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Genetic analysis of resistance to yellow rust in hexaploid wheat using a mixture model for multiple crosses

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Abstract DNA-based molecular markers have been used in numerous studies for tagging specific genes in wheat for subsequent use in marker-assisted selection. Usually in plant breeding, procedures for mapping genes are based on analysis of a single segregating population. However, breeding programmes routinely evaluate large numbers of progeny derived from multiple-related crosses with some parental lines shared. In most such related crosses, the number of progeny is quite small. Thus, statistical techniques for detecting quantitative trait loci (QTLs) using data from conventional multi-cross breeding programmes are interesting. The objective of this study is to present a mixture model for QTL mapping in crosses of multiple inbred varieties with non-normal phenotype distributions and to use this model to map QTLs for yellow rust resistance in elite wheat breeding material. Three doubled haploid populations consisting of 41, 42 and 55 lines, respectively, originating from four parental varieties were studied. Multi-cross QTL analysis with three specific pathogen isolates of *Puccinia striiformis* f. sp. *tritici* and a mixture of the isolates revealed QTLs for resistance at four different genomic locations. These QTLs were found on chromosome 2AL, 2AS, 2BL and 6BL and explained between 21 and 41% of the phenotypic variation. Two of these QTLs, one on the long arm of chromosome 2A and one on the short arm of chromosome 2A were identical to the known yellow rust resistance genes *Yr32* and *Yr17*, respectively, whereas the QTLs located on the long arms of chromosomes 2B and 6B may reflect types of resistance to yellow rust, which have not previously been mapped.

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Introduction

Wheat (*Triticum aestivum* L. em. Thell) is one of the most important crops worldwide. It is allohexaploid ($2n = 6x = 42$) and has a complex genetic structure with three genomes A, B and D (Bennett and Smith 1976). DNA-based molecular markers have been shown in numerous studies to be useful for tagging specific genes in wheat (Devos et al. 1995; Schachermayr et al. 1997; Buerstmayer et al. 2002; Ellis et al. 2002), which may be used for marker-assisted selection (MAS) to accelerate germplasm improvement. Usually such gene mapping [for example of quantitative trait loci (QTLs)] is based on a single segregating population derived from a cross between two lines. Such single-cross analysis may reveal limited information, if the segregating genes represent only part of the underlying genetics in the entire breeding material. Conventional breeding programmes generally evaluate large numbers of progeny derived from multiple-related crosses; that is, separate crosses may share a parental line. However, the number of progeny per cross is sometimes small compared with the 100–200 offspring most often needed for reliable mapping of QTLs in single-cross experiments.

Recently, there has been growing interest in extending statistical models for QTL mapping to multiple crosses. Xu (1998) developed fixed and random model procedures for mapping QTLs in independent F_2 populations. Liu and Zeng (2000) proposed a fixed-effect model that

extends composite interval mapping to combined crosses from multiple inbred lines and accomodates complex cross designs with overlapping or non-overlapping parental lines. Bink et al. (2002) proposed a Bayesian method for related outbred plant populations. These approaches may be advantageous since more allelic variation is sampled and no additional mapping population has to be produced for a specific trait of interest.

However, current multi-cross QTL methods assume normally distributed residual variation and may not be optimal if the phenotype distribution deviates markedly from a normal distribution. While careful transformation of the phenotypes may in some cases yield good approximations to a normal distribution, this is hard to do if, for instance, the phenotype distribution is multimodal. In the case of QTL analysis of single-cross data with a non-normal phenotype distribution, interval mapping can occasionally result in spurious LOD score peaks in regions of low genotype information (Broman 2003; Feenstra and Skovgaard 2004). The two-component mixture model of Feenstra and Skovgaard (2004) avoids such spurious LOD score peaks in single-cross analysis. Here, an extension of the two-component model to crosses from multiple inbred lines to map QTLs for yellow rust is presented.

Yellow rust is a common and important disease in wheat production (Chen et al. 2002) for which resistance remains an objective in many breeding programmes. During the last 25 years there has been a great interest in identifying yellow rust resistance genes. According to KOMUGI (http://www.shigen.nig.ac.jp/wheat/komugi), an integrated wheat science database, more than 40 resistance genes have been described and more than half of these genes have already been mapped. There are resistance genes for yellow rust in elite material of wheat, which are incompletely described and therefore difficult to manipulate in breeding. A new resistance gene designated *Yr32* was tagged by molecular markers in a population Senat × Savannah (Eriksen et al. 2004) probably originating from the variety Carstens V (CV), commercialized in 1921 (Zadoks 1961). This variety has been widely used as a source of yellow rust resistance during creation of new wheat varieties (Stubbs 1985). According to Chen and Line (1993), the yellow rust resistance of CV is complex, with one to four genes behaving as either dominant or recessive (Calonnec et al. 2002). In this paper, the proposed mixture model for multi-cross QTL analysis is used to map QTLs for yellow rust resistance in three related crosses derived from four inbred wheat cultivars involving resistance derived from the CV complex.

Material and methods

Plant material

The plant material consisted of double haploid (DH) populations of wheat (*T. aestivum*) derived from three different crosses of winter wheat. The experimental design consisted of related pairwise crosses among four widely used parental varieties Deben, Solist, Kris and Wasmo in the following way: Solist × Deben, Kris × Deben and Kris × Wasmo. All four parental varieties possess resistance to *Puccinia striiformis*, which causes yellow rust. However, the underlying genetic basis of their resistance is very different and incompletely characterized. The population Solist × Deben included 55 DH lines, Kris × Deben had 41 DH lines and Kris × Wasmo had 42 DH lines. All three populations were inoculated in the field with three different isolates of *P. striiformis*.

Pathogen isolates

Three *Puccinia striiformis* isolates were used, supplied by M.S. Hovmøller (submitted), Danish Institute of Agricultural Sciences, Flakkebjerg, Denmark. Isolate 71/93 possesses virulence matching resistance genes *Yr1, Yr2, Yr3, Yr32 and Sd*, 08/97 carries virulence for *Yr1, Yr2, Yr3, Yr4, Yr9*, *Yr17, Su* and *Sd* (Justesen et al. 2002) and 70/99 carries virulence for *Yr1, Yr2, Yr3, Yr4, Yr6, Yr9*, *Yr17, Su* and *Sd* (M.S. Hovmøller, personal communication). The isolates were multiplied on 'susceptible' cultivars ('spreaders'), that is, 71/93 on Skater (*Yr2, Yr32*), 08/97 on Brigadier (*Yr9, Yr17*, +) and 70/99 on Lynx (*Yr6, Yr9, Yr17*) (resistance genes adopted from M.S. Hovmøller, submitted).

