. The chromosomal position of the resistance gene designated *Rrs12* on 4H was inferred from its linkage (15.6 ± 3.8 cM) with isozyme locus *Acp2* (GARVIN et al. 1997).

Both qualitative and quantitative data may be used to map resistance loci relative to molecular and/or morphological markers in plant genomes. For example, using classical linkage analysis, ABBOTT et al. (1995) mapped the *Rrs13* gene on the short arm region of chromosome 6H, and GRANER and TEKAUZ (1996)

mapped the scald resistance gene *Rrs1*$_{Hudson}$ in the proximal portion of the long arm of chromosome 3H, close to the centromere. QTL analysis using maximum likelihood or multiple linear regression (MLR) also has been used to study resistance to scald (Backes et al. 1995; Thomas et al. 1995; Grønnerød et al. 2002). In the present paper we compare this latter approach with a relatively new statistical algorithm, partial least squares regression (PLSR, Wold et al. 1983; Martens and Næs 1989; Martens and Martens 2001). In another paper we describe the principles of PLSR as a simple and intuitively appealing tool with great potential in structural and functional QTL analysis (Bjørnstad et al. 2004). The theory of PLSR is described in more detail in the cited references.

In this paper we will compare PLSR with the MLR method-based on the programme 'PLAB-QTL' (Utz and Melchinger 2000)-to analyze the genetic control of resistance in a double haploid population of 'Ingrid' × 'Steudelli', supplemented by a very small population of 'Ingrid' × 'Jet'.

MATERIAL AND METHODS

Plant material

The *Rhynchosporium* sensitive cultivar 'Ingrid' was crossed to the resistant 'Steudelli'(CI2266) and ninety-seven doubled haploid (DH) lines were produced from the F_1. DH numbers 1–39 and 85 were derived by the *Hordeum bulbosum* L. method (Jensen 1976) and the remainder by anther culture (Bjørnstad et al. 1993), followed by chromosome doubling through colchicine treatment. The DH-lines 30, 74, 81 and 91 are not included due to failure to produce seeds. From the cross 'Ingrid' × 'Jet' (CI967) only 11 DH-lines were produced by the *H. bulbosum* method, due to very low regeneration capacity of the embryos.

Pathogen isolates and resistance tests

Plants were tested with two different *Rhynchosporium* isolates, '4004' and 'WRS 1872', over a 3-year period (Table 1). Since the first tests were made before all DH-lines were available, the tests comprised unequal numbers. Each experiment consisted of two replications, each with a minimum of 3–4 plants per line.

Barley plants were grown in Jiffy strips, in 30 × 20 cm plastic boxes with 55 "pots" in each box. In 1997 plants were grown in growth chambers ; in 1998 and 1999 a greenhouse, always at a temperature of 16–18°C, was used. The plants were inoculated at the two- to three-leaf stage, fourteen days after sowing. Inoculum was produced according to Salamati and Tronsmo (1997). The concentration of the inoculum was adjusted to about 2×10^5 spores ml^{-1}. The spore suspension was sprayed as evenly as possible onto the plants with a jet vaporiser until leaves were moist by droplets. One inoculum preparation was used on all seedlings in a given experiment. High humidity was maintained by covering the inoculated plants in the greenhouse with plastic hoods for 48 h. Plants were assessed visually by the same observer for signs of scald symptoms on the lamina of the second leaf 14 days after inoculation. A 0–5 scale, with '0' designating no visible symptoms, and '5' a collapse of 80–100% of the lamina, was used (Lyngs Jørgensen 1992; Grønnerød et al. 2002). Disease ratings ≤ 2 were classified as resistant reactions (R), 2.1–3.0 as moderately resistant (MR), 3.1–4.0 as moderately susceptible (MS) and 4.1–5.0 as susceptible (S).

Tests with the isolate 'WRS 1872' was done in Canada (A. Tekauz) and scored on a 4-category scale: R (resistant), MR (moderately resistant), MS (moderately susceptible) and S (susceptible). These qualitative scores were converted to numbers (1–4 scale) for QTL analysis.

The 11 lines from the cross 'Ingrid' × 'Jet' were included in only two of the tests, since results were highly consistent in both.

DNA preparation

Genomic DNA for RFLP (restriction fragment polymorphism) and the different PCR reactions was extracted from leaf tissue of 14 days old seedlings according to the CTAB protocol of Kleinhofs et al. (1993) or with use of a Plant DNA Isolation Kit from Boehringer Mannheim (cat. no. 1667319).

Table 1. *Overview of isolates used and tests carried out.*

Isolate	Experiment/year tested	DH-line tested	Reference
'4004', syn. '103'	March 1997	1–40, 85	Lyngs Jørgensen 1992
	August 1997	1–59, 85	
	May 1998	1–85	
	October 1998	1–101	
	May 1999	1–101	
'WRS 1872'	October and December 1997	1–59, 85	Graner and Tekauz 1996

AFLP analyses

AFLP marker analysis was performed essentially as described by Vos et al. (1995) with some modifications, as described by Grønnerød et al. (2002). Briefly, the following 14 combinations of primers were used for selective amplification: *M*+CAA/*E*+AAC, *M*+CAA/*E*+AAG, *M*+CAA/*E*+ACA, *M*+CAA/*E*+ACC, *M*+CAA/*E*+ACG, *M*+CAC/*E*+AAC, *M*+CAC/*E*+ACG, *M*+CAG/*E*+AAC, *M*+CAG/*E*+ACA, *M*+CTA/*E*+ACA, *M*+CTA/*E*+ACG, *M*+CTT/*E*+ACA, *M*+CTG/*E*+ACG, *M*+CTT/*E*+ACT. Denaturing 5% polyacrylamide gels were silver stained and polymorphisms were scored between the parents and screened across the DH lines. Segregating AFLP markers were scored for each DH line as either 'Ingrid'-type (a) or 'Steudelli' or 'Jet'-type (b). All the genotypes were scored for presence and absence of polymorphic AFLP fragments. Two persons scored polymorphisms independently.

The selective primer combinations used are designated by normal AFLP marker nomenclature, i.e. the three selective nucleotides of the *Mse*I primer are written first followed by the three selective nucleotides of the *Eco*RI primer. A number corresponding to the polymorphic band analysed follows the six letters. The polymorphic bands were mostly numbered serially in decreasing order of molecular weight.

Anchor markers

RFLP, STS and SSR analysis. — Restriction digestions of extracted DNA, gel electrophoresis, Southern blot hybridisation, probe preparation, and ^{32}P-dCTP labelling were carried out according to standard procedures (Kleinhofs et al. 1993). The appropriate enzymes (*Eco*RI, *Eco*RV, *Hind*III and *Bam*HI) revealing polymorphisms for the RFLP markers in the parental survey were used for surveying the DH-population. RFLP probes were selected from libraries of barley genomic DNA (ABG and MWG) and oat (*Avena sativa* L.) cDNA (CDO). The following authors described the probes: CDO, Heun et al. (1991); MWG, Graner et al. (1991) and ABG, Kleinhofs et al. (1993).

