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Inheritance and localisation of resistance to *Mycosphaerella graminicola* causing septoria tritici blotch and plant height in the wheat (*Triticum aestivum* L.) genome with DNA markers

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Abstract Resistance to the disease septoria tritici blotch of wheat (*Triticum aestivum* L.), caused by the fungus *Mycosphaerella graminicola* (Fuckel.) J. Schrot in Cohn (anamorph *Septoria tritici* Roberge in Desmaz.) was investigated in a doubled-haploid (DH) population of a cross between the susceptible winter wheat cultivar Savannah and the resistant cultivar Senat. A molecular linkage map of the population was constructed including 76 SSR loci and 244 AFLP loci. Parents and DH progeny were tested for resistance to single isolates of *M. graminicola* in a growth chamber at the seedling stage, and to an isolate mixture at the adult plant stage, in field trials. A gene located at or near the *Stb6* locus mapping to chromosome 3A provided seedling resistance to IPO323. Two complementary genes, mapping to chromosome 3A, one of which was the IPO323 resistance gene, were needed for resistance to the Danish isolate Risø97-86. In addition, a number of minor loci influenced the expression of resistance in the growth chamber. In the field, four QTLs for resistance to septoria tritici blotch were detected. Two QTLs, located on chromosomes 3A and 6B explained 18.2 and 67.9% of the phenotypic variance in the mean over two trials. Both these QTLs were also detected at the seedling stage with isolate Risø97-86, whereas isolate IPO323 only detected the QTL on 3A. Additionally, two QTLs identified in adult plants on chromosomes 2B and 7B were not detected at the seedling stage. Four QTLs were detected for plant height located on chromosomes 2B, 3A, 3B and on a linkage group not assigned to a chromosome. The major QTLs on 3A and on the unassigned linkage group were consistent over two trials, and the QTL on 3A seemed to be linked to a QTL for septoria tritici blotch resistance.

Keywords *Septoria tritici* · *Mycosphaerella graminicola* · Qualitative and quantitative resistance · Plant height · Doubled-haploid

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Introduction

The disease septoria tritici blotch of wheat (*Triticum aestivum* L.) is caused by the ascomycete fungus *Mycosphaerella graminicola* (Fuckel.) J. Schrot in Cohn (anamorph *Septoria tritici* Roberge in Desmaz.). This disease is widespread in wheat-growing regions all over the world and yield losses are often severe (Eyal et al. 1987; Hardwick et al. 2001). Plant breeders in Northern Europe have shown an increasing interest in breeding wheat for resistance to septoria tritici blotch. The result has been an increasing number of wheat cultivars possessing a good level of resistance, being released commercially (Anonymous 2002).

In the field, wheat cultivars rarely express complete resistance to septoria tritici blotch, but on highly resistant cultivars the disease development is delayed to a point where the impact on yield is considerably reduced (Eyal 1999). This resistance has been described as non-specific with no interactions between genes in pathogen and host (Van Ginkel and Scharen 1988; Johnson 1992). Later work provided evidence of specificity in the interaction between genotypes of the host and isolates of the pathogen when tested in the growth chamber and in the field (Kema and Van Silfhout 1997). Recently, a gene-for-gene relationship involving the gene *Stb6* in the cultivar Flame and an avirulence gene in the *M. graminicola* isolate IPO323, was described (Kema et al. 2000; Brading et al. 2002). The *Stb6* gene is one of the six genes for resistance to *M. graminicola* that have been designated, *Stb1, Stb2, Stb3, Stb4, Stb5* and *Stb6* (Wilson 1985; Somasco et al. 1996; Arraiano et al. 2001; Brading

et al. 2002). Only two of these genes, have been mapped to a chromosomal location, this is *Stb5* on 7D (Arraiano et al. 2001) and *Stb6* on 3A (Brading et al. 2002) in wheat. Resistance provided by single genes with a major effect is often relatively short-lived, as examples with powdery mildew and stripe rust have shown (Švec and Miklovičová 1998; Bayles et al. 2000). Recently, an example of a septoria tritici blotch-resistant wheat cultivar that lost the effectiveness of resistance over relatively few years was described (Cowger et al. 2000). Continuing efforts to identify and characterize new and effective sources of resistance to *M. graminicola* seem to be essential to ensure the availability of resistant wheat cultivars in the future.

The main source of inoculum of *M. graminicola* during spring and summer are the splash-dispersed pycnidiospores (Shaw and Royle 1993; Eriksen et al. 2001; Eriksen and Munk 2003). These spores are spread from the base and upwards in the crop canopy, and the amount of inoculum transported decreases rapidly with height (Shaw and Royle 1993). A relationship between short stature and susceptibility to septoria tritici blotch has been reported (Baltazar et al. 1990).

The aim of this study is to investigate the genetic control of the qualitative and quantitative resistance to septoria tritici blotch of the winter wheat cultivar Senat. Senat expresses almost complete resistance to several *M. graminicola* isolates in the growth chamber and has a high level of resistance in the field. The chromosomal location of genes providing the near immune response to specific isolates in the growth chamber was compared with the location of QTLs detected using data from field trials. The identification of such genes for quantitative and qualitative resistance, together with the elucidation of the relationship between them, will provide important information for plant breeders trying to combine different types of resistance in a cultivar.

Materials and methods

Plant material

At Sejet Plantbreeding, a cross was made between Senat and the highly susceptible winter wheat cultivar Savannah, with Savannah as the female parent. Using the wheat × maize method (Laurie and Bennett 1988), a population of 106 DH (doubled-haploid) lines was produced from the F_1 generation. Of these lines 71 were available for trials in the first season, 2000/01, and all 106 lines were available for trials in 2001/02.

Growth chamber test

The parents and the DH progeny of the cross Savannah × Senat, were tested in a growth chamber with isolates of *M. graminicola*. Of each line, 15 seeds were sown in a pot arranged in a straight line next to a net. After 10–12 days, the fully developed primary leaves were placed in a horizontal position by fixation to the net with a piece of string, and with the adaxial side of the leaves facing upwards. The inoculum of *M. graminicola* was produced by growing the fungus in liquid yeast glucose medium for 5 to 8 days. The seedlings were inoculated by applying a spore suspension of 10^6 spores/ml, with four drops of Tween 20 per 100 ml, to the horizontally fixed leaves. The pots were subsequently kept at high humidity in bags for 48 h, and then moved to transparent plexiglass boxes in which the relative humidity was above 80%. Growth chamber conditions were 20 °C with 18 h of light. Assessment of disease severity as percent coverage with necroses on ten leaves per pot was performed 21–22 days after inoculation.

Field experiments

Field experiments were performed in 2000/01 with 71 DH lines at one location, Sejet (Jutland, Denmark), and in 2001/02 with 106 DH lines at two locations, Sejet and Nr. Aaby (Fyn, Denmark). The parents and DH progeny were grown in two rows of 1 m in the 2000/01 season and in three rows in the 2001/02 season. Due to lack of seed, replicates were only possible in 2001/02 where the trials consisted of two randomised replicates. The experiments were inoculated twice with 12 days in-between inoculations. The first inoculation was performed after the flag leaves were fully expanded. The inoculum consisted of a mixture of 11 *M. graminicola* isolates, collected in Denmark in 1997 from plots of different cultivars untreated with fungicides. These 11 isolates were selected because tests on seedlings of Savannah and Senat in a growth chamber showed them to be virulent on both cultivars. Inoculum was produced in liquid medium as described above and applied as an aqueous spore suspension using a knapsack sprayer. The spore suspension had a concentration of 10^6 spores/ml and 0.5 ml/l of Tween 20 was added. The trial at Sejet was mist-irrigated for 5 min morning and evening for a few days after the inoculations.

Disease severity was assessed as percent coverage with lesions bearing the fruit bodies of *M. graminicola* on ten leaves per plot and leaf layer. In 2001, flag leaves were assessed once, 27 days after the second inoculation. In 2002, flag leaves and leaf 2 were assessed three times at an interval of 6–7 days, and starting from 9 days after the second inoculation at Sejet and 13 days at Nr. Aaby. In the following, these data are designated StbSejet01, StbSejet02 and StbNrAaby02, and the mean AUDPC over the two trials in 2002 is designated StbMean02. In the 2002 trials, plant height was measured from the soil surface to the base of the spike on the main tiller, in the following designated HtSejet02, HtNrAaby02 and the mean over the two trials HtMean02.