Phenotype data

Disease resistance of the 138 DH lines in the study was assessed in field experiments during two consecutive years, 2000/2001 and 2001/2002 at one location, Sejet (Jutland, Denmark). Lines were evaluated in 1 m×1 m plots of six rows with two rows of each of two different lines to be evaluated surrounded by two rows of disease spreader varieties. In the first experimental year the 138 DH lines were infected with a mixture of all three pathogen isolates. The second year, three replications of the experiment were conducted each of which was infected with one of the pathogen isolates separately. To reduce inappropriate pathogen isolate contamination among replications, seven m-guard rows of rye were sown between replications of the experiment. Resistance of double rows in each plot was scored on a 0–9 scale with 0 as completely resistant and 9 as total infection (McNeal et al. 1971). For further data analysis these scores were divided by 10 and arc sine square root transformed to improve variation homogeneity.

Molecular marker analysis

DNA was extracted from seeds using a slightly modified CTAB method (Saghai-Maroof et al. 1984). The seeds were milled in a mixer-mill (Retsch MM300) by adding a

steel ball in each sample. Markers employed for mapping were simple sequence repeats (SSR) markers developed by the Wheat Microsatellite Consortium (*wmc*) (Somers et al. 2004) and by Röder et al. (1998) (*gwm*). PCR reactions were performed on a Primus Multiblock from MWG Biotech. PCR reactions with modifications (0.2 units of Taq and 25 ng of template DNA) and thermo cycling conditions for SSRs were as described in detail by Eriksen et al. (2003). The primers used for PCR amplification were fluorescently labelled with either 6-FAM, TET, HEX, NED, VIC or PET and the fragment length were detected using either a TAMRA or LIZ fluorescently labelled internal size standard of 500 base pair on an ABI310 (Applied Biosystem) automatic DNA sequencer.

Linkage map

A reference map with genetic map positions for most of the employed SSR markers was retrieved from the International Triticeae Mapping Initiative (ITMI) map based on data from Röder et al. (1998) and M.J. Christiansen (unpublished). Maps based on data from the individual populations as well as an integrated map were constructed and were compared to the ITMI map.

Individual linkage map

Individual linkage maps of SSR markers for each-cross were constructed using the computer software G-MENDEL (Holloway and Knapp 1993) for calculation of linkage groups and ordering of markers. The reliability of the locus order was checked using Monte Carlo simulation. The three maps generated were compared with one another and also with the ITMI map. Markers that did not map to unique positions, according to Monte Carlo simulation were removed. Furthermore, markers having conflicting linkage group assignment in the four individual maps were removed. The remaining reliable markers were used for construction of an integrated map.

Integrated linkage map

Linkage grouping and locus ordering was conducted with CARTHAGENE (Schiex and Gaspin 1997), a software package especially designed to produce integrated maps from data made up of several distinct inbred line crosses. Initial grouping of markers used a minimum LOD score of 3 and a maximum Kosambi distance of 25 cM as linkage thresholds. This produced 27 initial linkage groups of three or more markers, which is more than the 21 chromosomes in wheat; therefore some linkage groups were subsequently joined based on the chromosomal location of markers in the ITMI map. Remaining markers and pairs of markers not meeting the initial grouping criterion were tentatively assigned to linkage groups using a less stringent criterion of a minimum linkage LOD score of 2 to a marker in an existing linkage group or based on their chromosomal location in the ITMI map. Within each linkage group, markers

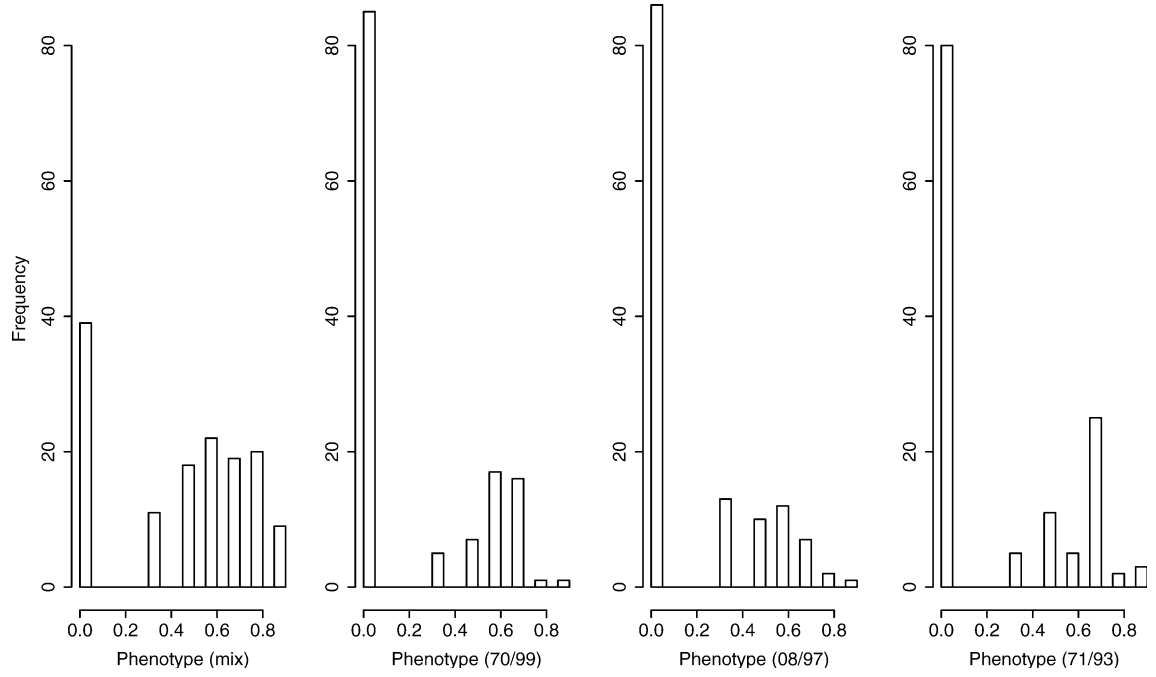

Fig. 1 Histograms of transformed disease resistance scores. From left to right the *histograms* correspond to the mixture of pathogen isolates, isolate 70/99, isolate 08/97 and isolate 71/93, respectively

were ordered to maximize the multipoint likelihood of the map. The marker order was improved by permuting subsections of the map. Some markers were very tightly linked, which produced several possible maps with very similar likelihoods. In these cases, the map in best agreement with the ITMI map was used. Markers with positions deviating clearly from the ITMI map and markers showing poor linkage to any other marker were discarded. Final maps of the linkage groups corresponded very well with the ITMI map and were used for QTL analysis. Figures of linkage groups and LOD scans for QTL mapping were produced with MAPCHART (Voorrips 2002).