Sequence-tagged site (STS) analysis was done using the marker 'YLM' (Paltridge et al. 1998), 'STS-MWG680' (Graner and Tekauz 1996) and 'agtc-17' (Grønnerød et al. 2002).

The DH-populations were mapped using the microsatellite or simple sequence repeat primers (SSRs) described previously (Becker and Heun 1995; Liu et al. 1996; Russell et al. 1997; Ramsay et al. 2000). Segregation of SSR alleles was analysed by electrophoresis on denaturing polyacrylamide gels (5%) followed by silver staining.

Linkage map construction. — Segregating AFLP, RFLP, SSR and STS marker data were analysed using JoinMap 2.0 (Stam 1993; Stam and van Ooijen 1995) with the parameters set for DH-derived progeny. JoinMap was also used to group the linked markers and to construct the genetic map. Anchor markers were used to assign linkage groups to the corresponding barley chromosomes. Kosambi's mapping function was applied for map distance calculation (Kosambi 1944). Segregation of markers in the doubled-haploid progeny was tested against an expected 1:1 ratio by χ^2 analysis using JoinMap.

QTL mapping. — QTL analysis was performed with the package PLABQTL (Utz and Melchinger, 2000) based on simple and composite interval mapping (SIM and CIM, respectively). Both individual experiments with isolate '4004' and mean values averaged over these five experiments were analyzed. For the isolate 'WRS 1872' only mean values over the two tests were analyzed, since the transformation from qualitative classes to scores was done on the basis of both tests. The appropriate threshold LOD score for significance was determined by permutation tests of the orginal data (Churchill and Doerge 1994), and an average value of 2.65 was chosen as the 5% significance threshold value for declaring a QTL. Two QTL positions on the same chromosome were regarded as different when at least two markers and a minimum distance of 20 cM separated them. The explained phenotypic variance in the multiple regression model including all detected QTL was calculated. The QTL were validated by cross validation (CV), dividing the genotypes into five subsets (in turn, using four for calibration, the last one for validation in each independent run). Only the average variances after validation are reported. In the region of the three putative QTL, the markers with the highest LOD values were used as cofactors for CIM singly or in combination.

For PLSR we used the software programme The Unscrambler v. 8.6 (Computer-aided Modelling Inc, Oslo, Norway, e-mail: camo@camo.no). The data from each cross were analyzed separately. In 'Ingrid' × 'Steudelli' an X matrix (97 genotypes × 304 markers (266 AFLP, 3 STS, 10 RFLP, 25 SSR)) and Y matrix (97 genotypes × 6 disease tests) were analyzed. In 'Ingrid' × 'Jet' the corresponding matrices were (11 × 484) and (11 × 2), respectively.

Each input variable in X and Y was standardized, i.e. centred to a mean of 0, and scaled to a standard deviation of 1. In each model the markers corresponding to each principle component (PC) were determined through bi-plots and their regression coefficients inspected. The ones with the highest b-values and

best significance levels were determined after cross validation and jack-knife uncertainties (based on the sums of squared differences in values predicted in each sub-model and the full model containing all samples (MARTENS and MARTENS 2001). In the 'Unscrambler' different options for CV were tested, e.g. a chosen number of random subsets (like the PLAB-QTL method of 80% for calibration, 20% for validation), a chosen number of fixed groups, or full CV (leave out one at a time). The markers included in the final model were selected on the basis of % validated variance (R^2). The plots of scores, loadings and outliers were inspected and interpreted.

RESULTS

Map construction

By using 14 primer combinations 265 AFLP markers were generated in the 'Ingrid' × 'Steudelli' population. A total of 309 markers (265 AFLP, 25 SSR, 16 RFLP and 3 STS) were first grouped using a 10.5 LOD threshold. At lower thresholds it was impossible to split chromosome 3H and chromosome 6H markers. Groups were assigned to chromosomes based on previous information of anchor-marker location, and the most likely ordering of groups was determined by comparison of common markers with published maps. JoinMap 2.0 (STAM and VAN OOIJEN 1995) was re-run for each chromosome group with only those markers that belonged to same chromosome number. Chromosome 7H and 2H markers were split into two groups each at all LOD threshold grouping values >2, and two separate linkage maps were constructed for each of these chromosomes (Fig. 3). Markers from the remaining five chromosomes linkage groups created one group (Fig. 3). A total of 164 markers are shown in the map of the 'Ingrid' × 'Steudelli' population. Cosegregating AFLP markers were numerous and skipped from the map construction. Certain AFLP markers that either had many missing scores, appeared unlinked or their inclusion produced inconsistencies in a linkage group were excluded.

The linkage map covered a total map distance of 884 cM compared with the total length of 1060 cM of the barley consensus map (QI et al. 1996). The map had an average interval length of 5.4 cM. On chromosome 3H seventeen markers mapped exactly to the same position as *MWG680. STS*/*MWG680* cosegregated with agtc17. Only 11 markers could be mapped to chromosome 6H, and gaps existed on other chromosomes. Other areas were well covered with closely linked markers (Fig. 3).

Of the 309 markers analysed in the doubled-haploid progeny, 95 (31%) showed distorted segregation (χ^2-test, $P \leq 0.05$) (data not shown). Of the 164 markers included in Fig. 3, 45 (27.3%) showed distorted segregation (locus-wise χ^2-test, $P \leq 0.05$). Fourteen of these loci were located on chromosome 3H in a 30-cM segment surrounding *MWG680*. 'Ingrid' alleles were over-represented. Other markers with distorted segregation (not shown) included chromosomes 1H and 5H. In the latter case 'Steudelli' alleles were over-represented.

Assessment of resistance

'Ingrid' × 'Steudelli'. — Figure 1 shows the reaction score after the inoculation of *R. secalis*, isolate '4004' for the individual tests and the mean score for the five experiments. The strong variation between tests is evident, despite carefully controlled inoculation conditions. The symptom severity was very high for the 'May 98' test, which showed no resistant plants. The same can be seen for the 'March 97' test, whereas 'August 97' showed the greatest phenotypic variance and also differentiated best between 'Ingrid' and 'Steudelli'. The mean score shows 17 DH-lines with moderate or fully resistant reaction with isolate '4004' (Fig. 2). 45 lines were moderately susceptible, intermediate between the two parents 'Ingrid' (S) and 'Steudelli' (MR).

Only 58 of the DH-lines were inoculated with the isolate 'WRS 1872'. The pattern was the same as for isolate '4004', about four times more moderate susceptible to susceptible plants than resistant to moderately resistant (Fig. 2). The distribution of R-MR and MS-S differed between isolate '4004' and 'WRS 1872'.