Molecular marker analysis

DNA was extracted from 2–3 leaves of 10-day old seedlings. The leaves were freeze-dried in 2 ml reaction tubes, two steel balls were added and the samples were milled in a ball-mill (Retsch MM200). DNA was extracted from the milled samples using the CTAB method (Saghai-Maroof et al. 1984). The molecular markers employed for the mapping were SSR (simple sequence repeat) markers developed by the WMC (Wheat Microsatellite Consortium) and Röder et al. (1998). SSR markers from the two sources were designated *Xwmc* and *Xgwm* respectively. In addition the AFLP (amplified fragment length polymorphism) technique was employed to generate sufficient marker information for a linkage map of the Savannah × Senat cross (Vos et al. 1995).

PCR reactions were performed on Perkin Elmer 2700 or 9700 thermal cyclers. Cycling conditions for microsatellites were, 1 min denaturation at 94 °C, 1 min annealing at 50, 55 or 60 °C (depending on the microsatellite) and 1 min extension at 72 °C, for 31 cycles. The samples were initially denatured for 5 min and the cycling was followed by an extension step of 7 min. The PCR reaction mix contained 0.25 μM of each primer, 2.0 mM of $MgCl_2$, 200 μM of each deoxynucleotide, 1 × *Taq* buffer, 0.5 units of *Taq* polymerase and 50 ng of template DNA, in a reaction volume of 10 μl. The SSR primers were fluorescently labelled with either 6-FAM, TET or HEX, and the fragment lengths were determined on a Megabace 1,000 automatic DNA sequencer. AFLPs were generated according to the protocol for the Perkin-Elmer AFLP Plant

Mapping Kit (Anonymous 2000). The restriction enzymes used were *Mse*I, *Pst*I and *Eco*RI. The initial screening of the parents with *Mse*I/*Eco*RI primer combinations resulted in very few AFLPs compared to *Mse*I/*Pst*I combinations, and only *Mse*I/*Pst*I primer combinations were screened on the DH lines. Pre-amplification was performed with one selective nucleotide (C) on the 3′ end of the *Mse*I primer and without selective nucleotides on the *Pst*I primer. In the main amplification, primers with three selective nucleotides on the 3′ end of the *Mse*I and two or three selective nucleotides on the 3′ end of *Pst*I primers were employed. The *Mse*I primers for the main amplification were fluorescently labelled with either 5-FAM, JOE or NED, and the fragment lengths were detected on an ABI377 automatic DNA sequencer. The AFLP fragments were named as MNN/PNN-###, where M represents the *Mse*I restriction enzyme, P represents the *Pst*I restriction enzyme, NN is the number of the primers with a core sequence matching the *Mse*I or *Pst*I adapters (primer sequences can be found at http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html) and ### is the length of the amplified fragment in base pairs.

Data analysis

A logit transformation was applied to the field assessments of septoria tritici blotch from 2001. For the 2002 field trials, data from the flag leaf and leaf 2 was combined, and the area under the disease progress curve (AUDPC) was calculated, using the formulae of Shaner and Finney (1977). The AUDPC data and the plant heights from 2002 were subjected to analysis of variance (ANOVA) according to the following model:

$$y_{ijk} = \mu + g_i + l_j + r(l)_k + (lg)_{ij} + \varepsilon_{ijk},$$

where y_{ijk} = phenotypic trait value of individual i (i = 1, 2, …, 106) at location j (j = Sejet, Nr. Aaby) in replicate k (k = 1, 2), μ = population mean, g_i = main effect of genotype, l_j = main effect of location, $r(l)_k$ = main effect of replicate nested within the location, $(lg)_{ij}$ = interaction effect between the location and the genotype and ε_{ijk} = random error assumed as N(0, σ^2). The heritability (h^2) of septoria tritici blotch resistance and plant height for 2002, was estimated using the formula of Schön et al. (1993) and the variance components estimated in the analysis of variance.

The linkage map with the molecular markers was generated using the computer software JOINMAP 2.0 for the calculation of linkage groups (Stam and Van Ooijen 1995). The order of markers on the linkage groups was determined with G-MENDEL, using the KSAR function and adjustment for segregation distortion (Holloway and Knapp 1993). The reliability of the locus order was checked using Monte Carlo simulation in G-MENDEL and a reduced map for QTL (Quantitative Trait Locus) analysis was constructed. On this map markers that did not map to unique positions, according to Monte Carlo simulation, were removed. Linkage groups were assigned to chromosomes using WMC SSR markers mapped in several different populations (Chalmers et al. 2001; Gupta et al. 2002; M. J. Christiansen, personal communication). Linkage groups were oriented with the short arm up, and distances in cM were calculated from the short toward the long arm. Figures of linkage groups were produced with MAPCHART (Voorrips 2002).

Due to the discrete distribution of the data, analysis of QTL on the disease data from growth chamber tests was performed using non-parametric Kruskal-Wallis analysis with the software MAPQTL 3.0 (Van Ooijen and Maliepaard 1996). QTLs were declared at markers with a peak in the K-statistic exceeding 13.5 corresponding to a 5% error level with 210 markers (210 statistical tests), using a standard Bonferroni correction, and assuming a chi-square distribution of the test statistic with one degree of freedom (Lynch and Walsh 1998).

On field data, QTL analysis was performed using CIM (Composite Interval Mapping) with PLABQTL (Utz and Melchinger 1996). A few outliers identified by PLABQTL were removed before the analysis. Based on a Bonferroni correction a threshold of LOD 3.6 for the 5% error level was assumed for CIM. A QTL was declared when the Bonferroni threshold was exceeded and the QTL was selected in the final stepwise regression procedure performed in PLABQTL. Estimates of phenotypic variance explained R^2 and additive effects of the QTLs were obtained from a multiple regression, including the QTLs selected during the stepwise regression procedure. This led to the exclusion of a few QTLs with LOD > 3.6; however, the models excluding these QTLs only had marginally lower R^2 values than the models including all QTLs. Cross validation (CV) was performed with PLABQTL to obtain estimates of the phenotypic variance explained by all the detected QTLs together that were less biased by model selection, and to estimate how stable the QTLs were against genotypic sampling (Utz et al. 2000). For CV the data was split in five groups, 4/5 of the genotypes were used for estimation of QTLs and the remaining 1/5 was used for validation of the estimated QTL model, i.e. estimation of QTL effects. Estimation and validation was done five times, each time using a new 1/5 of the data for validation. The split of the data and CV was repeated 200 times for each data set and the average R^2 values over the validation runs were calculated.

Results

Molecular linkage map

Out of a total of 152 SSR primer pairs 73 (48%) showed polymorphism between Savannah and Senat. SSR markers were chosen to represent as many of the 21 wheat chromosomes as possible, 65 were tested on the whole population, resulting in 77 SSR loci. A total of 56 *Mse*I/*Pst*I AFLP primer combinations were tested on the population, resulting in 268 polymorphic AFLPs, i.e. an average of 4.8 AFLP per primer combination. Of these 345 loci, 76 SSR loci, 244 AFLP loci and in addition one resistance gene locus (see below) were placed on the linkage map. The markers that were not placed on the map, were either of bad quality, unlinked AFLP markers, or were co-segregating with other markers. The numbers of AFLP markers on the three genomes provide a measure of diversity between the genomes of Savannah and Senat. Of the mapped AFLP loci, 26.9% were assigned to the A genome, 68.6% to the B genome and 4.5% was assigned to the D genome (Table 1). Chromosome group 4 was under represented with only three AFLP and five SSR markers. Markers were mapped on chromosomes 1A, 1B, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4D, 5A, 5B, 5D, 6B, 7A, 7B and 7D; 17 of the 21 wheat chromosomes were represented on the map. Eleven of the chromosome maps consisted of more than one linkage group, or included unlinked SSR markers. Six linkage groups consisting of 23 markers in total could not be assigned to a chromosome. The map length was 2,300 cM, with an average interval length of 7.9 cM, and any point on the map was within 20 cM of a marker.