QTL analysis

Quantitative trait loci analysis used interval mapping with an extended version of the two-component mixture model (Feenstra and Skovgaard 2004), accommodating cross designs involving multiple inbred lines. The statistical model can be regarded as a generalization of the model of Liu and Zeng (2000) for multiple crosses with increased robustness against non-normal phenotype distributions. In this study, the phenotype distribution deviated markedly from a normal distribution (Fig. 1) and separate analyses of the data from each of the three crosses with an interval mapping model resulted in spurious LOD score peaks (Feenstra and Skovgaard 2004). Also, in simulated multi-cross data similar problems were encountered when the model of Liu and Zeng (2000) was used (Fig. 2). The extended two-component mixture model overcame the problem of spurious LOD score peaks when single crosses were analysed. Here the extended version of the two-component mixture model was used to achieve similar robustness during analysis of multiple crosses.

A wide variety of experimental designs involving multiple inbred lines may be contemplated. The experimenter may, for example, have a mixed collection of backcross, doubled haploid or F_2 populations in which the parental lines may overlap in complex patterns. Also, in practical plant breeding it often occurs that not all possible combinations of parental lines are exhausted, since crosses are usually only made between parental lines that differ in the trait of interest. In the presentation of the statistical models that follow, the experimental design of the yellow rust study presented in this paper will be assumed. However, the approach applies equally well to more complex combinations of different crosses and to more complete designs.

Statistical model

In the following, let the four parental lines, Solist, Deben, Kris and Wasmo, be denoted as P_1, P_2, P_3 and P_4, respectively. Let DH(i,j) be the DH population originating from crossing P_i and P_j, that is, in the present study the DH populations are DH(1,2), DH(2,3), and DH(3,4). Also, let y_{ijk} denote the trait value of the kth individual in population DH(i,j) with $k = 1,\ldots,n_{ij}$. Following Liu and Zeng (2000), first the statistical model with the following likelihood function of the parameter vector $\theta = (\alpha_1, \alpha_2, \alpha_3, \beta_{12}, \beta_{23}, \beta_{34}, \sigma)^{\mathrm{T}}$ is considered.

$$L(\theta) = \prod_i \prod_j \prod_{k=1}^{n_{ij}} \sum_{l=1}^{2} p_{ijkl} f\left(y_{ijk}; \mu_{ijkl}, \sigma\right). \quad (1)$$

Here p_{ijkl}, ($l = 1,2$) are the conditional probabilities, given the marker data and the QTL position, that individual k in population DH(i,j) has the allele from P_i and P_j, respectively, and $f(y; \mu, \sigma)$ is the density function for a normal distribution with mean μ and standard deviation σ. The systematic part of the model may be expressed as $\mu_{ijk1} = \alpha_i + \beta_{ij}$, if the allele at the putative QTL comes from P_i, and $\mu_{ijk2} = \alpha_j + \beta_{ij}$, if it comes from P_j. Here α_i and α_j are additive effects from parental lines P_i and P_j, respectively, and β is a column vector of fixed effect parameters; it holds three parameters, one

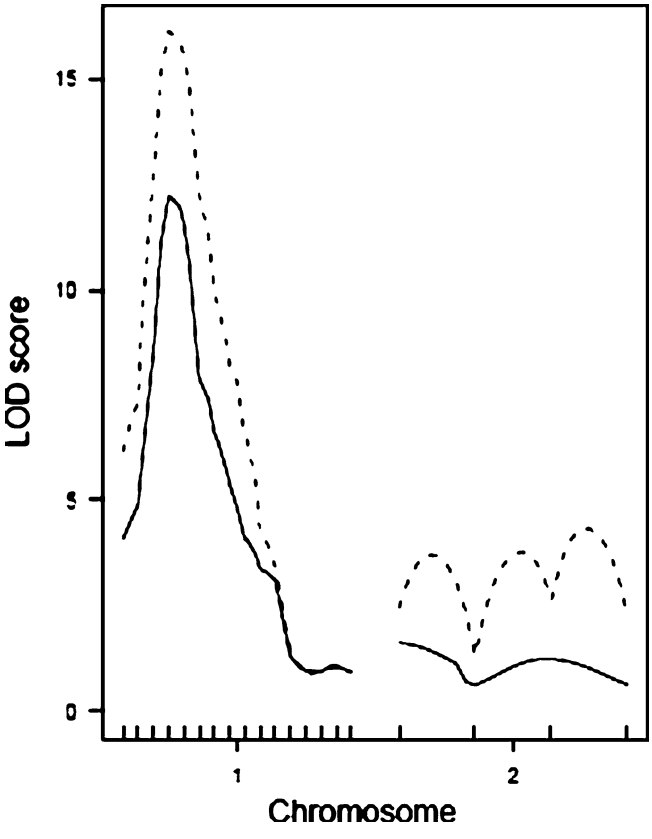

Fig. 2 A simulation example of multi-cross quantitative trait loci (QTL) mapping on three populations of 50 DH lines derived from pairwise crosses of four parental strains. Two chromosomes each 120-cM long were simulated with a QTL at position 25 cM on chromosome 1. The QTL segregated in two out of three crosses, and was detected by both the extended two-component model (*solid line*) and the model of Liu and Zeng (2000) (*broken line*). The latter model produced the largest peak on chromosome 1, but also produced spurious peaks on chromosome 2, falsely indicating the presence of a QTL on this chromosome. This artefact was avoided by the extended two-component model

for the effect of each of the crosses. Note that the additive effects $\alpha_1,\ldots,\alpha_4$ cannot be uniquely estimated. Therefore the constraint $\alpha_4 = -\alpha_1 - \alpha_2 - \alpha_3$ is used to make the additive effects at the locus sum to 0.