'Ingrid' × 'Jet'. — Generally 'Jet' had a stronger resistance than 'Steudelli' (scores 1–2, approx. 1 unit better on the 0–5 scale). The resistance was also less influenced by subtle changes in environmental conditions. The same applied to the DH-lines, of which three had scores between 1 and 2 (reaction type R), two between 2 and 3 (MR) and the remaining 6 were susceptible (S, between 3.5 and 5). Although the number of DH- lines clearly is too low to assess segregation ratios, it suggests a 1:1 segregation and a qualitative inheritance.

QTL analyses

'Ingrid' × 'Steudelli'. — A simultaneous PLSR analysis of all 5 tests with '4004' was performed. The results showed – not surprisingly – great differences between tests. 'August 97' had loadings almost 10-fold those of 'May99'. Only one PC was consistently detected, associated with significant markers such as *YLM* and *MWG680* all located close to *Rrs1*. However,

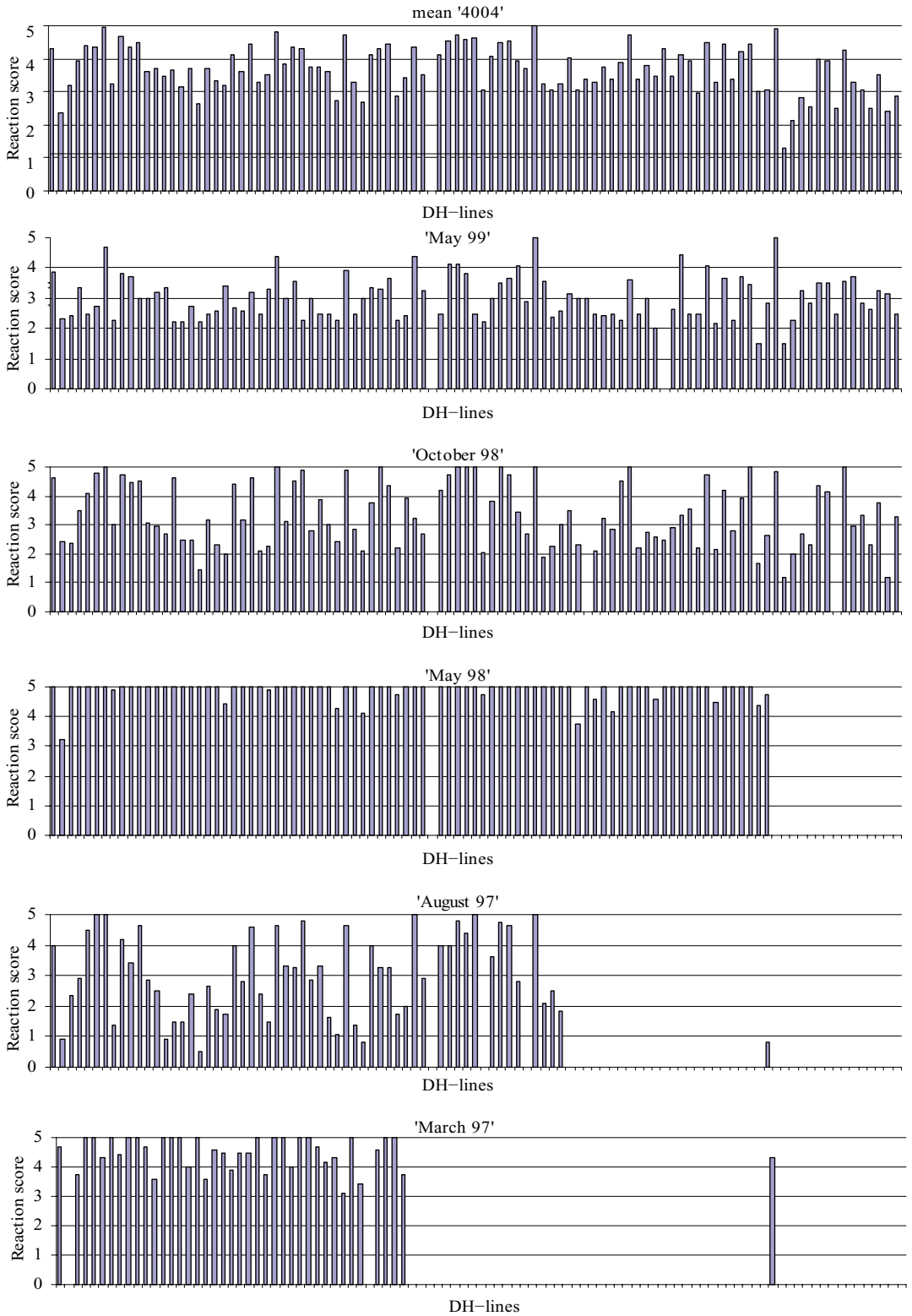

Fig. 1. Reaction score after inoculation of *R. secalis*, isolate '4004' from five individual tests and average values of the tests.

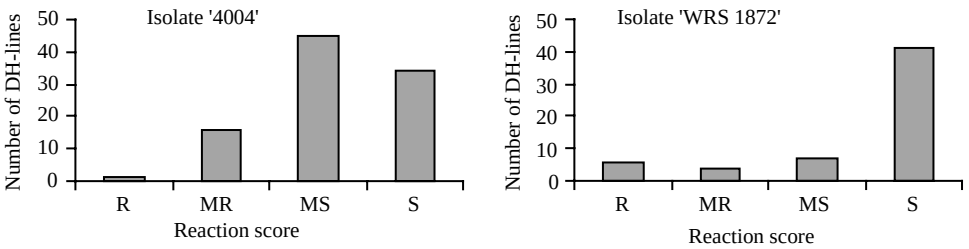

Fig. 2. Reaction to *R. secalis*, isolate '4004' and 'WRS 1872' in a DH-population of 'Ingrid' × 'Steudelli' ('Ingrid' = MR and 'Steudelli' = S with both isolates). Mean values across different experiments.

the validated R^2 was only 36%, and the model detected a number of significant outliers.

Separate PLSR analysis of all 5 tests. Joint analysis of data with such differences between replications introduces a lot of noise rather than easing the interpretation. As shown in Table 2, the validated R^2 in the different tests varied between 11.0% and 64.9%. Two QTL were identified in 4 out of 5 tests: one on 3H close to *YLM*/*MWG680* and another on 7H close to *MWG2018*/*Bmag0206* (alternatively the closely linked *MWG555*). In two tests ('May98' and 'May99') only one of them was detected The 7H QTL was the stronger factor in both 'October98' and 'August 97' and corresponds to the well-known locus *Rrs2*. Significant markers on chrom. 4H were detected in tests 'May99' and 'March97', whereas a 6H cluster was only detected in 'October 98'.