Segregation distortion was evaluated using the chi-square test (χ^2). In total 79 loci (25%) showed significant distortion ($P \leq 0.05$), 71 toward the Savannah allele and eight loci toward the Senat allele. Clusters of distortion ($\geq$3 distorted markers) were found on chromosomes 2A, 3A, 3B, 4D, 6B, 7A and 7D, and on three unassigned linkage groups. Of the distorted loci 57% were found in such clusters. There was no significant difference in the

Table 1 Distribution of AFLP and SSR markers over the three genomes A, B and D and the seven chromosome groups of the molecular linkage map of the Savannah × Senat cross

Chromosome group	AFLP			SSR			Total		
	A	B	D	A	B	D	A	B	D
1	12	27	–	4	3	–	16[a]	30	–
2	20	22	1	4	9	6	24[a]	31[a]	7[a]
3	8	33	2	10	5	3	18[a]	38[a]	5
4	1	–	2	2	–	3	3[b]	–	5
5	11	21	–	2	5	2	13	26[a]	2[c]
6	–	21	–	–	5	–	–	26	–
7	8	29	5	3	3	5	11	32[a]	10[d]
Total	60	153	10	25	30	19	85	183	29
%[e]	26.9	68.6	4.5	33.8	40.5	25.7	28.6	61.6	9.8

[a] Chromosome consists of two linkage groups
[b] One SSR marker unlinked
[c] Two unlinked SSR markers
[d] Chromosome consists of four linkage groups
[e] Fraction of total AFLP markers, total SSR markers or total markers on a genome

level of distortion between AFLP and SSR loci ($\chi^2 = 2.3$; $P = 0.13$). For the whole map, more alleles originated from Savannah, 54.7%, than from Senat, due to the large number of alleles scored in the population (33,000) this difference was highly significant, $\chi^2 = 287$ corresponding to $P \ll 0.000$. Closely linked markers are difficult to map in a unique sequence due to a lack of recombinants in mapping populations. Furthermore, a marker spacing of less than 10 cM on the linkage map does not add much additional statistical power or accuracy to the QTL analysis (Darvarsi et al. 1993). Therefore, a reduced map with higher confidence in marker positions was produced for QTL analysis. The reduced map covered 1,660 cM, with an average interval length of 9.2 cM. On this map, 67 SSRs, 142 AFLPs and one resistance gene locus, remained. Here 18% of the markers showed distortion, again with no difference in distortion between SSR and AFLP markers ($\chi^2 = 0.58$; $P = 0.45$).

Means, heritability and correlations of disease resistance and plant height

There was a large difference in disease level between Savannah and Senat in growth chamber and field. The mean disease severities for Savannah and Senat in the growth chamber were 90.2 and 6.1% for isolate IPO323, and 93.1 and 10.0% for Risø97-86, with the progeny lines in-between, apart from a few lines expressing slightly lower or higher disease severity (Fig. 1A and B). In the field trial in 2001, the logit-transformed mean disease severities for Savannah and Senat were 1.6 (untransformed = 83.5%) and –2.6 (untransformed = 6.9%). In 2002, the mean AUDPC over both trials were 697.4 percent-days for Savannah and 43.6 percent-days for Senat (Table 2). For both the 2001 trial and the mean over the 2002 trails, the disease scores on the progeny were distributed between the parental cultivars, with very few lines exhibiting higher or lower disease levels (Fig. 1C and D). Thus there was no clear transgressive segregation for disease resistance. The plant height differed slightly between the parents with Savannah being the tallest. Savannah measured 66.4 cm as a mean over the two trials against a mean height of 65.3 cm for Senat, and the standard errors of the two estimates overlapped (Table 2). For plant height there was a clear transgressive segregation in the progeny ranging from 55 to 77 cm (Fig. 1E).

Table 2 Means of Savannah and Senat and their DH progeny, and estimates of variance components and heritability from the analysis of variance of the data from field trials at Sejet and Nr. Aaby in 2002. Analysed traits are AUDPC for infection with septoria tritici blotch (Stb) and plant height (Ht). $^{**}P<0.01$ and $^{***}P<0.001$ based on a *F*-test of the effect associated with the variance component

Parameter	Stb (AUDPC)	Ht (cm)
Means with standard deviations		
Sejet		
Savannah	654.7 ± 4.5	68.3 ± 0.7
Senat	33.0 ± 8.2	67.5 ± 1.5
DH lines	318.9 ± 24.2	69.3 ± 0.7
Nr. Aaby		
Savannah	740.2 ± 2.7	64.5 ± 2.2
Senat	54.2 ± 6.8	63.2 ± 2.5
DH lines	367.9 ± 14.2	64.7 ± 0.8
Total		
Savannah	697.4 ± 42.9	66.4 ± 2.5
Senat	43.6 ± 13.0	65.3 ± 3.0
DH lines	343.4 ± 31.5	67.0 ± 2.4
Variance components[a]		
σ_g^2	147,990***	98.8***
σ_{lg}^2	5,260**	7.0
σ_e^2	3,360	6.9
Heritability		
h^2	0.98	0.95

[a] σ_g^2: genotypic variance, σ_{lg}^2: location × genotype interaction variance and σ_e^2: error variance

Analysis of variance of the two field trials for 2002 attributed a very high proportion of the variance to an effect of the genotype for both disease and plant height. For the disease data there was a significant location × genotype interaction variance, but the contribution to the total variance was low. The heritability of both AUDPC

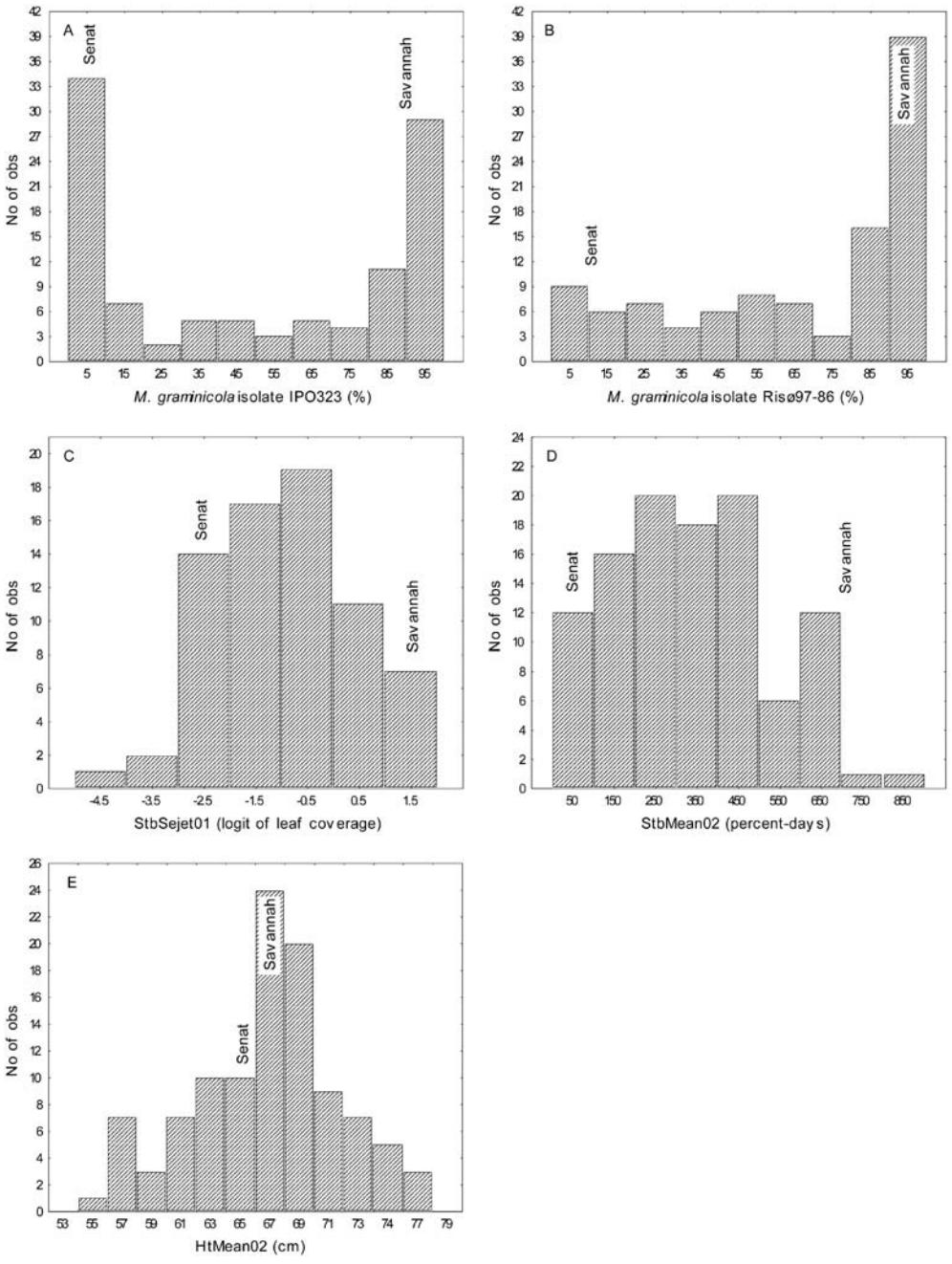

Fig. 1 Histograms showing the results of disease test and plant height measurements on the DH progeny of Savannah × Senat. Results from the growth chamber after inoculation with *M. graminicola* isolates IPO323 (**A**) and Risø97-86 (**B**). Field trials artificially inoculated with *M. graminicola* at Sejet in 2001 (**C**) and the mean of field trials at Sejet and Nr. Aaby in 2002 (**D**). Mean of plant height measurements in the 2002 trials (**E**). The reaction of the parents is indicated on the graphs