A two-component model extension

The statistical model defined by Eq. 1 rests on the assumption of a normal distribution of the residual errors. Here a two-component mixture model is presented that is robust against non-normal errors and which contains the original model as a special case. The two-component mixture model extends the model of Feenstra and Skovgaard (2004) to cross designs involving multiple inbred lines. In constrast to the original model (Eq. 1), this new model is a mixture of two normal distributions, whether a QTL is present or not. Although this model may also not fit the data well it avoids different types of distributions under the null hypothesis compared to under the alternative and hence is more robust towards distributional deviations. For this model the likelihood function of the parameter vector $\theta = (\alpha_1, \alpha_2, \alpha_3, \beta_{12}, \beta_{23}, \beta_{34}, \pi_{121}, \pi_{122}, \pi_{231}, \pi_{232}, \pi_{341}, \pi_{342}, \sigma)^T$ is given by

$$L(\theta) = \prod_i \prod_j \prod_{k=1}^{n_{ij}} \sum_{l=1}^{2} p_{ijkl}\left[\pi_{ijl} f\left(y_{ijk}; \mu_{ijk1}, \sigma\right) + \left(1 - \pi_{ijl}\right) f\left(y_{ijk}; \mu_{ijk2}, \sigma\right)\right]. \quad (2)$$

Here p_{ijkl}, $f(y; \mu, \sigma)$ and μ_{ijkl} are defined as given previously. Also, α_i and α_j are additive effects with $\alpha_4 = -\alpha_1 - \alpha_2 - \alpha_3$ and β_{12}, β_{23}, and β_{34} are cross effects. In this model there are six extra mixing parameters; π_{ij1} and π_{ij2} are the probabilities in the population DH(i,j) that phenotypes from individuals with the QTL allele from P_i and P_j, respectively, are drawn from the first part of the mixture distribution.

Estimation and hypothesis testing

Maximum likelihood estimates of the parameters in Eqs. 1 and 2 were obtained with forms of the expectation-maximization (EM) algorithm (Dempster et al. 1977). The asymptotic covariance matrix for the maximum likelihood parameter estimator was found using the EM algorithm (Louis 1982; Kao and Zeng 1997).

The null hypothesis of no QTL effect is formulated differently for the two models (model 1 vs. model 2). In both cases, the null hypothesis should imply that the unobserved genotype at a putative QTL does not influence the phenotype y_{ijk}. In the statistical model, the null hypothesis is H_0: $\alpha_i - \alpha_j = 0$, for all pairs (i, j), whereas the alternative is that in at least one of the crosses, α_i differs from α_j. Estimation under the null hypothesis is simple in this case, since the model is a multiple linear regression model. In the case of the two-component model, the null hypothesis of no QTL effect is H_0: $\pi_{ij1} = \pi_{ij2} = \pi_{ij}$, for all pairs ($i$,$j$), whereas the alternative is that in at least one of the crosses $\pi_{ij1} \neq \pi_{ij2}$. The two-component model estimation under the null hypothesis is still done with the EM algorithm, but only once since the estimates do not depend on genotypes at putative QTL.

For both models, LOD scores were found by calculating ratios between the maximized likelihood function under the full model versus under the null hypothesis. This was done at positions every 2 cM throughout the genome. Significance of LOD score peaks was assessed by comparison with genome-wide LOD thresholds obtained by analysis of 2,000 data sets with permuted phenotype values (Churchill and Doerge 1994). For locations with significant LOD score peaks, the QTL effect of a particular allele was expressed as the deviation from the mean phenotypic value in a hypothetical average reference population of all four parents. Also, for locations with significant LOD score peaks, predicted values were back transformed to the original disease score scale for each allele in each of the populations: Solist × Deben, Kris × Deben and Kris × Wasmo. Further detection of significance of resistance segregation in different crosses used likelihood ratio tests of hypotheses of no QTL effect in one or two of the three subpopulations. For the final model the residual sum of squares (RSS) was calculated and compared to the RSS under the null hypothesis model with no QTL effect to estimate the proportion of phenotypic variability explained by the QTL. The two-component mixture model

Table 1 Marker and mapping information for the three populations Solist × Deben, Kris × Deben and Kris × Wasmo

	Solist × Deben	Kris × Deben	Kris × Wasmo
Population size	55	41	42
Number of loci screened	463	463	463
Number of polymorphic loci	189	167	192
Discarded loci	56	43	36
Mapped loci	133	124	156
	Integrated map		
Number of loci	239		
Discarded loci	23		
Mapped loci	216		
Average number of loci/chromosome	10		
Map in cM	2734		
Average interval length in cM	12,7		

