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ORIGINAL PAPER

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Identification and validation of QTL for *Sclerotinia* midstalk rot resistance in sunflower by selective genotyping

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Abstract Midstalk rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is an important cause of yield loss in sunflower (*Helianthus annuus* L.). Objectives of this study were to: (1) estimate the number, genomic positions and genetic effects of quantitative trait loci (QTL) for resistance to midstalk rot in line TUB-5-3234, derived from an interspecific cross; (2) determine congruency of QTL between this line and other sources of resistance; and (3) make inferences about the efficiency of selective genotyping (SG) in detecting QTL conferring midstalk rot resistance in sunflower. Phenotypic data for three resistance (stem lesion, leaf lesion and speed of fungal growth) and two morphological (leaf length and leaf length with petiole) traits were obtained from 434 F_3 families from cross CM625 (susceptible) × TUB-5-3234 (resistant) under artificial infection in field experiments across two environments. The SG was applied by choosing the 60 most resistant and the 60 most susceptible F_3 families for stem lesion. For genotyping of the respective F_2 plants, 78 simple sequence repeat markers were used. Genotypic variances were highly significant for all traits. Heritabilities and genotypic correlations between resistance traits were moderate to high. Three to four putative QTL were detected for each resistance trait explaining between 40.8% and 72.7% of the genotypic variance ($\tilde{p}_{TS}$). Two QTL for stem lesion showed large genetic effects and corroborated earlier findings from the cross $NDBLOS_{sel}$ (resistant) × CM625 (susceptible). Our results suggest that SG can be efficiently used for QTL detection and the analysis of congruency for resistance genes across populations.

Keywords *Helianthus annuus* · *Sclerotinia sclerotiorum* · Selective genotyping · Artificial infection · Simple sequence repeats · QTL · Cross validation

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Introduction

White rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major yield-limiting factor of sunflower in the temperate regions of the world. Several wild *Helianthus* species were described as potential sources of genes for resistance to *S. sclerotiorum* (Seiler and Rieseberg 1997) and thus have been used to produce interspecific hybrids (Kräuter et al. 1991; Jan 1997; Schnabl et al. 2002). Complete resistance to *S. sclerotiorum* could not be achieved in cultivated sunflower, but lines derived from interspecific crosses showed improved resistance when infected with *S. sclerotiorum* (Degener et al. 1999a; Köhler and Friedt 1999; Rönicke et al. 2004). However, enhanced resistance to *S. sclerotiorum* in elite germplasm should be possible by simultaneously introgressing different resistance genes from well-characterised donor lines.

Based on earlier screenings (Degener et al. 1999b), two sunflower lines, $NDBLOS_{sel}$ and TUB-5-3234, were selected as promising sources of resistance against *S. sclerotiorum* due to a significant reduction of lesion length on the stem after mycelium infection with *S. sclerotiorum*. The source of resistance in line $NDBLOS_{sel}$ is unknown, because the germplasm pool NDBLOS was derived by bulking 49 B lines selected for high oil content (Roath et al. 1987). Resistance of line TUB-5-3234 was considered to be regulated by genes different from those identified for $NDBLOS_{sel}$. Resistance genes in TUB-5-3234 most likely originated from *Helianthus tuberosus*, because this line was derived from

an interspecific hybrid with the susceptible inbred line HA89 (Degener et al. 1999b).

Earlier studies suggested that inheritance of resistance against *S. sclerotiorum* is polygenic (Bert et al. 2004), and that large mapping populations would be required for detection of the underlying quantitative trait loci (QTL). To assess congruency of QTL for resistance against *S. sclerotiorum* in the two sunflower inbred lines $NDBLOS_{sel}$ and TUB-5-3234, we analysed large mapping populations derived from crosses with a common susceptible parent (CM625). Results for the cross with $NDBLOS_{sel}$ were presented in a companion paper (Micic et al. 2004). To reduce the costs of marker analyses, we used selective genotyping (SG) to identify QTL for resistance to midstalk rot in line TUB-5-3234. SG is a trait-dependent method used to increase the statistical power of QTL detection and was first proposed by Lebowitz et al. (1987). The method exploits the fact that most of the information for QTL effects is in the 'tails' of the quantitative trait distribution. Thus, the power of QTL detection can be markedly increased for quantitative traits (Lander and Botstein 1989).

In the present study, we mapped QTL for resistance to midstalk rot based on phenotypic data from 434 F_3 families derived from cross CM625 × TUB-5-3234 and a selective genotyping approach. Our objectives were to: (1) estimate the number, genomic positions and genetic effects of QTL for resistance to midstalk rot in line TUB-5-3234, (2) make inferences about the efficiency of SG in detecting QTL conferring *Sclerotinia* midstalk rot resistance in cultivated sunflower and (3) determine the presence of common QTL between this population and the previous mapping population with $NDBLOS_{sel}$.

Materials and methods

Plant material

Line TUB-5-3234, an inbred line developed from an interspecific cross between *H. tuberosus* and HA 89 (*H. annuus*), highly resistant against artificial leaf infection with *S. sclerotiorum* (Degener et al. 1999a) was chosen as resistant parent (P_R). Inbred line CM625 was used as susceptible parent (P_S). One F_1 plant derived from the cross $P_S \times P_R$ was self-pollinated to produce F_2 plants. Randomly chosen F_2 plants were selfed to produce 434 F_3 families.