and plant height in 2002 was high, for AUDPC $h^2 = 0.98$ and for plant height $h^2 = 0.95$ (Table 2).

The phenotypic correlations between the disease assessments from the field trials was generally high 0.89–0.94. The correlations between field and growth chamber data and between the data for the two isolates IPO323 and Risø97-86, was generally lower, 0.45–0.54, but significant (Table 3). The correlation between the plant height measurements of the two trials was high, 0.87, but there was no correlation between plant height and disease level.

Specific resistance to isolates of *M. graminicola* in the growth chamber

A total of 16 *M. graminicola* isolates collected in Denmark and one Dutch isolate IPO323 (obtained from G.H.J. Kema, Plant Research International, Wageningen, The Netherlands), were tested on Savannah and Senat in the growth chamber. Five isolates were avirulent on Senat and all 17 isolates were virulent on Savannah (data not shown). Two of the avirulent isolates, IPO323 and Risø97-86, gave a particularly good differentiation between Savannah and Senat. These two isolates were found to differ in their reaction pattern on the cultivar Flame,

Table 3 Phenotypic correlations between, septoria tritici blotch infection (Stb) in field trials, at Sejet and Nr. Aaby, and from growth chamber tests with *M. graminicola* isolates IPO323 and Risø97-86 and plant height (Ht). ***$P < 0.001$

Type	StbSejet01	StbSejet02	StbNrAaby02	StbMean02	IPO323	Risø97-86	HtSejet02	HtNrAaby02
StbSejet01								
StbSejet02	0.89***							
StbNrAaby02	0.90***	0.94***						
StbMean02	0.88***	0.97***	0.97***					
IPO323	0.51***	0.48***	0.45***	0.48***				
Risø97-86	0.47***	0.54***	0.48***	0.51***	0.53***			
HtSejet02	–0.16	–0.04	–0.07	–0.09	–0.18	–0.11		
HtNrAaby02	–0.12	–0.09	–0.13	0.15	–0.16	–0.13	0.87***	
HtMean	–0.14	–0.07	–0.14	–0.12	–0.18	–0.12	0.96***	0.97***

Table 4 Segregation, based on growth chamber experiments, of specific resistance to the *M. graminicola* isolates IPO323 and Risø97-86 in the doubled-haploid population from the cross Savannah × Senat

Isolate	Observed number		Total	Expected ratio	χ^2	*P*
	Resistant	Susceptible				
IPO323	43	52	95	1:1	0.85	0.36
Risø97-86	22	74	96	1:3	0.22	0.64

Table 5 QTLs for resistance to the *M. graminicola* isolates IPO323 and Risø97-86 detected in the DH progeny of Savannah × Senat, in the growth chamber

QTL	Chromosome arm	Marker	Position (cM)	K[a]	Effect (%)[b]
IPO323					
QStb.risø-3A.1	3AS	IPO323[c]	10.8	67.3	–70.0
QStb.risø-3A.2	3AS	*Xwmc505*	60.0	20.5	–37.0
QStb.risø-6B.1	6BS	*M48/P32-112*	80.8	17.3	–31.8
Risø97-86					
QStb.risø-3A.1	3AS	IPO323[c]	10.8	33.5	–40.4
QStb.risø-3A.2	3AS	*Xwmc388a*	58.0	20.2	–30.6
QStb.risø-3B	3BL	*M62/P38-373*	4.9	13.6	–23.4
QStb.risø-6B.2	6BC[d]	*Xwmc341*	101.5	14.2	–30.0

[a] K-statistic for Kruskal-Wallis analysis with MAPQTL, 5% level of significance $K \geq 13.5$
[b] The difference in percentage disease between the means of the two marker classes, a negative value indicates that Senat contributes the resistance allele
[c] Locus for resistance to IPO323 when the data is treated as qualitative data
[d] C: close to centromere

IPO323 being avirulent and Risø97-86 being highly virulent (data not shown). The isolates IPO323 and Risø97-86 were tested on the progeny of the cross Savannah × Senat (Fig. 1A and B). With isolate IPO323, the progeny lines segregated into resistant and susceptible classes. Lines with coverage of necroses of less than 30% in growth chamber tests were classified as resistant and lines with more than 50% necroses as susceptible. The classification of lines with between 30 and 50% necrosis was considered uncertain, and these lines were treated as missing values in the qualitative analysis. Segregation for resistance to IPO323 was in agreement with a 1:1 ratio, suggesting that one gene was responsible for the resistance (Table 4). This gene was mapped to the short arm of chromosome 3A, 4.9 cM from the microsatellite marker *Xgwm369* (Fig. 2). The segregation of resistance to Risø97-86 was more quantitative (Fig. 1B). However, a large number of lines were clearly susceptible with more than 80% necrosis and fewer lines were clearly resistant. The same classification into resistant and susceptible lines, as for IPO323, resulted in a segregation ratio not significantly different from 1:3, suggesting the involvement of two complementary genes.

Detection of QTLs for resistance to septoria tritici blotch

The growth chamber data for IPO323 and Risø97-86 was treated as quantitative data, and using the molecular linkage map QTL analysis was performed. Due to the non-normal distribution of the data, a non-parametric analysis of single markers was applied using the Kruskal-Wallis test. For resistance to IPO323, a very highly significant QTL (*QStb.risø-3A.1*) was found at the IPO323 resistance gene locus, K = 67.3 (Table 5). Another peak in the K-statistic was found at a position 49.2 cM further down on the short arm of chromosome 3A at the SSR locus *Xwmc505* (*QStb.risø-3A.2*), and finally a QTL was found on chromosome arm 6BS, at the AFLP marker *M48/P32-112* (*QStb.risø-6B.1*). The two