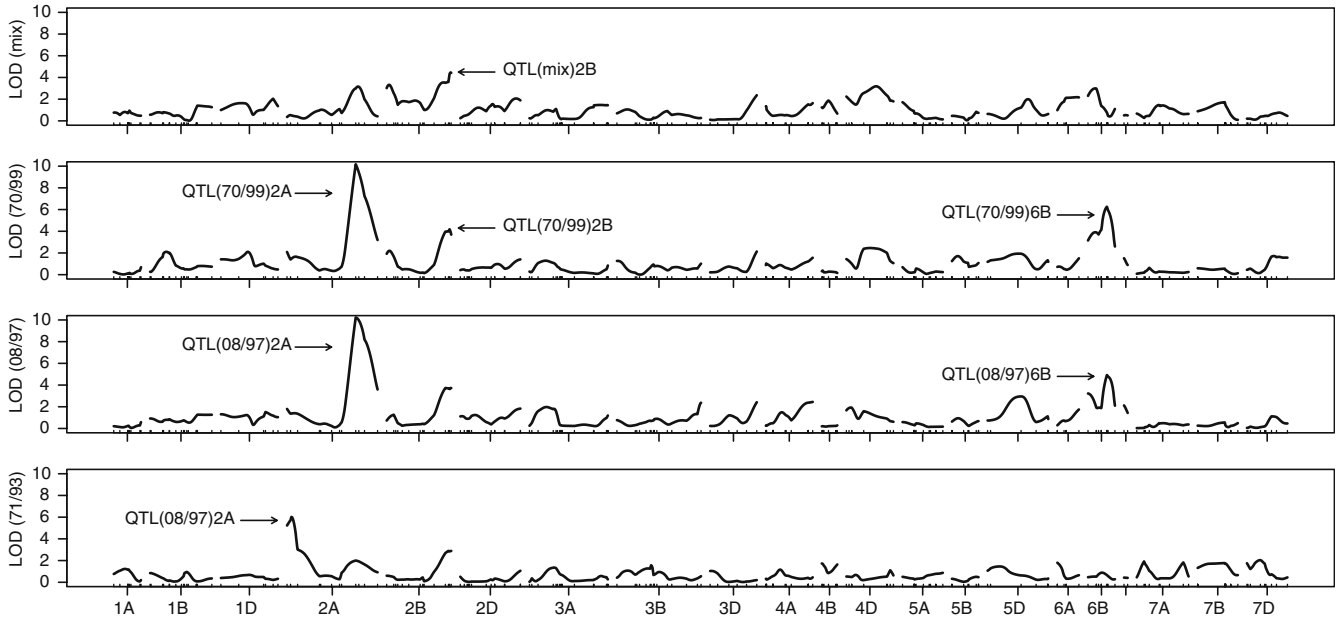

Fig. 3 LOD scan for each of the four pathogen isolate treatments across all wheat chromosomes. The first three LOD scans from the bottom represent the specific pathogen isolates 71/93, 08/97 and 70/99, respectively. The LOD scan at the top represented a mixture of the same three pathogen isolates. For each of the four treatments the identified QTLs are indicated

for multiple crosses was implemented with new functions building on the framework of R/qtl (Broman et al. 2003), an add-on package for the general statistical software **R** (Ihaka and Gentleman 1996; R Development Core Team 2005).

Results

Linkage map

Among 360 primer pairs, 201 primer pairs showed polymorphism in at least one of the three populations. The 360 primer pairs revealed a total of 463 marker loci. The proportion of polymorphic loci in the three populations ranged from 36 to 41%, whereas the proportion of discarded loci ranged from 19 to 30%. The number of loci suitable for mapping in the individual populations ranged from 27 to 34%. Of the 201 polymorphic primer pairs, 182 primer pairs corresponding to 216 loci (47%) were mapped on the integrated marker map with the following distribution for the three populations: Solist × Deben, Kris × Deben and Kris × Wasmo: 133 loci (29%), 124 loci (27%) and 156 loci (34%), respectively. SSR loci were mapped on all of the 21 wheat chromosomes. The map length was 2734 cM, with an average interval length of 13 cM (Table 1).

QTL identification

Multi-cross QTL analysis with three specific pathogen isolates and a mixture of the isolates revealed six significant QTLs for resistance to yellow rust (Fig. 3). Two of the six QTLs were localized to the same position in the analysis of two different pathogen isolates, that is a total of four different genomic positions harbouring QTLs were identified in the three mapping populations (Table 2).

Identification of yellow rust resistance genes Yr32 and Yr17

A major QTL was found on chromosome 2AL at position 189 cM (Fig. 4), providing resistance to the isolates 70/99 and 08/97. This QTL explained 41 and 40% of the phenotypic variation for the isolates 70/99 and 08/97, respectively. The resistance originated primarily from Solist and Wasmo. No QTL effect was found in the population Kris × Deben on the same chromosomal position (Table 2), which is in agreement with individual QTL analysis for each population separately (Table 3). This QTL is tightly linked to microsatellite wmc 198a at position 189 cM. According to the virulence phenotype of the isolate, chromosome location and previous report by Eriksen et al. (2004), this QTL is believed to be *Yr32*, known to be present in both Solist and Wasmo according to disease test by M.S. Hovmøller (submitted). This is also supported by fingerprint data of Solist and Wasmo, since both varieties show the same alleles for the three closely linked SSRs: wmc 198a, wmc 170b and wmc 181 (data not shown). The largest effect is seen in the Solist × Deben population, where allele substitution from the Deben allele to the Solist allele is estimated to reduce disease score from 2.30 to 0 and from 1.87 to 0 for the isolates 70/99 and 08/97, respectively (Table 4).

Table 2 Maximum LOD scores of detected quantitative trait loci (QTLs) and their position in cM

	Max. LOD	Position (cM)	Effect in Solist × Deben (*P* value)	Effect in Kris × Deben (*P* value)	Effect in Kris × Wasmo (*P* value)	$\tilde{a}_{Deben}$	$\tilde{a}_{Solist}$	$\tilde{a}_{Kris}$	$\tilde{a}_{Wasmo}$	PVE_{QTL} (%)
QTL(70/99)2A	10.17	189	6×10^{-9}	0.44[a]	4×10^{-4}	0.21	−0.28	0.21	−0.15	41
QTL(08/97)2A	10.23	189	9×10^{-9}	0.84[a]	2×10^{-4}	0.20	−0.25	0.20	−0.16	40
QTL(71/93)2A	6.20	12	0.4[a]	0.005	1×10^{-5}	−0.02	−0.02	−0.19	0.23	27
QTL(mix)2B	4.46	176	0.002	0.001	0.50[a]	−0.25	0.07	0.09	0.09	21
QTL(70/99)6B	6.26	52	2×10^{-6}	0.90[a]	0.01	−0.02	0.29	−0.02	−0.24	25
QTL(08/97)6B	4.92	52	8×10^{-6}	0.43[a]	0.16[a]	−0.07	0.21	−0.07	−0.07	29

Test of QTL effect (*P* values given) in each of the three crosses, the QTL effect estimated for the four varieties ($\tilde{a}_{variety}$) and the proportion of the phenotypic variation explained by the QTL (PVE_{QTL})
[a]The null hypothesis of no QTL effect in this population was accepted

In the Kris × Wasmo population, the disease score is estimated to be reduced from 1.48 to 0.01 and from 1.49 to 0.01, respectively for the same two isolates when the Kris allele is substituted with the Wasmo allele at the QTL (Table 4).