Field experiments

Resistance of F_3 families against midstalk rot caused by *S. sclerotiorum* was evaluated under artificial inoculation in two environments (2000 and 2001) at Eckartsweier, located in the Upper Rhine Valley (140 m above sea level, 9.9°C mean annual temperature, 726 mm mean annual precipitation) in south-west Germany. The experimental unit was a one-row plot, 2 m long, with 12 plants and row spacing of 0.75 m. Plots were overplanted and later thinned to a final plant density of about eight plants/m^2. In each environment, the experimental design was a 21 × 21 lattice, with three replications. Parental lines were included as triplicate (P_R) and quadruple entries (P_S).

The *S. sclerotiorum* isolate used in this study was collected in 1995 from naturally infected sunflower plants at Eckartsweier. The inoculum was cultured as described by Micic et al. (2004). Briefly, the leaf test of Degener et al. (1998) was used to assess *S. sclerotiorum* resistance in sunflower after artificial infection. Five plants per plot were inoculated with *S. sclerotiorum*. Three resistance and two morphological traits were recorded: (1) leaf lesion measured in centimetres as the length of the brown rotted zone along the leaf vein beginning around the explant 1 week after inoculation; (2) speed of fungal growth reflecting fungal progression inside the leaf and petiole tissue, estimated from the ratio between leaf length with petiole in centimetres and the time in days from leaf infection until the lesion of the fungus reached the base of the petiole; (3) stem lesion measured in centimetres as length of the tan to gray rotted zone on the stem, 1 month after inoculation; (4) leaf length measured in centimetres from the leaf apex to the base of the petiole one week after inoculation; and (5) leaf length with petiole measured in centimetres.

Marker analyses

Leaf tissue from 434 F_2 plants was collected in 2000 and dried. Based on means across environments, the 60 most resistant and the 60 most susceptible F_3 families were identified, and genomic DNA from the corresponding 120 F_2 plants was extracted as described in detail by Köhler and Friedt (1999). A total of 1,109 simple sequence repeat (SSR) primer pairs were screened for polymorphism between the two parent lines, 1,089 of them were developed by the Department of Crop and Soil Science, Oregon State University (Gedil 1999; Tang et al. 2002; Yu et al. 2003), and 20 SSR primers were taken from the publication by Paniego et al. (2002). Out of the 1,109 tested primer pairs 78 high-quality marker loci were chosen for construction of the genetic linkage map. SSR marker analyses were performed as described by Tang et al. (2002) and Paniego et al. (2002). Genotyping was conducted on an ALF Express sequencer (Amersham Pharmacia Biosciences, Germany) using fluorescence (Cy5)-labelled primers. The software package Allele Link (Amersham Pharmacia Biosciences) was used for allele scoring.

Statistical analyses

Field data

Lattice analyses of variance were performed with data from each environment, using plot means calculated

from individual plant measurements for each trait. Inoculated plants showing no symptoms were considered as escapes and therefore excluded from the calculation of plot means. Adjusted-entry means together with effective error mean squares from individual analyses were used in the combined analyses of variance to estimate variance components and correlation coefficients. Components of variance were estimated considering all effects in the statistical model as random. Estimates of variance components for the genotypic variance ($\hat{\sigma}_g^2$), genotype × environment interaction variance ($\hat{\sigma}_{ge}^2$) and error variance ($\hat{\sigma}^2$), as well as their standard errors were calculated as described by Searle (1971, p. 475). Heritabilities ($\hat{h}^2$) on an entry-mean basis were calculated according to Hallauer and Miranda (1981). Phenotypic (r_p) and genotypic correlation (r_g) coefficients were estimated among traits in F_3 families by applying standard procedures (Mode and Robinson 1959). All necessary computations for the field trials were performed with software package PLABSTAT (Utz 2000).

Marker data

At each of the 78 SSR marker loci, deviations of observed frequencies from the expected Mendelian segregation (1:2:1 or 3:1) and allele frequency (0.5) were tested using the χ^2 test (Weir 1996). Owing to multiple tests, appropriate type I error rates were determined by the sequentially rejective Bonferoni procedure (Holm 1979). A linkage map for cross $P_S \times P_R$ based on the 120 F_2 plants and 78 SSR marker loci was constructed by using software package JoinMap, version 3.0 (van Ooijen and Voorrips 2001). Linkage between two markers was declared significant in two point analyses when the $\log_{10}$ of the likelihood odds ratio (LOD) score exceeded the threshold of 3.0. The remaining ungrouped markers were then assigned to linkage groups according to previously published genetic maps (Tang et al. 2002; Burke et al. 2002; Yu et al. 2003; Micic et al. 2004) by using the *move selected loci* command in JoinMap and a reduced LOD stringency (1.0). Recombination frequencies between marker loci were estimated by multi-point analyses and transformed into centiMorgans (cM) using Haldane's (1919) mapping function.