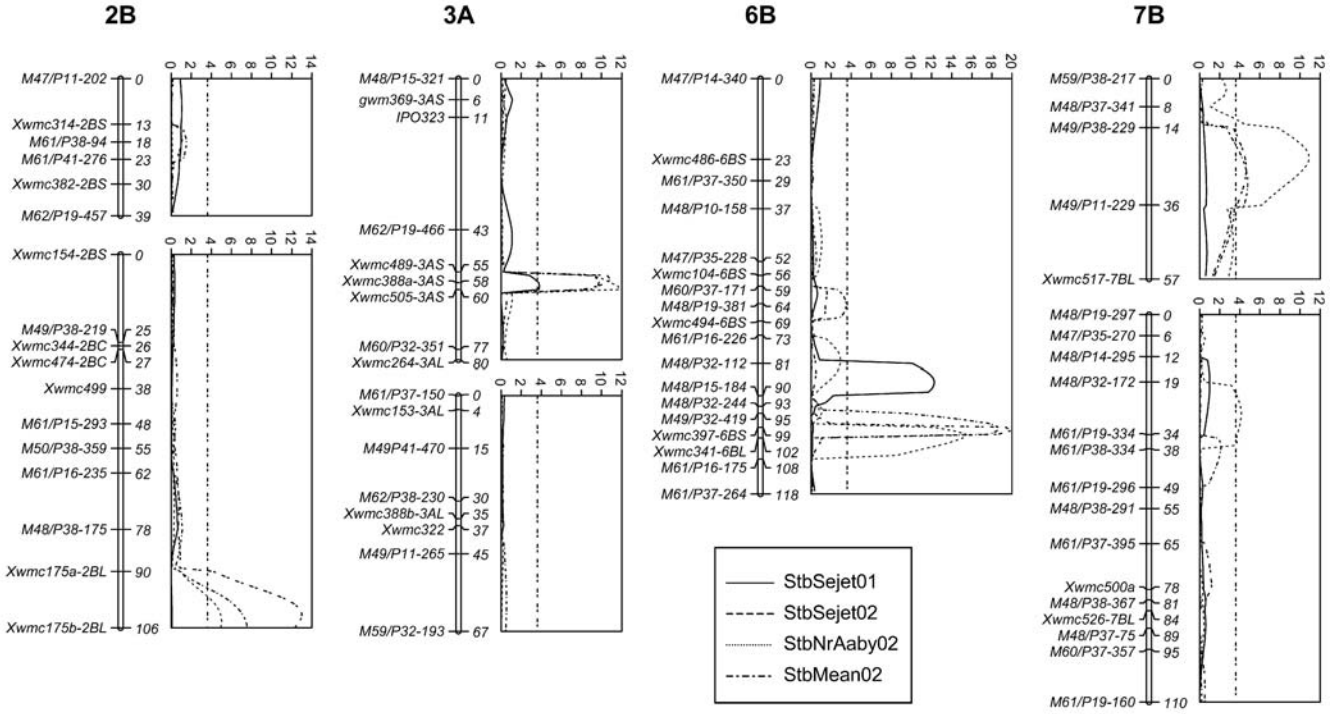

Fig. 2 Linkage map and LOD scans for composite interval mapping for chromosomes where QTLs for resistance against septoria tritici blotch was detected in DH progeny of Savannah × Senat. The constant line at LOD 3.6 represents the 5% significance threshold. C: close to the centromere

latter QTLs had K values of 20.5 and 17.3, though much lower than K = 67.3 for the resistance gene locus; these values were still highly significant. For Risø97-86 four QTLs were found. The two most-significant QTLs were located on chromosome arm 3AS at the IPO323 resistance gene locus and at *Xwmc388a* closely linked to *Xwmc505*. These two QTLs are thus located on the same positions as the QTLs detected on 3AS with isolate IPO323. The remaining two QTLs for resistance to Risø97-86 were just significant and located on chromosome arm 3BL at the AFLP marker *M62/P38-373* (*QStb.risø-3B*) and chromosome 6B, close to the centromere, at the SSR marker *Xwmc341* (*QStb.risø-6B.2*).

Composite interval mapping was performed on disease data from the three field trials and on the mean of the two trials in 2002 (Table 6). A QTL (*QStb.risø-6B.2*), with a very high effect on disease resistance, was detected on chromosome 6B in all four data sets (StbSejet01, StbSejet02, StbNrAaby02 and StbMean02). For StbSejet02, StbNrAaby02 and in their mean, StbMean02, this QTL was located at approximately the same position, 99–101 cM, flanked by the SSR markers *Xwmc397* at the position 98.5 cM and *Xwmc341* at the position 101.5 cM, close to the centromere of chromosome 6B (Table 6; Fig. 2). This QTL was detected with LOD scores of 19.8, 15.5 and 18.7, explaining 66.6, 67.0 and 67.9% of the phenotypic variance in the two trials and their mean. The QTL on 6B detected with the StbSejet01 data was located at the position 86 cM, and had a LOD score of 12.3 and R^2 = 58.2%. However, this QTL was detected in only 38.3% of the estimation runs of the CV analysis, whereas in 30.5% of the estimation runs a QTL was detected in the interval 97–103 cM on 6B (data not shown). This may have been caused by the low number of progeny tested in the Sejet 2001 trial, which made the results quite sensitive to genotypic sampling. Consequently, the QTL on 6B detected in StbSejet01 was probably identical to the QTL at 99–101 cM detected in the other two trials. The QTL on chromosome 6B was located at the same position as the QTL detected with the isolate Risø97-86 in the growth chamber. Another QTL with a large effect on resistance to septoria tritici blotch was detected on chromosome 3A (*QStb.risø-3A.2*) at approximately the same location in all four data sets, 57–60 cM, closely linked to *Xwmc388a* and *Xwmc505* at 58 and 60 cM, on the short chromosome arm. The LOD scores were 3.8, 10.8, 11.8 and 9.7 and the QTL explained 23.9, 24.0, 11.5 and 18.2% of the phenotypic variance in the three trials and in the mean of the 2002 trials, respectively. A QTL was detected at the same position with the isolates IPO323 and Risø97-86 in the growth chamber. A QTL on 2BL (*QStb.risø-2B*), at 102–106 cM, was detected in StbSejet02, StbNrAaby02 and StbMean02, with LOD scores of 13.0, 5.0 and 7.6, and with R^2 values of 35.7, 17.4 and 25.9%. Finally, on 7B a QTL (*QStb.risø-7B*) was detected at 22–30 cM with LOD scores of 4.6, 10.9 and 4.8 and with R^2 values of 10.8, 15.8 and 12.2%, in StbSejet02, StbNrAaby02 and StbMean02, respectively. Interactions between the detected QTLs were not significant in the final multiple regression. The detected QTLs explained a high propor-

Table 6 QTLs for resistance to septoria tritici blotch (Stb) and plant height (Ht) detected by composite interval mapping for the individual trials in 2001 and 2002, and in addition for means over locations in 2002

QTL	Chromosome arm[a]	Position (cM)	Support interval[b]	LOD	R^2 (%)[c]	Additive effect[d]
StbSejet01						
QStb.risø-3A.2	3AS	59	55–61	3.8	23.9	–0.46
QStb.risø-6B.2	6BS	86	82–90	12.3	58.2	–1.05
Total					62.1	
Total CV					37.7	
StbSejet02						
QStb.risø-2B	2BL	102	98–106	13.0	35.7	–73.5
QStb.risø-3A.2	3AS	57	55–59	10.8	24.0	–47.5
QStb.risø-6B.2	6BC	100	98–101	19.8	66.6	–126.0
QStb.risø-7B	7B	26	15–37	4.6	10.8	–34.8
Total					77.5	
Total CV					56.7	
StbNrAaby02						
QStb.risø-2B	2BL	104	98–106	5.0	17.4	–50.8
QStb.risø-3A.2	3AS	60	57–61	11.8	11.5	–36.2
QStb.risø-6B.2	6BC	101	100–103	15.5	67.0	–148.9
QStb.risø-7B	7B	22	18–28	10.9	15.8	–50.8
Total					73.2	
Total CV					42.4	
StbMean02						
QStb.risø-2B	2BL	106	100–106	7.6	25.9	–55.9
QStb.risø-3A.2	3AS	58	55–61	9.7	18.2	–43.8
QStb.risø-6B.2	6BC	99	97–102	18.7	67.9	–138.8
QStb.risø-7B	7B	30	19–37	4.8	12.2	–39.7
Total					75.5	
Total CV					51.2	
HtSejet02						
QHt.risø-3A	3AS	62	59–67	17.3	35.0	2.84
QHt.risø-3B	3BL	12	7–14	6.3	14.2	–1.54
QHt.risø-1X	1X	25	20–38	4.9	23.2	–2.19
Total					44.4	
Total CV					19.5	
HtNrAaby02						
QHt.risø-2B	2BL	44	38–55	4.8	8.0	–1.30
QHt.risø-3A	3AS	80	77–80	6.3	24.0	2.27
QHt.risø-1X	1X	41	36–46	11.1	28.6	–2.91
Total					40.4	
Total CV					11.2	
HtMean02						
QHt.risø-3A	3AS	62	58–69	10.5	23.0	2.07
QHt.risø-1X	1X	41	35–47	8.3	31.8	–2.87
Total					42.9	
Total CV					16.5	

[a] C: close to centromere; 1X: Linkage group not assigned to a chromosome
[b] Support interval with a LOD fall of 1 expressed as a position on the chromosome in cM
[c] The phenotypic variance explained by the individual QTLs in a simultaneous multiple regression, the total adjusted phenotypic variance explained and the mean phenotypic variance explained, estimated using the validation data sets in the Cross Validation (CV) analysis
[d] Additive effects, calculated as half the difference between the genotypic values of the two marker genotypes. A negative effect indicates that the QTL allele from Senat contributes the resistance, or reduces the plant height. Units are StbSejet01: logit(leaf coverage), StbSejet02, StbNrAaby02 and StbMean02: percent-days (AUDPC) and HtSejet02, HtNrAaby02 and HtMean02: cm

tion of the total phenotypic variance in the data, R^2 = 62.1–77.5%. These R^2 values were reduced by approximately 1/3 when estimated in the validation data sets of the CV analysis (Table 6).