A second QTL was detected on the short arm of chromosome 2A at position 12 cM (Fig. 4) explaining 27% of the phenotypic variation in resistance to the isolate 71/93. The multi-cross analysis showed significant effects of this QTL in populations Kris × Deben and Kris × Wasmo, but not in Solist × Deben (Table 2). Analysis of the individual subpopulations also indicated effects of this QTL in populations Kris × Wasmo and Kris × Deben, however, only the effect from the Kris × Wasmo population was significant (Table 3). These findings are in agreement with the estimated allele effects for which the allele from Kris is chiefly responsible for reducing the disease score (Table 2). The maximum LOD score of 6.2 was found at position 12 cM only 2 cM from the SSR wmc 407 (Table 2). According to the virulence phenotype of the isolate and the mapping position, this QTL is believed to be *Yr17*. Fingerprint data of Kris and the variety Savannah (data not shown) known to carry the translocation containing the cluster of resistance genes *Lr37-Yr17-Sr38* show null alleles for both surrounding markers (wmc 382 and wmc 407) for both varieties indicating the presence of an interspecific translocation. The idea of *Yr17* as the primary gene responsible for resistance in Kris is in agreement with disease tests of M.S. Hovmøller (submitted) and has been verified by the allele-specific molecular marker ventriup/LN2 (Helguera et al. 2003). The effect of this QTL was highest for the Kris × Wasmo population where the estimated mean disease score was reduced from 1.69 to 0 when the QTL allele from Wasmo was substituted for the Kris allele. A more modest disease score reduction from 0.33 to 0 was estimated for the Kris × Deben population when the Deben allele was substituted with the Kris allele (Table 4).

Additional QTLs for resistance to yellow rust

In addition to the QTLs on chromosome 2A that are believed to represent the resistance genes *Yr32* and *Yr17*, a QTL explaining 21% (Table 2) of the phenotypic variation was detected on chromosome 2BL at position 176 cM (Fig. 5), with the resistance allele originating from Deben. This QTL with flanking SSR markers, wmc 149 (173 cM) and wmc 317a (179 cM) conferred resistance to the mixture of the pathogen isolates, but was not found to significantly reduce disease scores for the individual isolates. There was however LOD score peaks at this position in the analyses of experiments with the individual isolates (Fig. 3) with the LOD score peak for isolate 70/99 approaching significance ($P = 0.066$). The QTL effect in terms of resistance to the mixture of pathogen isolates was highest in the Kris × Deben population, where substitution of the Kris allele for the Deben allele was estimated to reduce the disease score from 3.38 to 0.81. In Solist × Deben, substitution of the Solist allele with the Deben allele produced an estimated reduction of disease score from 2.95 to 0.63 (Table 4). No QTL effect was indicated for the population Kris × Wasmo (Table 2).

A QTL explaining 25% of the phenotypic variation was detected on chromosome 6BL at position 52 cM, providing resistance to isolate 70/99 (Fig. 6). In the Solist × Deben population substituting the Solist allele for the Deben allele was estimated to reduce the disease score from 1.00 to 0, while in the Kris × Wasmo population, substituting the Kris allele for the Wasmo allele reduced disease score from 0.88 to 0.06 (Table 4). No QTL effect was found for the population Kris × Deben (Table 2). The same QTL on chromosome 6BL was detected using isolate 08/97 (Fig. 3) and explained 29% of the phenotypic variation (Table 2). For this isolate, however, effects were significant only for the Solist × Deben population, where substitution of the Solist allele with the Deben allele reduced disease score from 0.80 to 0 (Table 4).

Discussion

Identification of QTLs for yellow rust resistance genes

All four parental varieties in this study have previously been reported to possess some resistance to *P. Striiformis* causing yellow rust (M.S. Hovmøller, submitted). Solist,

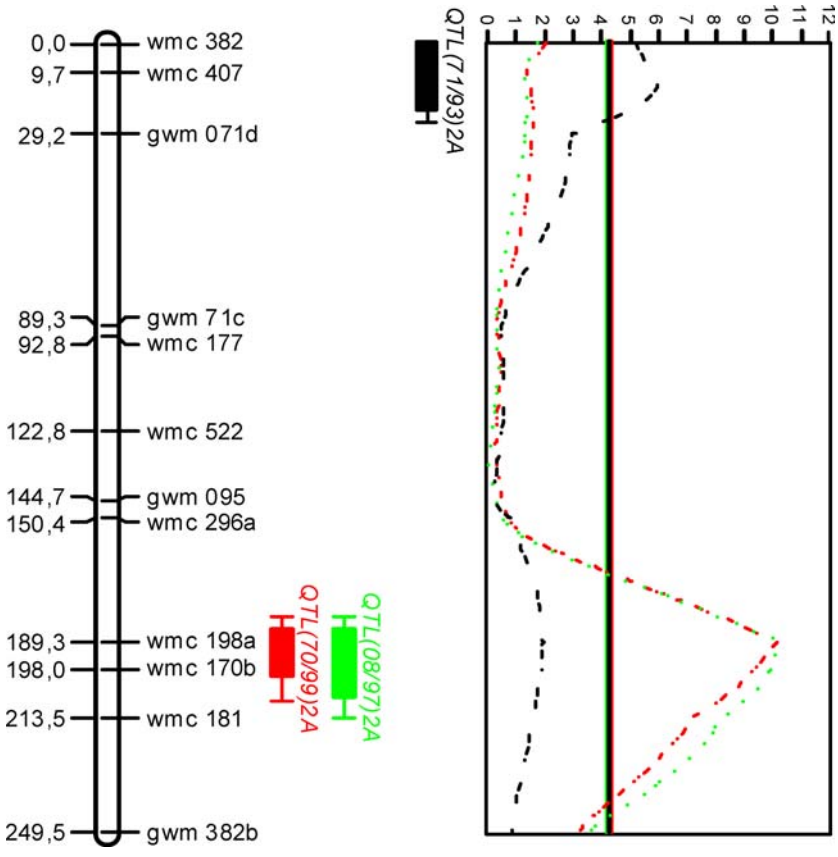

Fig. 4 Linkage map of chromosome 2A showing the QTLs identified for each of the three pathogen isolates, 71/93 (*black box*), 70/99 (*red box*) and 08/87 (*green box*) used. For each QTL identified support intervals for significant LOD score peaks are shown. The *boxes* correspond to one LOD intervals and the *antennae* to two LOD intervals. The *colour* of the *boxes* correspond to the *dotted coloured lines* of the LOD score scan while the *solid coloured lines* represent 5% genome-wide significance thresholds for each isolate