QTL analyses

All necessary computations for QTL mapping and estimation of their effects were performed with software package PLABQTL (Utz and Melchinger 1996). Analyses were performed with means across environments of the 120 selected F_3 families. The method of composite interval mapping with cofactors (Jansen and Stam 1994) was used for the detection, mapping and characterization of QTL. Cofactors were selected by stepwise regression according to Miller (1990) with an F-to-enter and an F-to-delete value of 3.5. A LOD threshold of 2.5 was chosen to declare a putative QTL as significant. The type I error rate was determined to be $P_e < 0.44$ using 1,000 permutation runs (Doerge and Churchill 1996). QTL positions were determined at local maxima of the LOD-curve plot in the region under consideration. The proportion of the phenotypic variance ($\hat{\sigma}_p^2$) explained by individual QTL was determined by the estimator $\hat{R}_{adj}^2$ as described by Utz et al. (2000). Estimates of the additive (a_i) and dominance (d_i) effects for the ith putative QTL, the total LOD score, as well as the total proportion of the phenotypic variance explained by all QTL, were obtained by fitting a multiple regression model including all putative QTL for the respective trait simultaneously (Bohn et al. 1996). Following Bohn et al. (1996), the ratio $DR = (|d_i|/|a_i|)$ was used to describe the type of gene action at each QTL: additive for dominance ratio $(DR) < 0.2$, partial dominance for $0.2 \le DR < 0.8$, dominance for $0.8 \le DR < 1.2$, and overdominance for $DR \ge 1.2$. The proportion of the genotypic variance explained by all QTL ($\hat{p}$) was determined as described by Utz et al. (2000). Standard fivefold cross-validation (CV) implemented in PLABQTL was used for obtaining asymptotically unbiased estimates of the genotypic variance explained (Schön et al. 2004). The whole data set (DS) comprising the entry means across environments was divided into five genotypic subsamples. Four of these were combined in an estimation set (ES) for QTL detection and estimation of genetic effects. The remaining subsample was used as a test set (TS) to validate QTL estimates obtained from ES by correlating predicted and observed data. Two hundred randomizations were generated for assigning genotypes to the respective subsamples, yielding a total of 1,000 replicated CV runs. Two QTL were declared as congruent across traits and populations if they had the same sign and were within a 20-cM distance (Melchinger et al. 1998).

Results

Phenotypic data

Three days after artificial inoculation, the majority of plants showed *S. sclerotiorum* infection symptoms on the leaf. The infection rates estimated from the ratio between infected and inoculated plants amounted to 95% for the first and 91% for the second environment. Means of the parental inbred lines P_R and P_S differed significantly ($P < 0.01$) for all resistance and morphological traits (Table 1). Histograms of progeny means across environments for the resistance traits for all 434 and for the selected 120 F_3 families are presented in Fig. 1. For the resistance traits, means across environments of the 434 F_3 families were normally distributed. The means of parents ($\bar{P}$) differed significantly ($P < 0.01$) from means of F_3 families ($P < 0.01$) for all resistance traits. The means of the upper and lower selected fractions were significantly ($P < 0.01$) different from each other for all scored traits (Table 1).

Table 1 Means of parental inbred lines of resistant (P_R) and susceptible (P_S), F_3 families, and lower and upper tail of selected F_3 families from cross CM625 (P_S) × TUB-5-3234 (P_R), as well as estimates of variance components and heritabilities for 434 F_3 families for resistance and morphological traits measured in two environments

Parameters	No.	Resistance traits			Morphological traits	
		Stem lesion (cm)	Leaf lesion (cm)	Speed of fungal growth (cm/day)	Leaf length (cm)	Leaf length with petiole (cm)
Means						
P_R	3	9.7 ± 1.94	7.2 ± 0.40	1.5 ± 0.07	19.0 ± 0.53	30.0 ± 0.68
P_S	4	36.4 ± 1.77	8.1 ± 0.35	1.8 ± 0.06	16.0 ± 0.50	26.4 ± 0.63
$\bar{P}$	7	23.0 ± 1.31	7.7 ± 0.27	1.7 ± 0.05	17.5 ± 0.36	28.2 ± 0.46
F_3 families	434	19.3 ± 0.32	7.1 ± 0.05	1.6 ± 0.01	18.6 ± 0.06	29.6 ± 0.08
Mean of lower tail	60	8.7 ± 0.32	6.2 ± 0.05	1.5 ± 0.01	19.3 ± 0.06	30.9 ± 0.08
Mean of upper tail	60	30.2 ± 0.32	8.1 ± 0.05	1.8 ± 0.01	17.7 ± 0.06	28.2 ± 0.08
Range of F_3 families		0.0–52.0	0.8–10.8	0.8–2.6	11.8–24.0	15.9–43.4
Variance components						
F_3 families						
$\hat{\sigma}^2_g$		36.77 ± 3.14**	0.51 ± 0.07**	0.014 ± 0.002**	0.92 ± 0.10**	2.06 ± 0.22**
$\hat{\sigma}^2_{ge}$		2.52 ± 1.33*	0.04 ± 0.06	0.006 ± 0.001**	0.33 ± 0.07**	0.40 ± 0.14**
$\hat{\sigma}^2$		44.93 ± 1.82	2.49 ± 0.10	0.036 ± 0.001	2.09 ± 0.10 +	4.66 ± 0.19
Heritability (F_3 families)						
$\hat{h}^2$		0.81	0.54	0.61	0.64	0.68
95% CI on $\hat{h}^2$ [a]		(0.77; 0.84)	(0.44; 0.62)	(0.53; 0.68)	(0.57; 0.70)	(0.61; 0.73)

**, *Variance component was significant at the 0.01 and 0.05 probability levels, respectively
[a]Confidence intervals (*CI*) on <@IEq34> were calculated according to Knapp et al. (1985)

Variances and heritabilities

Genotypic variances among F_3 families ($\hat{\sigma}^2_g$) were highly significant ($P < 0.01$) for all traits (Table 1). Estimates of genotype × environment interaction variance ($\hat{\sigma}^2_{ge}$) were not significant for leaf lesion, significant ($P < 0.05$) for stem lesion and highly significant ($P < 0.01$) for speed of fungal growth as well as for the morphological traits but relatively small compared with $\hat{\sigma}^2_g$. Heritability estimates for resistance traits were intermediate to high.