PLSR analysis of the 'August 97' test. This test was analyzed in detail, both because of its nice phenotypic distribution and the high R^2. The loading plot showing the preliminary result after the first selection of significant markers based on the highest regression coefficients (Fig. 4, BJØRNSTAD et al. 2004). Three clusters are apparent, corresponding to 2 PCs that

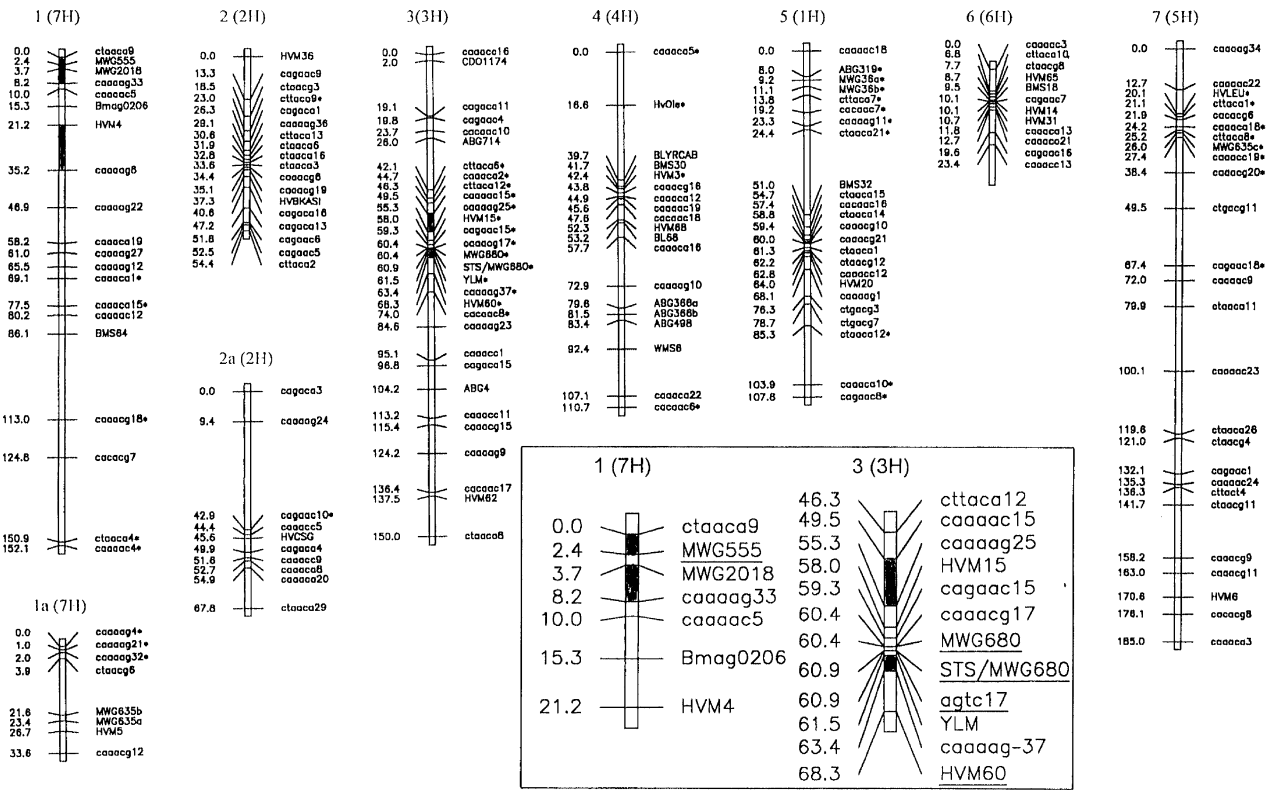

Fig. 3. Location of QTLs for resistance to leaf scald (*R. secalis*) on the skeletal map, based on 97 DHs from a cross 'Ingrid × Steudelli'. Chromosomes were oriented with the short arms to the top. Kosambi's mapping function was used. Shaded areas inside the chromosome bars are the QTLs for resistance to leaf scald. The approximate locations of race-specific resistance genes (*Rrs* genes) are estimated from the literature (see text).

Table 2. *Comparison of QTL for R. secalis resistance in the 'Ingrid' × 'Steudelli' population, detected through partial least squares regression (PLSR), simple or composite interval mapping (SIM, CIM) based on data from inoculations with isolate '4004' or 'WRS1872' in different replicated tests. * = sign at 5% level in the PLSR model, corresponds to LOD >2.65 in CIM. [1]Strongly affected by choice of cofactors in CIM.*

Test	QTL detected	PLSR (* = $b \pm$ 2SD >0)	Chrom.	LOD/SIM	LOD/ CIM	LOD/ PLS+SIM	LOD/ PLS+CIM
'4004' May 99	HVM4 – caaaag8	*	7H	3.2	n.s	–	–
	MWG2018 – Bmag0206	*	7H	4.5	(2.5)	–	–
	Caaaac19 – cacaac18	n.s.	4H	n.s.	5.4	–	–
	Caaaca16 – bl68	*	4H	6.3	5.1[1]	–	–
	R^2% (validated)	32.9		34.1	36.1	–	–
'4004' October 98	HVM4 – caaaag8	*	7H	8.6	4.8	7.9	n.s.
	MWG2018 –Bmag0206	*	7H	8.3	n.s.	10.1	10.6
	HVM31 – HVM14	*	6H	n.s.	n.s.	3.2	15.6
	YLM – MWG680	*	3H	4.6	n.s.	20.4	22.2
	R^2% (validated)	41.3		38.9	30.3	83.5	90.1
'4004', May 98	YLM-MWG680	*	3H	3.2	n.s.	–	–
	R^2% (validated)	11.0		10.7	0.0	–	–
'4004', August 97	MWG2018 – caaaag33	*	7H	10.0	7.7	n.s.	28.7
	Bmag0206 – HVM4	n.s	7H	8.6	n.s	16.5	14.9
	YLM – MWG680	*	3H	n.s[1]	n.s.	6.6	20.8
	cacaac18 – WMS6	n.s.	4H	n.s.	n.s.	2.9[1]	n.s.
	R^2% (validated)	64.9		38.9	43.7	91.8	97.3
'4004', March 97	HVM4-?	*	7H	n.s	n.s.	–	–
	BLRYCAB – HVM3	*	4H	n.s.	n.s.	–	–
	YLM – MWG680	(*)	3H	n.s.	n.s.	–	–
		20.6		0.0	0.0		
'4004', average	HVM4 – caaaag8	–	7H	4.5	–[1]	–	–
	MWG2018 – Bmag0206	*	7H	5.1	–[1]	–	–
	YLM-MWG680	*	3H	5.7	–[1]	–	–
	R^2% (validated)	40.6		29.4	–[1]	–	–
'WRS 1872'	MWG2018 – Bmag0206	*	7H	2.9	n.s.	–	–
	CDO1174-caaacc13	*	3H	2.7	4.0	–	–
	R^2% (validated)	37.0		0.0	0.0	–	–

explain 61% of the validated variance. The PC1 and PC2 highlight two clusters: one in the lower quadrant corresponding to markers close to *YLM*/*MWG680* on 3H, and another cluster in the upper quadrant, representing the *MWG2018*/*Bmag0206* locus on 7H. Both have high and positive loadings, indicating that the allele from 'Ingrid' (scored as 1) is positively correlated with high disease scores. The third cluster is only explained by this PC and represents loci close to *HVM65* on chrom 6H. The graph also shows some apparently 'loose' (through significant) markers, as well as the (non-significant) microsattelite *HV-LEU* on 5H.