Deben and Wasmo are all believed to possess resistance originating from the cultivar CV. Their resistance towards yellow rust, however, differs considerably with Deben as the most highly resistant cultivar. The yellow rust resistance properties of Solist and Wasmo are quite similar. However, tests with various pathogen isolates virulent to CV show that Solist may have fewer genes from CV compared to Wasmo. In addition to the CV genes, Deben is believed to have at least the resistance gene *Yr2*, while Solist is believed to have the resistance gene *Yr3* (M.S. Hovmøller, submitted). Little is known about yellow rust resistance in the variety Kris. In disease tests, Kris shows the same resistance properties as the variety Strubes Dickkopf, and is believed to carry at least the yellow rust resistance gene *Yr17*. From a large survey by M.S. Hovmøller (submitted) the following resistance genes have been postulated for the four varieties: Deben *Yr2*, *Yr32*, +; Solist *Yr3*, *Yr32*, +; Wasmo *Yr32*, +, + and Kris *Yr17*, where + indicates some additional unknown resistance.

In this study, using multi-cross QTL analysis, it was possible to verify two of the four postulated known resistance genes for the three varieties Solist, Wasmo and Kris; that is, *Yr17* and *Yr32*. The remaining two postulated resistance genes, *Yr2* and *Yr3*, could not be detected in this study since all the three isolates 70/99, 08/97 and 71/93 possess virulence for *Yr2* and *Yr3*. In addition to the resistance genes previously indicated by isolate analysis, one QTL on the long arm of chromosome 2B was identified with the disease reduction provided by the allele from Deben. This allele showed a clear tendency toward disease reduction for all three isolates and a significant effect using a mixture of the isolates. According to map position, two known resistance genes *Yr5* and *Yr7* are located in the same genomic region. However, going through the pedigree of Deben there are no indications of the presence of neither *Yr5* nor *Yr7*. The lack of the resistance genes *Yr5* and *Yr7* in Deben is also supported by isolate test (M.S. Hovmøller, submitted). Therefore this QTL may represent an unknown resistance gene to yellow rust different from the resistance genes found in Solist and Wasmo. Because LOD score peaks for this QTL were found for all three isolates, this resistance gene did probably not originate from CV, but may be a unique resistance gene previously indicated by M.S. Hovmøller (submitted) based on isolate analysis.

Another apparently unknown QTL was mapped to the long arm of chromosome 6B. It provided resistance primarily by the allele from the variety Wasmo and to a lesser extent by alleles from Deben and Kris when using isolate 70/99. However, when the isolate 08/97 was used, the allele effect of Wasmo did not differ from that of Deben and Kris and for this isolate the only population with a clear QTL effect is the cross of Solist and Deben. This leads to the conclusion that alleles of the QTL from Kris, Deben and Wasmo provide resistance to yellow rust relative to the allele of the QTL from Solist.

Table 3 Analysis of single crosses: LOD score peaks with *P* values less than 0.15 at the four QTL locations on chromosomes 2AS, 2AL, 2BL and 6BL for the three wheat crosses in the study

Cross	Postulated resistance genes[a]	Isolate mix.	Isolate 70/99 *Yr1,2,3,4,6,9,17, Su, Sd*[b]	Isolate 08/97 *Yr1,2,3,4,9,17, Su, Sd*[b]	Isolate 71/93 *Yr1,2,3, CV,Sd*[b]
Kris × Deben	*Yr17* × *Yr2, Yr32,* +	2BL, $P = 0.143$			
Solist × Deben	*Yr3, Yr32,* + × *Yr2, Yr32,* +		2AL, $P < 0.001$ 6BL, $P < 0.001$	2AL, $P < 0.001$ 6BL, $P = 0.003$	
Kris × Wasmo	*Yr17* × *Yr32,* +, +	2AL, $P = 0.056$	2AL, $P = 0.057$ 6BL, $P = 0.019$	2AL, $P = 0.013$ 6BL, $P = 0.041$	2AS, $P = 0.004$

+ indicates some additional unknown resistance, *Sd* Strubes Dickkopf, *Su* Suwon92/Omar
[a]M.S. Hovmøller (submitted)
[b]Justesen et al. (2002) and M.S. Hovmøller (personal communication)

Whether this previously unmapped QTL has originated from CV cannot be clarified from the present data.

Multi-cross QTL analysis

The present study using multi-cross QTL analysis for yellow rust in four parental wheat varieties has shown that such approaches are efficient for dissection of complex genetic traits to detect QTLs that segregate only a part of the breeding material and at the same time obtain information on allelic effects from the different parents included. The two-component mixture model used here is especially suitable when the phenotype distribution is clearly non-normal, because the model of Liu and Zeng (2000) in such cases may produce large LOD score peaks at positions unlinked to any QTLs (Fig. 2). The proposed two-component mixture model is expected to have somewhat lower power and precision compared to the model of Liu and Zeng (2000) due to the extra parameters in the model. A small simulation study comprising 800 data sets of three DH populations of size 50 indicated that this loss in power and precision is only minor (results not shown) and comparable to the pattern seen in single-cross analyses (Feenstra and Skovgaard 2004). In general, more studies are needed that explore the statistical properties of multi-cross QTL mapping methods over a wide range of data set structures and genetic models. Regarding the general applicability of multi-cross QTL methods, a number of points should be made.

First, in existing applied breeding programmes, breeders annually generate a large number of related crosses, each with too few progeny for reliable QTL analysis (Jansen et al. 2003). Thus, breeders wanting to map QTLs are faced with two choices: either to design a new single cross with a large number of progeny, or to integrate QTL mapping into existing breeding strategies with many related crosses using multi-cross QTL methods. The latter choice may be less costly than the former.

Second, in any single cross, the number of segregating QTLs may be limited, and QTLs for which the two parental lines share the same genotype cannot be detected. This is in contrast to multi-cross data, where chances are much better than any given QTL will segregate in at least one of the crosses. Thus, multi-cross QTL mapping strategies allow breeders to sample more allelic variation for the trait of interest.