Trait correlations

Correlations between resistance traits were moderate to high ($0.76 < r_g < 0.92$). Leaf length with petiole was tightly correlated with leaf length ($r_g = 0.85$, $P < 0.01$). Correlations of the latter traits were negative and medium with stem lesion ($-0.62 < r_g < -0.45$), negative and weak with leaf lesion ($-0.32 < r_g < -0.26$) and week to close to zero with speed of fungal growth ($-0.28 < r_g < -0.08$). Phenotypic correlations were generally lower than the corresponding phenotypic correlations.

Linkage map

Three out of the 78 loci (3.8%) showed a dominant segregation ratio. No significant deviations from the expected Mendelian segregation ratios or allele frequency 0.5 were observed. The proportion of the P_R genome among the selected F_2 families followed a normal distribution and ranged from 23.3% to 71.3%, with mean of $\bar{x} = 49.8\%$ and standard deviation (SD) of 7.8%. The proportion of the P_R and P_S genome in the resistant and susceptible tails followed a normal distribution. In the resistant tail, the mean proportion of P_R genome was $\bar{x} = 52.1\%$ (SD = 6.2%), whereas in the susceptible tail it was 47.6% (SD = 8.6%).

A genetic linkage map of the selected 120 F_2 plants was constructed based on 72 of the 78 polymorphic marker loci that coalesced into 13 linkage groups (Fig. 2). The linkage group (LG) nomenclature suggested by Tang et al. (2002) was followed. Presumably, each linkage group corresponds to one of the 17 chromosomes in the haploid sunflower genome ($x = 17$). Only one polymorphic locus was found for LGs 6, 7, 8, 11 and 12 (data not shown). The remaining linkage groups ranged in length from 15.3 cM to 156.9 cM (Fig. 2). The total map distance covered 1,005.2 cM, with an average interval length of 14.0 cM. About 87.2% of the mapped genome was located within a 20-cM distance to the nearest marker. For QTL analysis, six unlinked loci (ORS16, ORS57, ORS456, ORS502, ORS1146 and ORS1193) were assigned to an artificial linkage group with 50-cM interval length between markers.

QTL analyses

For stem lesion, three putative QTL were identified on LGs 4, 10 and 17, and one putative QTL was linked to the ungrouped marker ORS456, explaining between 16.1% and 24.0% of the phenotypic variance. All QTL displayed significant additive gene effects, except the QTL on LG17 with partial dominance. Alleles contributing to increased resistance originated from the resistant parent P_R, except the QTL on LG4. In a simultaneous fit of all putative QTL, 84.0% of the genotypic variance was explained by markers and this value was only slightly reduced with CV ($\tilde{p}_{TS} = 72.7\%$).