After inspecting the regression coefficients again a simplified model was constructed by selecting the marker pair with the highest b-coefficents and/or highest R^2 to represent each of the three putative QTL intervals. The result was revealing: only PC1 with two significant clusters remained, while the R^2 increased to 65%. The other markers could be sacrificed with no loss in explanatory power. The resulting two-locus model is described in detail on Figs. 5 and 6. In the loadings plot (Fig. 5) it may be seen that the deletion of the 6H cluster has reversed the ranking between QTL effects: the 7H cluster is now the stronger. Inspecting the scores (Fig. 6) four tight clusters are indicated, one in each quadrant, representing the 4 predominant DH-genotypes. The ones to the far right have susceptibility alleles from 'Ingrid' at both QTL, while those at the far left carry

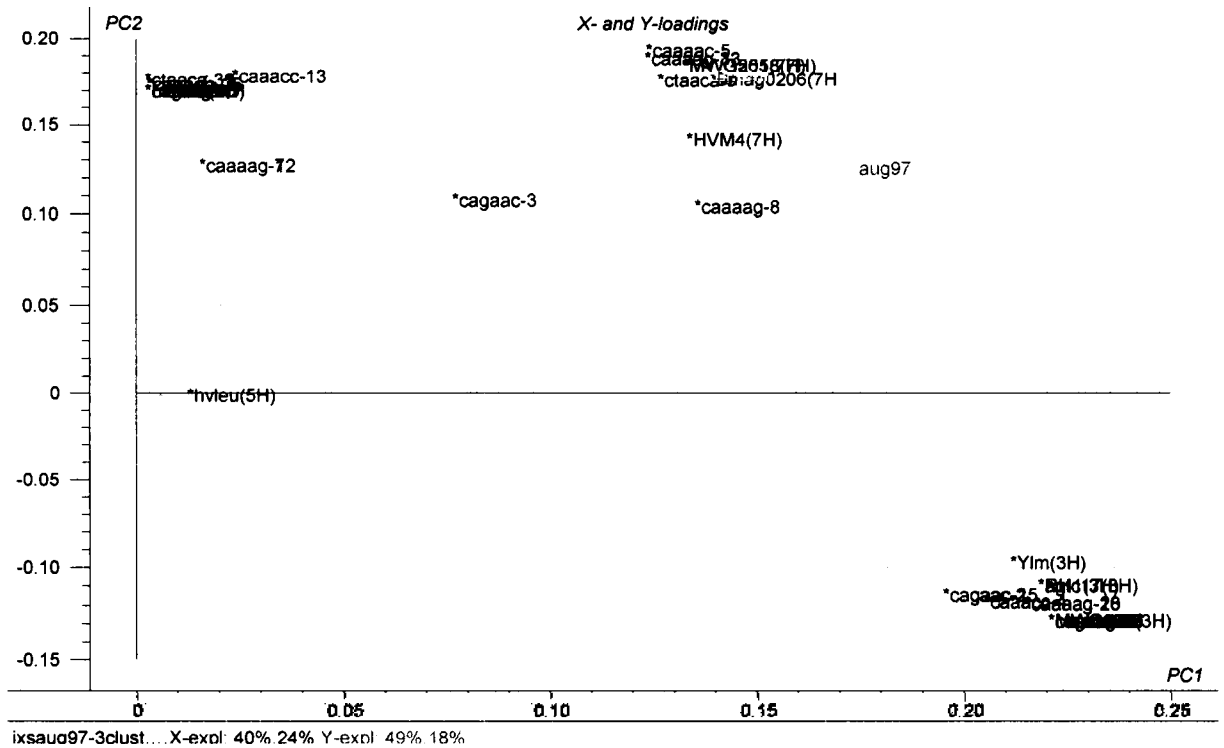

Fig. 4. PLSR loadings plot of the relationship between resistance in the test 'Aug97' and markers. Three clusters are depicted: one on 3H (PC1, lower right), one on 7H (PC1, upper right) and one on 6H (PC2).

both 'Steudelli' alleles. The intermediate clusters represent cross-over genotypes that carry one allele each, corresponding to the appropriate clusters in Fig. 5.

This graph also shows some interesting 'loners', apparently with looser connections to the main groups. Four of them (23, 27, 34 and 41) were detected by PLSR as significant outliers and the raw data were inspected in detail. Genotype 23 has a disease score of 2.4 and carries the 'Ingrid' alleles at the 3H locus. At 7H it carries the 'Steudelli' alleles at *MWG2018*, but the 'Ingrid' allele at *Bmag0206*, i.e. it represents a cross-over. Genotype 27 represents the opposite cross-