Detection of QTLs for plant height

The plant height was measured in the two trials at Sejet and Nr. Aaby in 2002. For this trait a relatively large QTL was detected on chromosome 3A (*QHt.risø-3A*) located at the position 62 cM (short arm) in the Sejet data, 80 cM

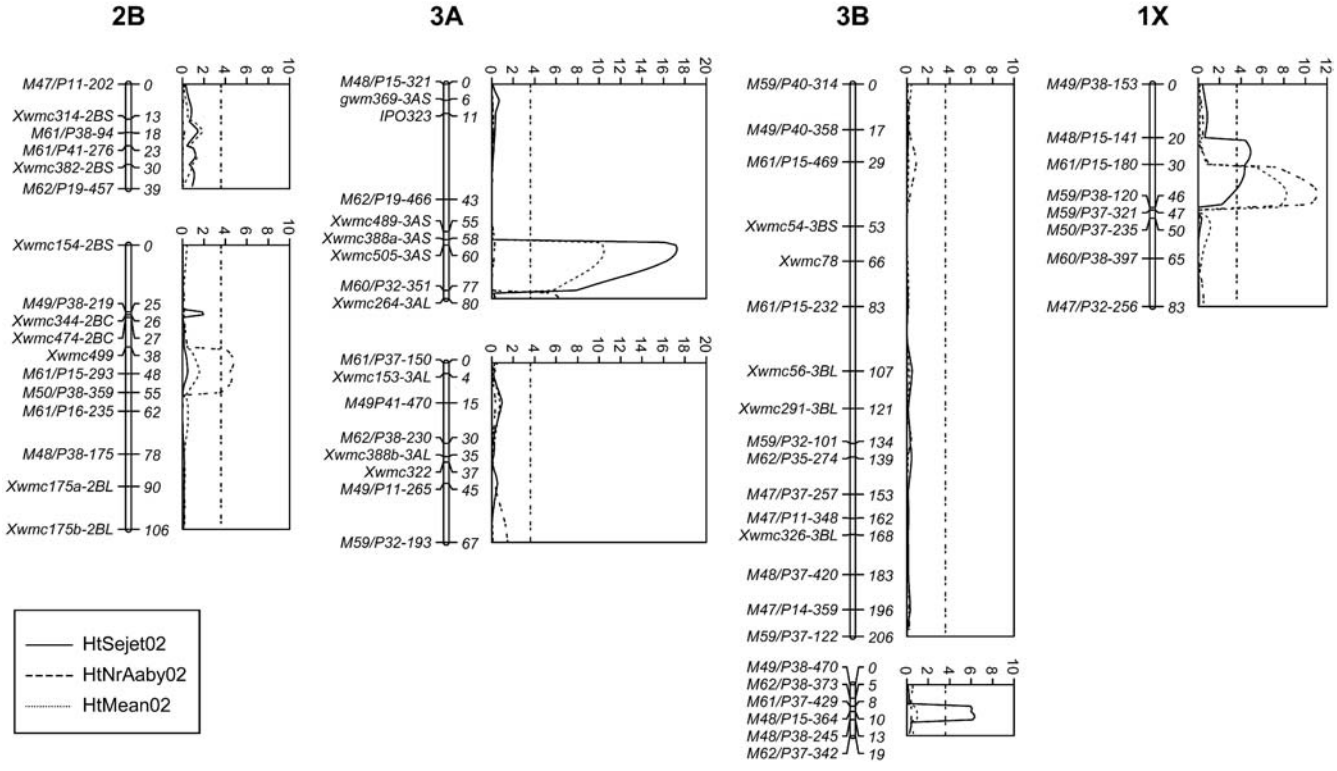

Fig. 3 Linkage map and LOD scans for composite interval mapping for chromosomes where QTLs for plant height was detected in the DH progeny of Savannah × Senat. The constant line at LOD 3.6 represents the 5% significance threshold. C: close to centromere

(long arm) in the Nr. Aaby data and at the position 62 cM (short arm) in the mean of the two trials (Table 6; Fig. 3). The LOD scores were 17.3, 6.3 and 10.5, and the effect on plant height was 2.84, 2.27 and 2.07 cm with the increasing allele being contributed by Senat. The QTL in the Nr. Aaby trial was detected within the support interval of 77–80 cM with a frequency of 45.2% in the CV analysis, but in the interval 58–69 cM a QTL was detected with a frequency of 41.0% (data not shown). The position of this QTL is thus affected by genotypic sampling and it is likely to be the same QTL as the one detected in the Sejet trial, at 62 cM. The only other QTL (*QHt.risø-1X*) detected on the same linkage group in both trials, was located on linkage group 1X at the position 25 cM in Sejet, 41 cM in Nr. Aaby and 41 cM in the mean of the two trials, with overlapping support intervals. The LOD scores were 4.9, 11.1 and 8.3 and the allele increasing the height was contributed by Savannah, and had the effects 2.19, 2.91 and 2.87 cm. Additional QTLs were detected on chromosome arms 3BL at Sejet and 2BL in Nr. Aaby. The effects of the QTLs for plant height was additive, and no interactions were significant. The phenotypic variance explained by these QTLs was 44.4, 40.4 and 42.9% for the two trials and their mean. This was reduced to 19.5, 11.2 and 16.5% in the validation data sets of the CV analysis.

Discussion

Molecular linkage map

The level of polymorphisms between Savannah and Senat was relatively high considering they are both modern commercial cultivars, and the generally low level of diversity usually found in bread wheat (Hazen et al. 2002). The level of SSR polymorphism was 48% of the tested markers, which is considerably higher than 15–30% found in the parents of four Australian mapping populations (Harker et al. 2001). The AFLP polymorphism was lower between Savannah and Senat, 4.8 AFLPs per primer combination, compared to a level of 7.7 between the parents of three of the Australian mapping populations (Chalmers et al. 2001). In agreement with many other studies, a very low level of marker polymorphism was detected on the D genome compared to the A and B genomes in the present study; the highest number of polymorphisms was detected on the B genome (Chalmers et al. 2001; Hazen et al. 2002; Huang et al. 2002). A part of the polymorphism of the B genome is caused by the presence of the IBL/IRS wheat-rye translocation in Savannah. The generally low level of polymorphisms of the D genome in bread wheat is probably caused by the recent introgression of the D genome, which has led to less time for genetic divergence of this genome (McFadden and Sears 1946). Furthermore, maybe only a fraction

of the diversity in the gene pool of the progenitor species of the D genome, *Aegilops tauschii*, is preserved in present-day bread wheat (Dvorak et al. 1998). The apparent low level of polymorphism on chromosome group 4 seen in this study has also been found for chromosomes 4B and 4D in other mapping populations (Chalmers et al. 2001; Hazen et al. 2002), and in a sample of 75 spring wheat cultivars (Christiansen et al. 2002).