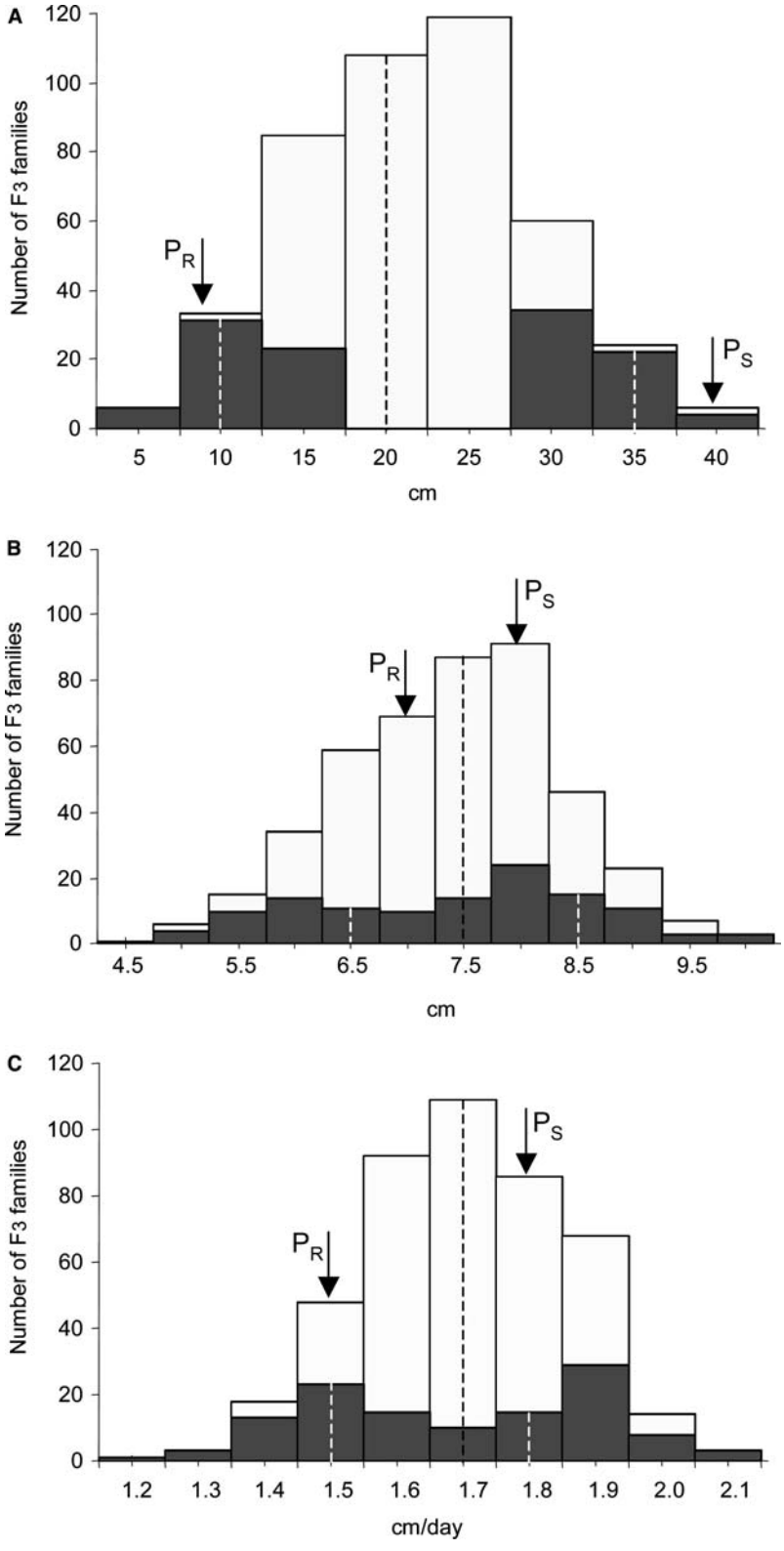

Fig. 1 Histograms for means of **a** stem lesion, **b** leaf lesion and **c** speed of fungal growth, measured in two environments in 434 F_3 families and 120 selected F_3 families derived from cross CM625 [susceptible parent (P_S)] ×TUB-5-3234 [resistant parent (P_R)]. *Dashed lines* indicate the overall means (*black lines*), as well as the means of selected fractions (*white lines*). *Arrows* indicate the means of parental lines P_R and P_S

The genomic regions located on LGs 4, 10 and 17 also carried QTL for leaf lesion and speed of fungal growth (Table 2). Compared to stem lesion, no additional genomic regions were identified and the resistance alleles on LG4 originated from the susceptible parent. All QTL had significant additive gene effects. The QTL explained up to 25.5% of the phenotypic variance and in a simultaneous fit 75.9% of the genotypic variance ($\hat{p}_{DS}$) was explained for leaf lesion and 84.6% for speed of fungal growth. Both estimates were reduced by CV to 40.8% and 53.7%, respectively.

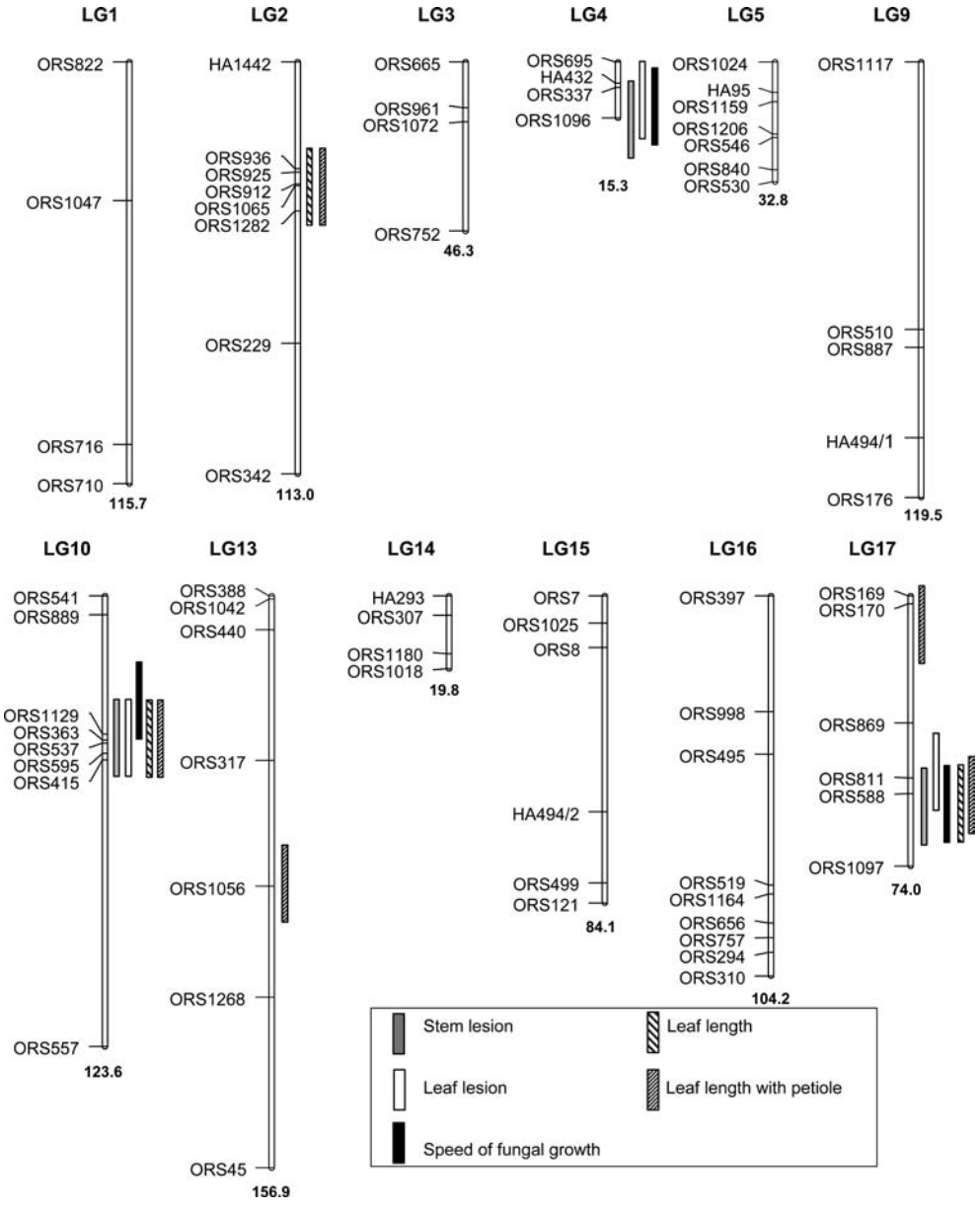

Fig. 2 Genetic linkage map of cross CM625 (P_S) × TUB-5-3234 (P_R) based on 72 simple sequence repeat marker loci analysed in 120 F_2 plants. Marker names are listed to the *left* of each linkage group. At the *bottom* of each linkage group the total length in centiMorgans (Haldane) is given. Chromosomal regions carrying quantitative trait loci for resistance and morphological traits are indicated by *boxes*. *Boxes* indicate congruency intervals. The box pattern (see *legend*) is associated with the respective trait. *LG* Linkage group