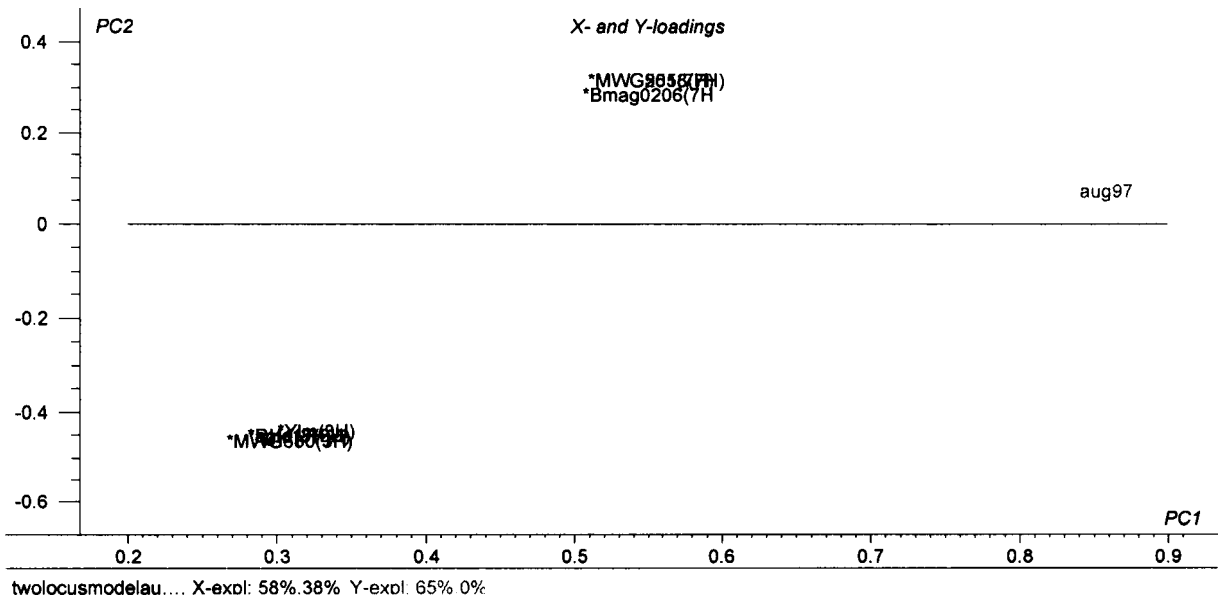

Fig. 5. PLSR loadings plot of the relationship between resistance in the test 'Aug 97' and two marker clusters, one on 3H (below PC1) and one on 7H (above PC1). Note that despite the 'loss' of the 6H factor, R^2 has increased.

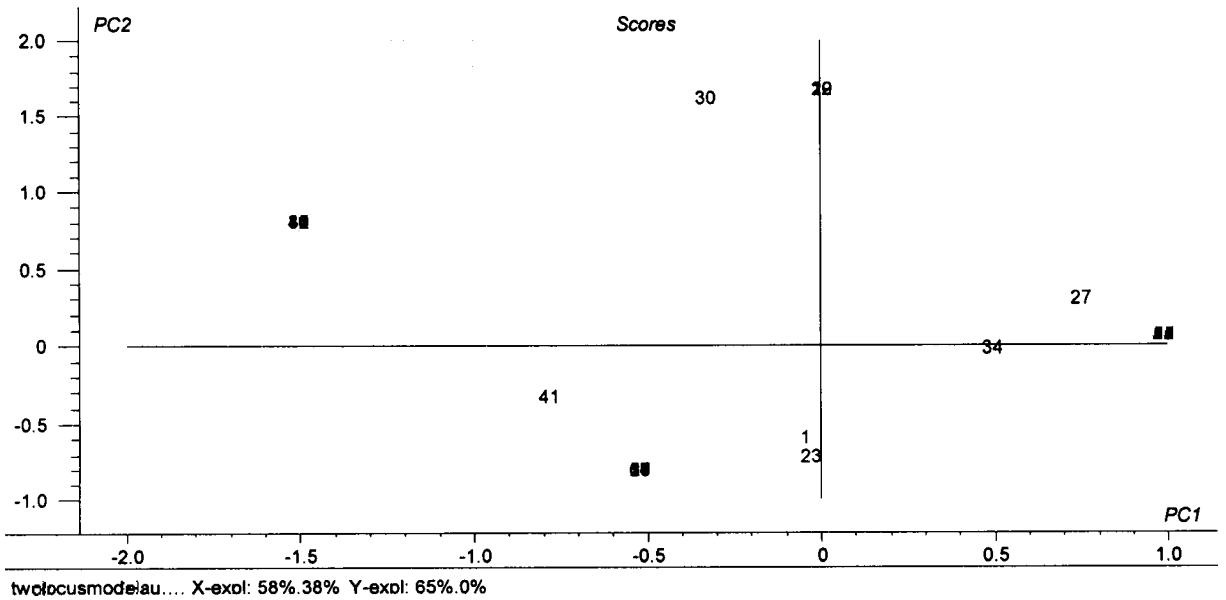

Fig. 6. PLSR score plot of the model in Fig. 5, showing the positions of the genotypes in the PC space, relationship between resistance in the test 'Aug97' and two marker clusters, one on 3H (below PC1) and one on 7H (above PC1). Note the 4 main DH classes, corresponding to genotypes having both QTL (far left), none (far right) or the one on 7H only (upper centre) or on 3H only (lower centre). Note also occasional outliers (discussed in the text).

over, with the 'Ingrid' alleles at *MWG2018*, but the 'Steudelli' allele at *Bmag0206*, and a resistance score of 4.7, indicating a loss of resistance at 7H. Similarly, genotype 34 represents a cross-over event at 3H ('Steudelli' allele at *MWG680* only), otherwise 'Ingrid' alleles and a close to susceptibility score of 3.3. Finally, genotype 41 carries 'Steudelli' alleles at both QTL, except for an apparent cross-over at *agtc17* and *STS-MWG680*. Its disease score was 2.0 and apparently no loss of resistance. Thus, in spite of their unexpected marker/trait combinations, all outliers tend to have reasonable explanations.

PLSR analysis of 'October 98' test. This was performed analogously to 'August 97'. Briefly, 3 QTL remained after variable selection: the two detected above, plus one on chromosome 6H, associated with *HVM31* and *HVM14*. The total validated variance was 46.5%, which was reduced to 42.1% if the 6H factor was removed. Only one outlier was detected, which was a cross-over for the 3H factor, had the resistance alleles at 7H and susceptibility alleles at 6H. Its phenotype was close to fully susceptible (3.9).

A joint PLSR analysis to predict 'October 98' based on 'August 97'. The resistance values of 'October 98' were first downscaled to 0.1% of its original value, so that effectively only 'August 97' contributed variance to the PLSR model. Next, by rescaling the degree of explanation of 'October 98' by the obtained model can be seen in Fig. 7. The positions of the clusters show that adding the less precise 'October 98' to the better 'August 97' model did not improve the explanation.

Role of epistasis. — PLSR allows easy testing of marker–marker interactions. This was one reason for choosing the Unscrambler software, since it was expected from previous studies that recessive epistasis was present in both crosses. However, no signs of such non-additive interactions between markers on 3H and 4H, nor 3H and 7H, could be detected.

Interval mapping. — As shown in Table 2 the detected QTL vary between individual tests and analysis methods (SIM or CIM). Except for 'March 1997' all tests were able to identify at least one QTL.

With simple interval mapping, the average of all tests with '4004' identified the same QTL region distally on 7H, but as two linked QTL. One was in the most distal 10 cM interval, between markers *MWG2018* and *Bmag0206*, the other more proximal, in a 14 cM interval between *caaaag8* and *HVM4*. This QTL had slightly weaker effect (Table 2). Also, the major QTL on 3H close to *YLM/MWG680* was identified.