Segregation distortion is a deviation from the expected Mendelian proportions of genotypes at a given locus in a segregating population. In the Savannah × Senat population, 25% of markers deviated from the 1:1 segregation expected for a DH population. A wide range of distortion levels has been observed in segregating populations of wheat. In three DH populations, with bread wheat cultivars as parents, produced by wheat × maize hybridisation, the levels of distortion ranged from 1.8% to 12.5% of the markers (Kammholz et al. 2001). In another bread wheat DH population, produced by microspore culture, 27% of markers deviated significantly from Mendelian expectations (Cadalen et al. 1997), and in a durum wheat recombinant inbred population 18.6% of markers showed distortion (Blanco et al. 1998). The level of distortion in the Savannah × Senat population is thus relatively high, but not unusual. However, the bias in the direction of distortion toward the one parent (Savannah) was not observed in the other studies. Competition between gametes caused by genes that act as partial lethal factors can distort the segregation at linked marker loci, by eliminating a proportion of the gametes possessing such a factor (Cheng et al. 1998). The SSR marker *Xwmc314* on chromosome arm 2BS, showed distortion toward the Savannah allele (χ^2 = 7.08; P = 0.008). Segregation distortion at *Xwmc314* has previously been found in two other wheat × maize DH populations (Kammholz et al. 2001), suggesting the presence of a distortion factor linked to this SSR locus. The bias of distortion toward the Savannah genome in the present study suggests that Savannah possess genes providing a superior ability to regenerate plants from the DH production process, or that Senat possess partial lethal factors resulting in reduced recovery of DH plants. Different steps during DH production could induce differential survivability, e.g. the recovery of green plants from embryo culture or recovery after chromosome doubling with colchicine. Such effects have been observed in an anther culture-derived DH population of wheat, where QTLs for green plant regeneration were located to chromosome arms 2AL, 2BL and 5BL (Torp et al. 2001). A region of distortion toward the Savannah genome was found on chromosome arm 2AL in the Savannah × Senat population, suggesting that QTLs for anther culture may also influence the regeneration of green plants from embryos.

Resistance to septoria tritici blotch

The resistance gene *Stb6*, providing resistance to *M. graminicola* isolate IPO323, has been mapped to the short arm of chromosome 3A, 2 cM from the microsatellite marker *Xgwm369* (Brading et al. 2002). The gene for resistance to IPO323 in Senat mapped to approximately the same location, 4.9 cM from *Xgwm369*. The allele of *Xgwm369* present in Senat is 300 bp in size, whereas the allele in Flame is 197 bp (Brading et al. 2002). These results can be interpreted as the same locus providing the resistance to IPO323 in the two cultivars, but a recombination event has associated *Stb6* with a different *Xgwm369* allele in Senat, or the SSR has mutated. Mutation frequencies in SSRs are high (Goldstein and Pollock 1997). Alternatively, different alleles at the *Stb6* locus or different genes at closely linked loci could be present in the two cultivars. When the data were treated as data for a quantitative trait additionally two loci were detected, *QStb.risø-3A.2* and *QStb.risø-6B.1*, with an effect on resistance to IPO323. However, these two loci had a minor effect compared to *QStb.risø-3A.1* at the IPO323 resistance gene, which was needed for the expression of resistance. The 1:3 segregation of resistance and susceptibility to Risø97-86 in the Savannah × Senat progeny, suggested that two complementary genes control resistance to Risø97-86 in Senat. A careful inspection of the genotypes of the resistant lines showed that the two loci *QStb.risø-3A.1* and *QStb.risø-3A.2* were required for expression of resistance to the Risø97-86 isolate (data not shown). For powdery mildew on barley, examples of resistance genes that require the presence of genes at different loci for expression of resistance have been described in detail. The *Rar1* and *Rar2* genes are often necessary for the expression of some of the race-specific resistance alleles at the *Mla* locus, and the *Ror1* and *Ror2* genes are necessary for the expression of the non-race-specific resistance at the *mlo* locus (Peterhansel et al. 1997). Further, two loci *QStb.risø-3B* and *QStb.risø-6B.2*, having a minor effect on resistance to Risø97-86 were also identified. The minor loci for resistance to IPO323 and Risø97-86 that was detected, is the likely reason for the expression of medium disease severity by a number of DH lines in the growth chamber tests (data not shown). The involvement of several loci in resistance to single isolates of *M. graminicola* has previously been reported (Simón et al. 2001). By analysing chromosome substitution lines, several chromosomes in two wheat lines, a synthetic hexaploid wheat line and a *Triticum spelta* line were identified as carrying resistance to single isolates of *M. graminicola*. Up to six chromosomes were found to contribute to resistance against the same isolate (Simón et al. 2001). In the tetraploid durum line Coulter (*T. turgidum*), resistance to a *M. graminicola* isolate was controlled by two independent genes (McCartney et al. 2002). In this case the genes were expressed independently over each other and only one was needed for resistance; however, against another isolate only one of the genes proved effective.

In the Savannah × Senat cross, some of the genes providing the resistance to single isolates in the growth chamber appear to be the same that are providing resistance in the field, whereas others are not. Three QTLs, *QStb.risø-3A.1, QStb.risø-3B* and *QStb.risø-6B.1*, were detected at the seedling stage with specific isolates but not at the adult plant stage in the field. One of these QTLs, *QStb.risø-3A.1*, located at the putative *Stb6* resistance-gene locus, was effective against both the IPO323 and the Risø97-86 isolate. The *Stb6* gene has been reported to be effective against the IPO323 isolate, at the adult plant stage (Brading et al. 2002). The lack of effect at the adult plant stage in this study is not necessarily surprising, as the isolates chosen for the inoculation of the field trials were virulent on Senat when inoculated as single isolates on seedlings. The other two seedling stage QTLs (*QStb.risø-3B* and *QStb.risø-6B.1*) are either not effective at the adult plant stage or they are isolate-specific. Two QTLs, *QStb.risø-2B* and *QStb.risø-7B*, detected in adult plants were not detected in seedlings, and these QTLs are maybe only effective at the adult plant stage. The QTLs, *QStb.risø-3A.2* and *QStb.risø-6B.2*, were effective at both the seedling and the adult plant stages. Kema and Van Silfhout (1997) similarly found examples of resistance to *M. graminicola* isolates that was expressed at both the seedling and adult plant stages, and resistance that was only expressed at the seedling plant stage. In mapping studies of quantitative resistance in barley, some QTLs for powdery mildew mapped using field data were located at the same position as known qualitative resistance genes, or at the same position as QTLs mapped with single isolates on detached leaves (Backes et al. 1996, 2003).

The total phenotypic variance explained by the detected QTLs in the field data ranged from 62.1 to 77.5%, which is high, and it is unlikely that any major QTLs are segregating in the population that was not detected in this study. The level of phenotypic variance explained for the individual QTLs summed to more than the total (Table 6). Such over-estimation of QTL effects is commonly observed, especially for QTLs detected with low statistical power (Lynch and Walsh 1998). Senat contributed all the alleles providing resistance to septoria tritici blotch at the detected loci, and this is in agreement with the absence of transgressive segregation.

QTLs for plant height

A large number of genes influence plant height. The height-reducing genes (*Rht*) located on the short arms of chromosomes 4B and 4D have a large effect and have been extensively used in wheat breeding (Börner et al. 1996). Chromosome 4B was not represented in the map of Savannah × Senat and only few markers mapped to 4D. Consequently, an effect of the height-reducing genes was unlikely to be detected in this study, even if the population segregates for these genes. A QTL for plant height, *QHt.risø-3A*, was detected at approximately the same position as the QTL *QStb.risø-3A.2* for resistance to septoria tritici blotch. One possible interpretation is that a QTL for plant height is located at this position, and this height QTL is having a pleiotropic effect on resistance, as Senat contributed the alleles increasing plant height and resistance. To remove the effect of plant height on disease development in the trials, artificial inoculations were performed after flag-leaf emergence. However, disease did occur naturally in the trials and natural inoculum may have affected disease levels on the flag leaf and leaf 2. The resistance QTL was also detected in the growth chamber, where plant height could not have had any influence on disease development. Consequently, linkage between a gene(s) with an effect on resistance and a gene(s) with an effect on plant height on chromosome 3A is the most-likely reason. This is an encouraging result, since it shows that it is possible to select for resistance to septoria tritici blotch without increasing plant height. This is also suggested by the lack of phenotypic correlation between plant height and resistance in this population. Both parents contributed alleles for increased plant height, Savannah at *QHt.risø-1X* and Senat at *QHt.risø-3A*, and this would be the reason for the transgressive segregation observed in the progeny. The phenotypic variance for plant height explained by the detected QTLs was 40.4–44.4%. This is low compared to the high heritability (95%) estimated from the 2002 trials. Other loci with an effect on plant height should be segregating in this population, perhaps at the *Rht* loci on 4B and 4D or in other parts of the genome that was not covered with markers. A major QTL for plant height has previously been located to the short arm of chromosome 3A, explaining 38.2% of the phenotypic variance for this trait (Shah et al. 1999). A minor QTL for plant height has been detected in the ITMI population located close to the centromere on the long arm of chromosome 3A (Börner et al. 2002).