For leaf length and leaf length with petiole, three and six putative QTL explaining between 10.0% and 37.5% of the phenotypic variance were detected (Table 2). The QTL on LG10 and LG17 also affected the three resistance traits. All putative QTL showed additive gene action except the QTL on LG2 exhibiting dominance. In a simultaneous fit, the detected QTL explained 78.8% and 85.6% of the genotypic variance ($\hat{p}_{DS}$) for leaf length and leaf length with petiole, respectively. Again, these estimates were reduced with CV.

Discussion

Several studies have demonstrated that SG is an efficient approach to detect QTL with reduced efforts and costs for genotyping (Foolad et al. 2001; Ayoub and Mather 2002; Zhang et al. 2003). In a study performed by Ayoub and Mather (2002), genotyping of only 10% of the population was sufficient to detect all major QTL. We evaluated two large populations originating from different resistant sources crossed to the same susceptible parent for their resistance against *S. sclerotiorum* to determine whether the same QTL were responsible for expression of resistance. Based on earlier findings, the resistance was known to be inherited by many genes with small effects. Therefore, and due to a limited budget for marker analyses, we decided to analyse QTL for *S. sclerotiorum* resistance in a large reference population $NDBLOS_{sel}$ × CM625 ($n = 354$) and to use SG in the second population rather than performing QTL analyses in two medium-sized populations.

Table 2 Parameters associated with putative quantitative trait loci (*QTL*) for three resistance and two morphological traits. Parameters were estimated from 120 selected F_3 families from the cross CM625 (P_S) × TUB-5-3234 (P_R) evaluated in two environments. *LG* Linkage group, *LOD* $\log_{10}$ of the likelihood odds ratio, *cM* centiMorgans

Resistance traits	Linkage group	Marker	Position on LG (cM)	LOD at QTL position	Genetic effect[a]		Variance[b] explained
					Additive	Dominance	
Stem lesion (cm)	4	ORS 337	10	6.37	−5.57	NS	22.2
	10	ORS 1129	38	7.17	5.09	NS	24.0
	17	ORS 588	56	4.56	4.40	3.36	16.1
		ORS 456[c]	0	5.18	3.92	NS	18.2
	$\hat{p}_{DS}$						84.0
	$\tilde{p}_{TS}$						72.7
Leaf lesion (cm)	4	ORS 337	14	5.55	−0.66	NS	19.6
	10	ORS 1129	38	2.50	0.47	NS	9.2
	17	ORS 811	50	2.66	0.35	NS	9.7
	$\hat{p}_{DS}$						75.9
	$\tilde{p}_{TS}$						40.8
Speed of fungal growth (cm/day)	4	HA 432	6	7.68	−0.13	NS	25.5
	10	ORS 889	26	3.37	0.09	NS	12.3
	17	ORS 811	52	3.56	0.07	NS	12.8
	$\hat{p}_{DS}$						84.6
	$\tilde{p}_{TS}$						53.7
Leaf length (cm)	2	ORS 912	34	3.56	0.38	NS	12.8
	10	ORS 1129	38	12.23	−0.92	NS	37.5
	17	ORS 811	50	3.82	−0.36	NS	13.6
	$\hat{p}_{DS}$						78.8
	$\tilde{p}_{TS}$						57.5
Leaf length with petiole (cm)	2	ORS 912	34	2.75	NS	−0.62	10.0
	10	ORS 1129	38	11.27	−1.07	NS	35.1
	13	ORS 317	78	3.90	−0.53	NS	13.9
	17	ORS 169	0	2.74	−0.48	NS	10.2
	17	ORS 811	52	3.85	−0.75	NS	13.8
		ORS 456[c]	0	3.38	−0.63	NS	12.3
	$\hat{p}_{DS}$						85.6
	$\tilde{p}_{TS}$						51.4

NS Not significant

[a]Genetic effects were estimated in a simultaneous fit using multiple regression

[b]For individual QTL, the proportion of the phenotypic variance (R^2) explained was estimated, for the simultaneous fit, the proportion of the genotypic variance explained by putative QTL in the data set (<@IEq45>) and test sets (<@IEq46>) using fivefold standard cross-validation was estimated

[c]Marker not assigned to linkage map. According to previous studies (Tang et al. 2002 and Micic et al. 2004) ORS456 is located on LG8.