In the 'August 1997' test the same 2 linked QTL were identified on 7H, one in the most distal 0–6 cM interval, at position 4 cM and support interval flanked by markers *MWG2018* and *caaaag33*. The more proximal one, at 16 cM in the 10–20 cM interval between *Bmag0206* and *HVM4*. The 3H locus was identified at position 62 cM, in the 58–68 cM interval.

Among individual tests results varied. The 'May 1998' was the only test not to identify a QTL on 7H,

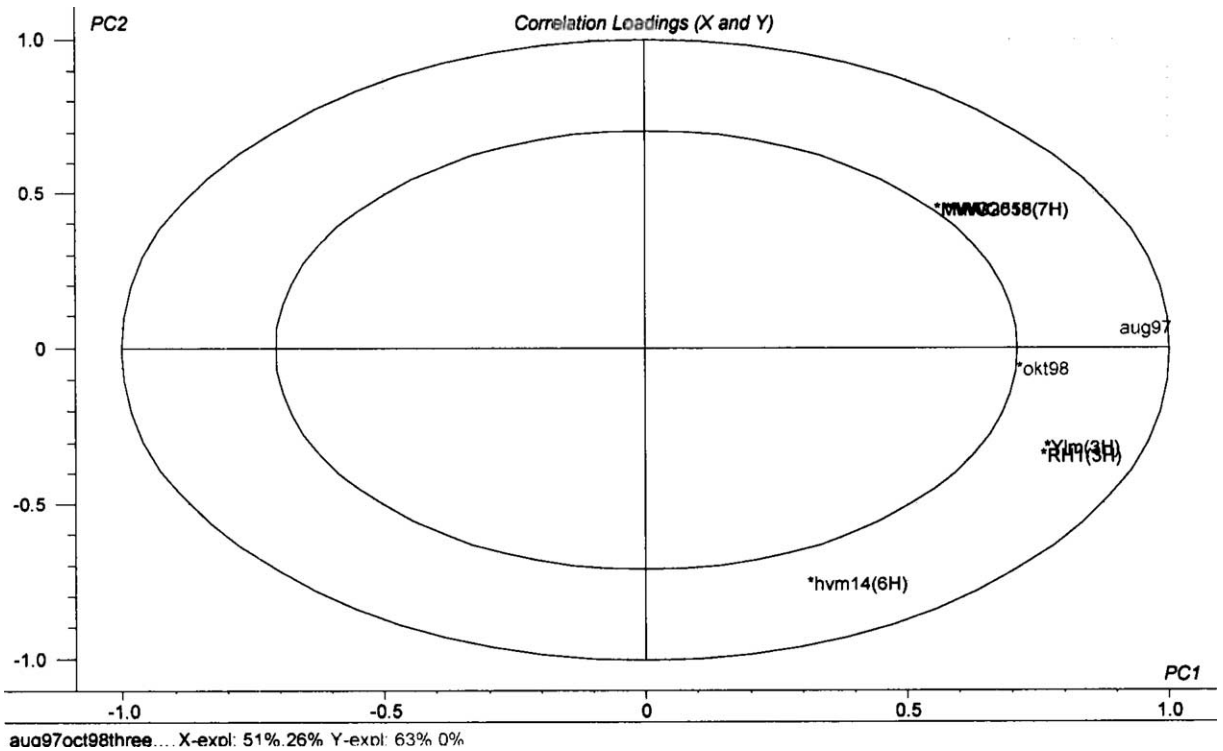

Fig. 7. PLSR correlation loadings plot of the relationship between resistance in the test 'Aug97' and 'Oct98'. The outer ellipse depicts 100% explained variance, the inner 50%. Three two-marker QTL on 7H, 3H and 6H were included a model of 'Aug97'. This model explains about the same% of the 'Oct98' variance as direct modelling.

and 'May 1999' was the only experiment that identified a QTL on other chromosomes (4H).

For composite interval mapping a number of alternative markers linked to or at the QTL detected by SIM were tested as co-factors, following the recommendations by Utz and Melchinger (2000). From the data in Table 2 three markers closely adjacent to the 3 main QTL (*MWG2018*, *caaaag8* and *YLM*) were tested individually or in combinations. In the analysis of the average of all tests, the QTL detection was very sensitive to the choice of co-factors and it was difficult to draw any safe conclusions. Hence, in this case no R^2 estimates are given in Table 2. In the individual tests the results were more stable, but it seemed erratic which of the 7H 'QTL' survived. Hence it is difficult to conclude which is the real position of the QTL. This also applies to the 'splitting' of the 4H QTL in the 'May 1999' test in CIM.

Contrary to expectations there was no consistent improvement in mapping precision due to CIM. By making the appropriate choices an 'improvement' was in many cases possible (even by including random markers as co-factors).

Interval mapping based on predicted values from PLSR. — In order to estimate the map positions of the PLSR factors we applied SIM and CIM with the predicted genotypic values estimated from the PLSR two-factor model in Fig. 5. Since the covariance structure in the X-data is already taken care of by PLSR, 'co-factors' and CIM might no longer be necessary. However, we tried both. The predicted values from 'October 1998' and 'August 1997' were analyzed to obtain the intervals and map positions associated with the QTL (Table 2, the two rightmost columns). The effect on R^2 was striking, as compared to the analysis based on the observed values. As expected, the same QTL were detected as with PLSR and in every cross-validation segment. The R^2 and LOD scores were generally higher and hence map confidence intervals more narrow in CIM. However, the question of one or two QTL on 7H could not be resolved. In 'August 1997' CIM indicated two clear QTL with positions at 2 (confidence interval 0–6) and 16 cM (confidence interval 14–18). In 'October 1998' the effect was opposite.

Isolate 'WRS 1872'. — The results with this isolate differed partly from '4004'. PLSR identified only two QTL, one on 7H in the *MWG2018-Bmag0206* region, the other in an interval between *CDO1174* and *caaacc13* on 3HL, approx. 40 cM distal compared to the *YLM*/*MWG680*-locus. PLABQTL (whether CIM

or SIM) identified only the latter. The R^2 were strikingly higher with PLSR. Neither indicated any QTL at the *YLM*/*MWG680*-locus, indicating that the isolate was virulent.

The QTL additive estimates for both isolates showed negative values, showing that the 'Steudelli' alleles contributed to lower scald scores, i.e. improved resistance.

Resistance in 'Jet'. — The low number of lines made a normal linkage-based analysis impossible, but not an analysis by PLSR. The score plot clearly differentiated the 3 resistant entries from the rest.

The resistance clearly was located close to the *MWG680* locus. However, there was also evidence of interaction between this locus and the RFLP marker *ABG498* on 4H (the latter had no individual effect). Together the two factors explained 69.4% of the explained variance, of which the interaction between 'Steudelli' alleles at the two loci contributed about 14.4%. However, when running both replicates instead of the mean, only the *MWG680* locus was significant, making the epistasis somewhat doubtful.