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References

Anonymous (2000) AFLP plant mapping protocol. Applied Biosystems, P/N 4303146C. www.appliedbiosystems.com

Anonymous (2002) Sortsforsøg 2002. Danish Institute of Agricultural Sciences and The Danish Agricultural Advisory Centre

Arraiano L, Worland AJ, Ellerbrook C, Brown JKM (2001) Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6*x*'. Theor Appl Genet 103:758–764

Backes G, Schwarz G, Wenzel G, Jahoor A (1996) Comparison between QTL analysis of powdery mildew resistance in barley based on detached primary leaves and on field data. Plant Breed 115:419–421

Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A (2003) Localisation of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor Appl Genet 106:353–362

Baltazar BM, Scharen AL, Kronstad WE (1990) Association between dwarfing genes 'Rht_1' and 'Rht_2' and resistance to *Septoria tritici* blotch in winter wheat (*Triticum aestivum* L. em Thell). Theor Appl Genet 79:422–426

Bayles RA, Flath K, Hovmøller MS, de Vallavieille-Pope C (2000) Breakdown of the *Yr17* resistance to yellow rust of wheat in northern Europe. Agronomie 20:805–811

Blanco A, Bellomo MP, Cenci A, De Giovanni C, D'Ovidio R, Iacono E, Laddomada B, Pagnotta MA, Porceddu E, Sciancalepore A, Simeone R, Tanzarella OA (1998) A genetic linkage map of durum wheat. Theor Appl Genet 97:721–728

Börner A, Plaschke J, Korzun V, Worland AJ (1996) The relationship between the dwarfing genes of wheat and rye. Euphytica 89:69–75

Börner A, Schuman E, Fürste A, Cöster H, Leithold B, Röder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105:921–936

Brading PA, Verstappen ECP, Kema GHJ, Brown JKM (2002) A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the septoria tritici blotch pathogen. Phytopathology 92:439–445

Cadalen T, Boeuf C, Bernard S, Bernard M (1997) An intervarietal molecular marker map in *Triticum aestivum* L. Em. Thell. and comparison with a map from a wide cross. Theor Appl Genet 94:367–377

Chalmers KJ, Campbell AW, Kretschmer J, Karakousis A, Henschke PH, Pierens S, Harker N, Pallotta M, Cornish GB, Shariflou MR, Rampling LR, McLauchlan A, Daggard G, Sharp PJ, Holton TA, Sutherland MW, Appels R, Langridge P (2001) Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust J Agric Res 52:1089–1119

Cheng R, Kleinhofs A, Ukai Y (1998) Method for mapping a partial lethal-factor locus on a molecular-marker linkage map of a backcross and doubled-haploid population. Theor Appl Genet 97:293–298

Christiansen MJ, Andersen SB, Ortiz R (2002) Diversity changes in an intensively bred wheat germplasm during the 20th century. Mol Breed 9:1–11

Cowger C, Hoffer ME, Mundt CC (2000) Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar. Plant Pathol 49:445–451

Darvasi A, Weinreb A, Minke V, Weller JI, Soller M (1993) Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134:943–951

Dvorak J, Luo MC, Yang ZL, Zhang HB (1998) The structure of the *Aegilops taushii* gene pool and the evolution of hexaploid wheat. Theor Appl Genet 97:657–670

Eriksen L, Shaw MW, Østergård H (2001) A model of the effect of pseudothecia on genetic recombination and epidemic development in populations of *Mycosphaerella graminicola*. Phytopathology 91:240–248 and Erratum 519

Eriksen L, Munk L (2003) The occurrence of *Mycosphaerella graminicola* and its anamorph *Septoria tritici* in winter wheat during the growing season. Eur J Plant Pathol 109:253–259

Eyal Z (1999) Breeding for resistance to *Septoria* and *Stagonospora* diseases of wheat. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria on cereals: a study of pathosystems. CABI Publishing, Cambridge, pp 332–344

Eyal Z, Scharen AL, Van Ginkel M (1987) The septoria diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico

Goldstein DB, Pollock DD (1997) Launching microsatellites: a review of mutation processes and methods of phylogenetic interference. J Hered 88:335–342

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder M, Gautier M-F, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Hayden MJ, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Hardwick NV, Jones DR, Slough JE (2001) Factors affecting diseases of winter wheat in England and Wales, 1989–98. Plant Pathol 50:453–462

Harker N, Rampling LR, Shariflou MR, Hayden MJ, Holton TA, Morell MK, Sharp PJ, Henry RJ, Edwards KJ (2001) Microsatellites as markers for Australian wheat improvement. Aust J Agric Res 52:1121–1130

Hazen SP, Leroy P, Ward RW (2002) AFLP in *Triticum aestivum* L.: patterns of genetic diversity and genome distribution. Euphytica 125:89–102

Holloway JL, Knapp SJ (1993) GMendel 3.0 users guide. Oregon State University, Department of Crop and Soil Science

Huang XQ, Börner A, Röder MS, Ganal MW (2002) Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor Appl Genet 105:699–707

Johnson R (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:3–22

Kammholz SJ, Campbell AW, Sutherland MW, Hollamby GJ, Martin PJ, Eastwood RF, Barcley I, Wilson RE, Brennan PS, Sheppard JA (2001) Establishment and characterisation of wheat genetic mapping populations. Aust J Agric Res 52:1079–1088

Kema GHJ, Van Silfhout CH (1997) Genetic variation for virulence and resistance in the wheat *Mycosphaerella graminicola* pathosystem III. Comparative seedling and adult plant experiments. Phytopathology 87:266–272

Kema GHJ, Verstappen ECP, Waalwijk G (2000) Avirulence in the wheat septoria tritici leaf blotch fungus *Mycosphaerella graminicola* is controlled by a single locus. Mol Plant Microbe Interact 13:1375–1379

Laurie DA, Bennett MD (1988) The production of haploid wheat plants from wheat × maize crosses. Theor Appl Genet 76:393–397

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, Massachusetts

McCartney CA, Brûlé-Babel AL, Lamari L (2002) Inheritance of race-specific resistance to *Mycosphaerella graminicola* in wheat. Phytopathology 92:138–144

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81–89, 107–116

Peterhansel C, Freialdenhoven A, Kurth J, Kolsch R, SchulzeLefert P (1997) Interaction analyses of genes required for resistance responses to powdery mildew in barley reveal distinct pathways leading to leaf cell death. Plant Cell 9:1397–1409

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Schön CC, Lee M, Melchinger AE, Guthrie WD, Woodman WL (1993) Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. Heredity 70:648–659

Shah MM, Gill KS, Baenziger PS, Yen Y, Kaeppler SM, Ariyarathne HM (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci 39:1728–1732

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051–1056

Shaw MW, Royle DJ (1993) Factors determining the severity of epidemics of *Mycosphaerella graminicola* (*Septoria tritici*) on winter wheat in the UK. Plant Pathol 42:882–899

Simón MR, Worland AJ, Cordo CA, Struik PC (2001) Chromosomal location of resistance to *Septoria tritici* in seedlings of a synthetic hexaploid wheat, *Triticum spelta*, and two cultivars of *Triticum aestivum*. Euphytica 119:149–153

Somasco OA, Qualset CO, Gilchrist DG (1996) Single-gene resistance to *Septoria tritici* blotch in the spring wheat cultivar 'Tadinia'. Plant Breed 115:261–267

Stam P, Van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Švec M, Miklovičová M (1998) Structure of populations of wheat powdery mildew (*Erysiphe graminis* DC f.sp. *tritici* Marchal) in Central Europe in 1993–1996: I. Dynamics of virulence. Eur J Plant Pathol 104:537–544

Torp AM, Hansen AL, Andersen SB (2001) Chromosomal regions associated with green plant regeneration in wheat (*Triticum aestivum* L.) anther culture. Euphytica 119:377–387

Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. J Quant Trait Loci 2

Utz HF, Melchinger AE, Schön CC (2000) Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154:1839–1849

Van Ginkel M, Scharen AL (1988) Host-pathogen relationships of wheat and *Septoria tritici*. Phytopathology 78:762–766

Van Ooijen JW, Maliepaard C (1996) MapQTL (tm) version 3.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wilson RE (1985) Inheritance of resistance to *Septoria tritici* in wheat. In: Scharen AL (ed) *Septoria* of cereals. Proceedings of a Workshop held at Montana State University, Bozeman, pp 33–35