Efficiency of selective genotyping

To validate the usefulness of SG for detecting QTL for *Sclerotinia* resistance, SG was performed *a posteriori* for population $NDBLOS_{sel}$ × CM625. Details on the experimental design and results from the entire population can be found in our companion paper (Micic et al. 2004). Based on the phenotypic data for stem lesion, the 60 most resistant and 60 most susceptible F_3 families from cross $NDBLOS_{sel}$ × CM625 were selected and a QTL analysis was performed to investigate the power of QTL mapping under SG. With LOD≥2.5, half the number of QTL detected for stem lesion in the reference population was detected using SG (Table 3). The two largest QTL in the reference population affecting resistance to *S. sclerotiorum* (LG8 and LG16) were also detected with SG. An additional small QTL on LG15 was found within a 24-cM distance with the two approaches. Owing to the very flat LOD profile, precise localisation of the QTL peak was difficult on this linkage group. An additional QTL was detected with SG in a region of the genome where no significant QTL was found in the reference population. Along most linkage groups, LOD curves ran parallel in both samples, but remained partly subthreshold with SG. When increasing the power of QTL detection by decreasing the LOD threshold (LOD = 1.5), two additional common QTL were detected without increasing the number of new QTL in SG.

Estimates of the genotypic variance explained by all detected QTL ($\hat{p}_{DS}$), as well as the validated proportion of the genotypic variance explained ($\tilde{p}_{TS}$), were considerably increased with SG as compared to the reference population despite fewer detected QTL. Both parameters are expected to be overestimated in SG due to the non-random genotypic sample and the limited sample size. With random sampling, $\tilde{p}_{TS}$ is expected to yield an asymptotically unbiased estimate of the genotypic variance explained by QTL. With SG, F_3 families in each tail of the distribution are expected to carry allele frequencies deviating from 0.5 at detected and non-detected QTL for stem lesion. Thus, prediction of genotypic values in TS based on the allelic state at detected QTL has a correlated response at non-detected QTL leading to overestimation of $\tilde{p}_{TS}$. As expected,

Table 3 Number of detected QTL in the entire population NDBLOS$_{sel}$ (P_R) × CM625 (P_S) and in 120 (60 most resistant and 60 most susceptible for stem lesion) F_3 families chosen by *a posteriori* selective genotyping (*SG*) and explained genotypic variance in the data set ($\hat{p}_{DS}$) and test sets ($\tilde{p}_{TS}$), as well as common QTL for the complete data set and SG in given traits

Parameters	Resistance traits			Morphological traits	
	Stem lesion	Leaf lesion	Speed of fungal growth	Leaf length	Leaf length with petiole
Reference population ($n = 351$)					
No. of QTL	8	9	6	7	9
$\hat{p}_{DS}$ (%)	50.4	45.4	40.1	38.4	51.2
$\tilde{p}_{TS}$ (%)	33.7	25.3	24.4	21.6	31.0
SG ($n = 120$)					
No. of QTL	4	11	6	6	5
$\hat{p}_{DS}$ (%)	69.3	83.7	80.1	55.0	63.6
$\tilde{p}_{TS}$ (%)	52.1	23.1	39.7	31.9	42.1
Common QTL[a]	3	7	4	3	4

[a]QTL were declared as common, if they were found within a 20-cM interval

overestimation of $\tilde{p}_{TS}$ was highest and the difference between $\hat{p}_{DS}$ and $\tilde{p}_{TS}$ estimates smallest for stem lesion compared to the traits leaf lesion and speed of fungal growth (Table 3).

Even though the objective of SG as trait-dependent method is to detect QTL for a single trait, it is useful to score correlated traits that provide additional information about the trait of interest. As expected, selecting the most resistant and susceptible fraction for stem lesion resulted in a correlated response for leaf lesion and speed of fungal growth. For both traits, two thirds of the QTL found in the reference population were also found using SG (Table 3). For leaf lesion, a relatively high number of new QTL were detected with SG. This tendency was even more pronounced when decreasing the LOD threshold (1.5). However, the validated proportion of the genotypic variance explained by QTL in SG was not increased accordingly, indicating that the additional QTL were most likely false positives due to the small sample size.

In general, our results confirmed the findings of other studies that the most important QTL can be detected by SG (Ajoub and Mather 2002; Foolad et al. 2001). We therefore concluded that SG can be efficiently used for analysis of congruency of resistance genes in an independent sample.

Comparison between segregating populations

We assessed the congruency of resistance genes identified in the phenotypically selected fractions of population CM625 × TUB-5-3234 with the results of population NDBLOS$_{sel}$ × CM625. Regarding the phenotypic data, both populations showed similar infection rates, distributions of means, variances and heritabilities. For stem lesion, a smaller number of QTL was detected in population CM625 × TUB-5-3234 compared to the reference population NDBLOS$_{sel}$ × CM625, but with SG four significant QTL were detected in each population (Tables 2, 3). One of the four QTL on LG4 was in common for the two crosses. Interestingly, this was the only QTL where the allele increasing resistance originated from the common, but susceptible, parent CM625. No QTL for leaf morphology was detected in this genomic region. A second QTL was identified linked to marker ORS456, in a genomic region previously identified in cross NDBLOS$_{sel}$ × CM625 to carry a QTL with a large significant effect on stem lesion. Despite the large number of SSR markers screened for polymorphism, no additional segregating marker could be found for population CM625 × TUB-5-3234 on LG8. The same large QTL could be present in both resistance sources, but with a severely underestimated genetic effect in cross CM625 ×T UB-5-3234 due to a large genetic distance from the marker. We therefore conclude that the genomic region surrounding ORS456 merits further analyses with respect to its importance for *Sclerotinia* resistance in different genetic backgrounds.

The two additional genomic regions (LG10 and LG17) with significant effects on stem lesion in population CM625 × TUB-5-3234 also had a significant effect on morphological traits (Fig. 2) and coincided precisely with two genomic regions detected in cross NDBLOS$_{sel}$ × CM625 affecting leaf morphology. Morphological differences between the susceptible parent CM625 and TUB-5-3234 were not as pronounced as between CM625 and NDBLOS$_{sel}$, but genotypic correlations between stem lesion and morphological traits were of similar magnitude in both crosses, corroborating the findings from the QTL analysis that leaf morphology can affect resistance against *S. sclerotiorum*.