Third, the results of a multi-cross QTL analysis can be presented in a unified and concise manner, with, for

Table 4 Predicted mean disease resistance scores on the original 0–9 scale for individuals with one or the other parental genotype, respectively, at the QTL position

	Deben × Solist		Deben × Kris		Kris × Wasmo	
	Deben	Solist	Deben	Kris	Kris	Wasmo
QTL 2AL at 189 cM						
70/99	2.30 [1.23, 3.58]	0 [0, 0.01]	0.73 [0.59, 0.89]	0.73 [0.59, 0.89]	1.48 [0.60, 2.65]	0.01 [0, 0.12]
08/97	1.87 [0.93, 3.06]	0 [0, 0.01]	0.33 [0.24, 0.43]	0.33 [0.24, 0.43]	1.49 [0.72, 2.49]	0.01 [0, 0.13]
QTL 2AS at 12 cM						
71/93	1.18 [0.97, 1.39]	1.18 [0.97, 1.39]	0.33 [0.02, 0.97]	0 [0, 0.03]	0 [0, 0.12]	1.69 [0.88, 2.69]
QTL 2BL at 176 cM						
Mix	0.63 [0.11, 1.54]	2.95 [2.07, 3.91]	0.81 [0.19, 1.81]	3.38 [2.48, 4.35]	1.92 [1.26, 2.68]	1.92 [1.26, 2.68]
QTL 6BL at 52 cM						
70/99	0 [0, 0.01]	1.00 [0.41, 1.81]	0.73 [0.33, 1.28]	0.73 [0.33, 1.28]	0.88 [0.30, 1.72]	0.06 [0, 0.26]
08/97	0 [0, 0.04]	0.80 [0.27, 1.59]	0.33 [0.10, 0.70]	0.33 [0.10, 0.70]	0.39 [0.11, 0.83]	0.39 [0.11, 0.83]

Results are given for all three crosses and for all four significant QTL locations; numbers in brackets are approximate 95% confidence intervals

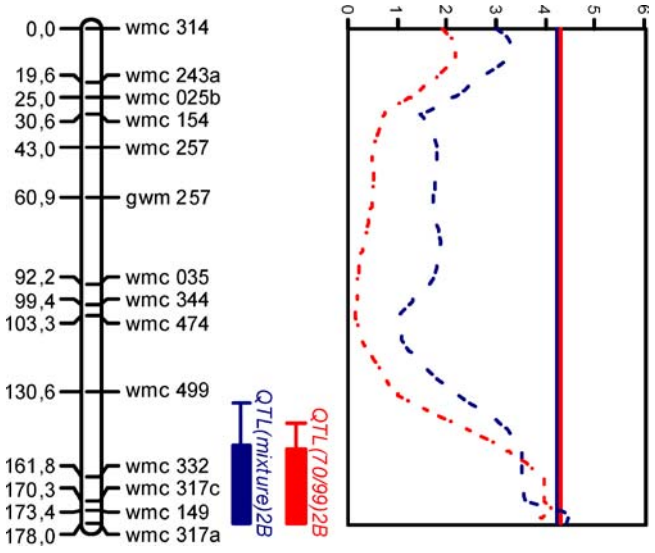

Fig. 5 Linkage map of chromosome 2B showing the QTLs identified for the pathogen isolate 70/99 (*red box*) and a mixture of all three pathogen isolates (*blue box*). For each QTL identified support intervals are shown. The *boxes* correspond to one LOD intervals and the *antennae* to two LOD intervals. The *colour* of the *boxes* correspond to the *dotted coloured lines* of the LOD score scan while the *solid coloured lines* represent 5% genome-wide significance thresholds for each isolate

example, one common LOD score curve, compared to presenting results from a number of different single QTL analyses.

Fourth, in order to allocate resources most efficiently, it would be valuable to breeders to compare the power of QTL detection and the accuracy and precision of parameter estimates from different QTL mapping strategies. Unfortunately, only few studies have considered this. Rebaï and Goffinet (1993) demonstrated analytically that analysing six related F_2 populations together is more powerful than using six independent populations of the same total sample size. On the other hand, Liu and Zeng (2000) found by computer simulation that parameter estimates from a multi-cross analysis of three F_2 populations from a

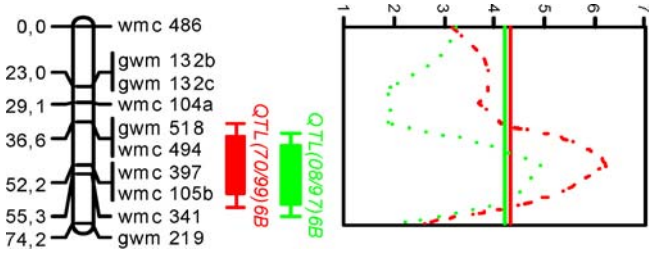

Fig. 6 Linkage map of chromosome 6B showing the QTLs identified for the pathogen isolate 70/99 (*red box*) and 08/97 (*green box*). For each QTL identified support intervals for significant LOD score peaks are shown. The *boxes* correspond to one LOD intervals and the *antennae* to two LOD intervals. The colour of the *boxes* correspond to the *dotted coloured lines* of the LOD score scan while the *solid coloured lines* represent 5% genome-wide significance thresholds for each isolate

diallel cross design had larger sampling variances than estimates from a single F_2 population with the same total number of individuals, reflecting the fact that more parameters are estimated in the diallel case. Thus, in order to achieve increased precision of QTL parameter and position estimates, it would be desirable to follow up on a multi-cross QTL analysis by constructing a single larger mapping population segregating for a given QTL. However, the strategy of using multiple smaller crosses first is important to allow breeders to identify those varieties that differ for the QTL to be mapped.

Finally, it should be noted that, the greater are the benefits of multi-cross QTL mapping, the more the parental lines overlap. In cases where the number of crosses equals or exceeds the number of parental lines, multi-cross QTL mapping may give breeders clues to how a particular QTL interacts with the rest of the genome (Jannink and Jansen 2001; Jansen et al. 2003). In the present study, three DH populations were derived from four parental lines; thus all six possible (unordered) pairwise combinations of parental lines were not exhausted. The full potential of multi-cross QTL analysis was therefore not reached in this study; still the multi-cross approach was able to detect QTL effects not found in separate analyses of the crosses (Table 2 vs. Table 3) and was able to dissect a rather complex type of disease resistance into a number of rather well-defined QTLs, some of which could be related to known genes, while others seem to be new genes.

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