DISCUSSION

Assessment of resistance and QTL

The expression of disease symptoms is determined by the combined influence of host genotype, pathogen genotype, and the prevailing environmental conditions. Sufficient moisture and moderate temperatures (13–18°C) are essential for the infection of barley by *R. secalis* (Salamati and Magnus 1997). Despite a high degree of standardization, the trait was quantitative and highly influenced by inoculation environments.

The tests 'August 97' and 'October 98' had the best differentiation and hence the best explanation of the phenotypic variance. 'May 99', however, showed least differentation, probably due to low infection pressure. 'Ingrid' scored just below 4 and there were few individuals with scores higher than 4. On the other hand, the inoculation pressure in 'May 98' and 'March 97' may have been too high. This shows the environmental sensitivity of this pathosystem and the resistance in 'Steudelli' in particular. It appeared much more sensitive than the resistances in 'Jet' and 'Abyssinian' (Grønnerød et al. 2002).

Resistance genes, markers and segregation

The present analysis showed that although quantitative the resistance donor 'Steudelli' was di-/oligogenic and that the two QTL were consistent. One of them is located on chromosome 7H close to the markers *MWG555*, *Bmag0206* and *MWG2018* (Fig. 3) and is probably an allele at *Rrs2*, mapped to the telomeric region of 7HS.

The study has given evidence of both one and two QTL in this region. The latter might correspond to an allele at the more proximal locus *Rrs3* (Bjørnstad et al. 2002). However, both from the direct PLSR results and from previous mapping studies (Schweizer et al. 1995) we tend to consider the one closer to HVM4 as an artifact. This also applies to the 'splitting' of the 4H QTL in the 'May 1999' test in CIM. The QTL found on chromosome 3H in the present study is obviously an allele at the *Rrs1* locus (Fig. 3).

The donor 'Jet' seems to carry only one factor, also located in this locus. Its different phenotype from 'Steudelli' progenies carrying only the 3H-resistance shows that the alleles in the two donors are not identical.

These results partly deviate from previous research by Baker and Larter (1963) and Habgood and Hayes (1971), confirming a QTL allele ('*rh7*') at *Rrs1* in both donors, but not their identity. Moreover, only weak traces of any '*rh6*' on 4H were found (in 'Jet'). There were no indications of any epistatic interactions in 'Steudelli', and the one in 'Jet' needs further confirmation.

The reasons for these discrepancies may be at least twofold. (1) The isolates used in the study were virulent to the 4H locus. (2) The resistant accessions are heterogeneous with regard to resistance, and only one genotype has been used here. Alemayehu and Parlevliet (1997) reported strong variation in resistance within Ethiopian landrace populations and hence heterogeneity in the individual lines chosen in the study to represent the original seed source. We have also observed this in previous studies (Grønnerød et al. 2002).

On the other hand it is also possible that the two-locus model in 'Steudelli' is wrong. It was suggested on the basis of segregation ratios, which may, however, be poor predictors of gene number. The markers around *Rrs1* (Fig. 3) all showed a strong deviation from a 1:1 ratio, like in the studies by Graner et al. (1991), Graner and Tekauz (1996) and Grønnerød et al. 2002. Performance not explained by the expected 'recessive epistasis' was also noted by Reitan et al. (2002) in breeding lines derived from 'Jet'.

The reaction to isolate '1872' indicates a QTL in another region on 3H than found with '4004'. This suggests that the two isolates may have differential virulence to the two loci mapped by Patil et al. (unpubl.) in the cross 'Ingrid' × 'CI11549'. Besides *Rrs1* they detected a locus ca 20 cM distal on 3HL (designated *Rrs4*). They also suggested its possible

identity with the gene '*Rhy*' found by BARUA et al. (1993) to be linked to CDO1174, an RFLP probe previously mapped to the short arm of 3H (HEUN et al. 1991) and cosegregating with *Rhynchosporium* resistance. Figure 3 shows the CDO1174 marker far away from the complex *Rh* area around MWG680. The map in Fig. 3 was mostly in agreement with previous literature (LIU et al. 1996). Some of the SSR markers have also moved compared to other maps (LIU et al. 1996).

Can PLSR contribute to improved QTL detection?

As shown in Table 2, PLSR tended to be better able to detect genetic patterns in these rather 'noisy' data. The R^2 values were higher in 5 of 7 tests and the QTL more consistent. Other advantages of PLSR that may be mentioned are:

- The visual ease of PLSR modelling, which allows the analyst to identify candidate QTL and test them in a user-friendly setting, emphasizing data interpretation and exploration rather than the need for a specialized statistical training. The approach resembles the graphical analyses of genotype × environment interactions as well as mapping data recently advocated by YAN and KANG (2003).
- The capacity of simultaneous analysis of multiple tests (or traits). This is analogous to the multiple interval mapping approach of KAO et al. (1999), rather than SIM or CIM. We have not tried their MIM Fortran programme, but it is interesting to note that they obtained higher precision in QTL location. PLSR can, however, not (yet) estimate the different epistatic components as outlined by KAO et al. (1999) and KAO and ZENG (2002).
- The option to test the influence of different marker putative markers and of outlier detection, a satisfaction not always provided by testing 'co-factors' in CIM.
- Moreover, PLSR may be an option where maps are unfinished, unavailable (like in 'Ingrid' × 'Jet'), difficult to construct (like in autotetraploids) or impossible (marker – trait analysis of random, unrelated genotypes).

Objections to the use of the method

- The genetic information inherent in the family type is not utilized during modelling. Mapping information is only used post hoc. – Although the method does not directly allow an 'interval' to be defined like in interval mapping, using predicted PLSR values in our case gave precise intervals and stability to perturbations during crossvalidation. We hence conclude that such interval mapping is a useful adjunct for estimating map positions of PLSR factors. The approaches are not mutually exclusive!
- PLSR is an unfamiliar method for most workers in the field. – We agree about the unfamiliarity of multivariate techniques in general. Of the >250 posters in 8th Barley Genetics Symposium (Adelaide 2000) only half a dozen used multivariate approaches (beyond cluster trees), in spite of multivariate data were presented in a large number of others). We believe the advent of new genomic techniques such as microarray data will rapidly convince our colleagues of the need for multivariate techniques (FEARN 2003; MARTENS 2003).
- PLSR does not give substantially different results. Multiple trait and epistasis are possible to analyze by existing programmes and PLSR does not offer anything new. – We agree that the features we have presented are more or less available in interval mapping programmes, but rarely simultaneously. The maximum likelihood approach of Kao and Zeng is an alternative, but according to ASINS (2002) it may "rapidly become computationally intractable as the number of QTL in the model increases". We have not had such experiences with PLSR, which we do believe is a real addition to the QTL toolbox. However, we expect its main strength will lie in linking gene expression data to phenotypes – a subject not treated here.

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