The five QTL detected for stem lesion in population NDBLOS$_{sel}$ × CM625 on LGs 2, 3, 6, 15 and 16 were not detected in this study. When testing for matching QTL in different populations, several reasons can account for lack of congruency. First, low power of detection and genotypic sampling can lead to low QTL consistency between populations. When marker intervals that have been selected based on earlier findings are tested for presence of QTL, it is adequate to increase the power of detection by lowering the significance threshold.

However, with LOD≥1.5, only one additional QTL for stem lesion was detected (LOD = 2.3) in cross CM625 × TUB-5-3234 on LG2, a genomic region that was not covered by markers in the other population. The four QTL detected in cross CM625 × TUB-5-3234 with LOD≥2.5 already accounted for a large proportion of the genotypic variance ($\tilde{p}_{TS}$ = 72.7). Even when considering that $\tilde{p}_{TS}$ is overestimated due to SG, it can still be assumed that more than half of the genotypic variance for resistance against *S. sclerotiorum* can be explained by the identified markers linked to genes influencing resistance and/or plant morphology. The unexplained variance is most likely due to QTL with genetic effects too small to be detected despite the fairly large phenotypic data set (434 F_3 families) on which SG was based.

A second prerequisite for finding congruency of QTL is an adequate linkage map coverage and a sufficient number of shared markers in both populations. In both populations polymorphism was low, but about half (47.4%) of the polymorphic markers found in population CM625 × TUB-5-3234 were in common with cross $NDBLOS_{sel}$ × CM625. As a consequence, all eight genomic regions exhibiting significant QTL for stem lesion in population $NDBLOS_{sel}$ × CM625 were either flanked by markers in population CM625 × TUB-5-3234 or at least at less than 10 cM distant from the nearest marker. Thus, lack of congruency between the two populations was in general not due to insufficient marker coverage.

A third reason for inconsistent results across populations could also be the different environmental conditions under which experiments were conducted. Different resistance mechanisms could be activated in the different environments. However, we found only small, barely significant genotype × environment interaction variances in both investigations.

The same genomic regions that affected stem lesion in SG of cross CM625 × TUB-5-3234 were identified to also affect leaf lesion and speed of fungal growth. When the significance threshold was lowered to LOD = 2.0, two additional significant QTL were detected on LG1 and nine that were in common with QTL for leaf lesion in population $NDBLOS_{sel}$ × CM625. Mycelial extension on leaves was also used by Mestries et al. (1998) and Bert et al. (2002) to assess resistance against *S. sclerotiorum*. To compare the chromosomal positions of QTL detected in their studies and our two populations, the linkage groups of Tang et al. (2002) were cross-referenced to the nomenclature of the maps of Mestries et al. (1998) and Bert et al. (2002) (A. Leon, personal communication). Bert et al. (2002) detected three QTL for lesion length on leaves on LGs 1, 9 and 13. Mestries et al. (1998) detected five QTL for this trait in different selfing generations, which coincided with our LGs 3, 8, 10 and 16. We found QTL for leaf lesion length on LGs 1, 4, 6, 8, 9, 13 and 15 for population $NDBLOS_{sel}$ × CM625 and on LGs 1, 4, 9, 10 and 17 for population CM625 × TUB-5-3234 (LOD≥2.0). Thus, six linkage groups carried QTL for leaf lesion in more than one population, LG1 and nine had a significant effect in three of the four populations.

In addition to LGs 1, 4, 8 and 9, LG10 is particularly interesting with respect to resistance against *S. sclerotiorum*. Mestries et al. (1998) detected a QTL for leaf lesion and capitulum index on LG10 and Bert et al. (2002) found a QTL for mycelium on the capitulum. Similar results were obtained by Rönnicke (personal communication), who also identified a QTL for head rot on LG10. In this study, all three-resistance traits were affected by a QTL on LG10. An integrated genetic map with data from all available SSR markers is currently being established (L. Gentzbittel, personal communication) and will enable the alignment of genomic regions identified to carry QTL for resistance within linkage groups.

Conclusions

We have identified two genomic regions with a major effect on resistance against *S. sclerotiorum*. On LG8, a large QTL was identified in both crosses of CM625 with lines TUB-5-3234 and $NDBLOS_{sel}$. Further research will be undertaken to analyse the genomic region on LG8 in more detail. For both crosses, data on a large number of recombinant inbred lines will become available in the near future. In addition, the two sources of resistance, TUB-5-3234 and $NDBLOS_{sel}$ show ample polymorphism on LG8 for the markers used in this study, thus allowing further genetic dissection of this genomic region. The genomic region on LG10 will also be analysed in more detail with respect to its importance for resistance in multiple plant parts (head and stalk) and to verify its association with leaf morphology.

The genetic effects of the QTL on LG8 and on LG4 are large enough to form a starting point for a marker-assisted selection program combined with phenotypic selection for *Sclerotinia* resistance. Based on our results, it is questionable whether TUB-5-3234 can contribute new alleles for resistance with sufficiently large genetic effects to be useful in marker-assisted introgression that have not already been identified in line $NDBLOS_{sel}$ and are not strongly correlated with morphological characters. Therefore, the identification of additional sources of genes conferring resistance against *S. sclerotiorum* in exotic material and genetic resources will be of crucial importance for future successes in resistance breeding of sunflower against this important disease.

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