Theor Appl Genet (2005) 110: 1490–1498
DOI 10.1007/s00122-005-1984-x

ORIGINAL PAPER

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QTL mapping of resistance to *Sclerotinia* midstalk rot in RIL of sunflower population $\mathrm{NDBLOS_{sel}} \times \mathrm{CM625}$

Received: 27 October 2004 / Accepted: 26 February 2005 / Published online: 20 April 2005

Abstract Midstalk rot caused by *Sclerotinia sclerotiorum* is an important disease of sunflower in its main areas of cultivation. The objectives of this study were to (1) verify quantitative trait loci (QTL) for midstalk-rot resistance found in F_3 families of the $\mathrm{NDBLOS_{sel}} \times$ CM625 population in recombinant inbred lines (RIL) derived from the same cross; (2) re-estimate their position and genetic effects; (3) draw inferences about the predictive quality of QTL for midstalk-rot resistance identified in the F_3 families as compared to those in the RIL. Phenotypic data for three resistance (leaf lesion, stem lesion, and speed of fungal growth) and two morphological traits (leaf length and leaf length with petiole) were obtained from 317 RIL following artificial infection in field experiments across two environments. For genotyping the 248 RIL, we selected 41 simple sequence repeat (SSR) markers based on their association with QTL for *Sclerotinia* midstalk-rot resistance in an earlier study. The resistance traits showed intermediate to high heritabilities ($0.51<\hat{h}^2<0.79$) and were significantly correlated with each other ($0.45<\hat{r}_g<0.78$).Genotypic correlations between F_3 families and the RIL were highly significant and ranged between 0.50 for leaf length and 0.64 for stem lesion. For stem lesion, two genomic regions on linkage group (LG) 8 and LG16 explaining 26.5% of the genotypic variance for *Sclerotinia* midstalk-rot resistance were consistent across generations. For this trait, the genotypic correlation between the observed performance and its prediction based on QTL positions and effects in F_3 families was surprisingly high ($\hat{r}_g(\mathrm{M_{iF3}}, \mathrm{Y_{iRIL}}) = 0.53$).The genetic effects and predictive quality of these two QTL are promising for application in marker-assisted selection to *Sclerotinia* midstalk-rot resistance.

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is an omnivorous and non-specific plant pathogen that is both common and widespread in most of the sunflower growing regions of the world. Under severe infection and depending on the plant organs infected by the fungus, *Sclerotinia* disease can cause serious yield losses in sunflower, reaching as high as 100% following a severe infestation (Sackston 1992). Of the three distinct types of diseases caused by *S. sclerotiorum* (wilt, midstalk rot, and head rot), midstalk rot is of particular importance in Germany. Midstalk rot typically originates from a leaf infection caused by airborne ascospores landing on wounded leaf tissue and colonizing the leaf. The infection progresses down the petiole, producing a stem lesion. The stalks ultimately break at the point of infection and the tissues above the lesion die.

The development of resistance against *S. sclerotiorum* is a major aim of sunflower breeding programs and has also become a major research objective. In several studies, genetic variability for partial resistance to *S. sclerotiorum* has been observed (Tourvieille de Labrouhe et al. 1996; Degener et al. 1998; Micic et al. 2004). Resistance to *S. sclerotiorum* was generally quantitatively inherited with predominantly additive gene action (Castaño et al. 1993; Genzbittel et al. 1998; Bert et al. 2002).

Results from studies of quantitative trait loci (QTL) for *Sclerotinia* resistance using molecular markers have corroborated the complex inheritance of *Sclerotinia* resistance (Mestries et al. 1998; Bert et al. 2002; Micic et al. 2004). Generally, QTL with small effects explaining only a small proportion of the phenotypic variance were detected. In a QTL mapping population derived from

Communicated by G. Wenzel

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the cross between the resistant line NDBLOS$_{sel}$ and the susceptible line CM625, Micic et al. (2004) identified 15 genomic regions affecting resistance against midstalk rot, which could be partially verified in a second population derived from a different resistance source (Micic et al. 2005). To be of use in marker-assisted breeding, the QTL detected in the early generations must be of predictive value for later generations. In maize, Groh et al. (1998) found only a low number of QTL for corn borer resistance to be in common between two recombinant inbred line (RIL) populations and their corresponding F_3 populations. For *Sclerotinia* resistance in sunflower, no data on the correlation between early- and late-generation resistance and the congruency of QTL across generations is available. We therefore conducted a study on *Sclerotinia* midstalk-rot resistance in 248 RIL developed by single seed descent from the cross NDBLOS$_{sel}$ × CM625. In addition to verifying QTL detected in F_3 families, the use of RIL should allow greater precision in estimating genetic effects due to reduced genetic variation within lines and the absence of dominance. As a result of the additional recombination during line development, an improved assessment of the association of resistance and morphological traits caused by linked QTL should be possible.

The objectives of our study were to (1) verify QTL for midstalk-rot resistance in RIL of the NDBLOS$_{scl}$ × CM625 population; (2) re-estimate their position and genetic effects; (3) make inferences about the predictive quality of QTL for *Sclerotinia* midstalk-rot resistance identified in F_3 families as compared to RIL.

Materials and methods

Plant material

From the cross between the resistant line NDBLOS$_{scl}$ (P_R) and the susceptible line CM625 (P_S), one F_1 plant was self-pollinated to generate 354 F_3 families used in a previous QTL mapping study on *Sclerotinia* midstalk-rot resistance (Micic et al. 2004). Generation advance from F_2 to F_6 was accomplished by single-seed descent, and a total of 317 F_6 RIL were produced.

Field experiments

Resistance of the RIL against midstalk rot caused by *S. sclerotiorum* was evaluated in 2002 and 2003 at Eckartsweier, located in the Upper Rhine Valley (140 m a.sl.; mean annual temperature: 9.9°C; mean annual precipitation: 726 mm) in southwest Germany, under artificial inoculation. The experimental unit was a one-row plot, 2 m long, with 12 plants and row spacing of 0.75 m. Plots were over-planted and later thinned to a final plant density of about eight plants per square meter. The experimental design was a 18 × 18 lattice design with three replications. Five plants per plot were inoculated with *S. sclerotiorum*. Parental lines were not tested in 2002 due to technical problems. In 2003, the parents were included as quadruple entries in each replicate.

Leaf infection method

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). The leaf test of Degener et al. (1998) was used to determine the midstalk rot of sunflower following artificial infection with *S. sclerotiorum*. Briefly, on five plants per plot the tip of one leaf of the fifth fully-grown leaf pair was inoculated. The *S. sclerotiorum* explant was placed at the extremity of the main vein and fixed with a self-sticky label. The inoculated leaf was covered with a transparent plastic bag, and about 10 ml water was added into the bag to maintain sufficient air humidity. Three resistance (leaf lesion, stem lesion, and speed of fungal growth) and two morphological (leaf length and leaf length with petiole) traits were recorded as described by Micic et al. (2004).

Marker analyses

About 5–10 g of fresh young leaf tissue from 317 RIL was collected at the star-bud stage and dried. The leaf material was ground to a fine powder, and genomic DNA was extracted as described in detail by Köhler and Friedt (1999). Genotyping was performed with 41 selected polymorphic simple sequence repeat (SSR) markers (Fig. 1). The selected markers covered the seven linkage groups (LGs) where significant QTL for stem lesion were detected in the study on 354 F_3 families from the cross NDBLOS$_{sel}$ × CM625 (Micic et al. 2004). One additional linkage group (LG1) containing two QTL for leaf lesion but none for morphological traits was included in the analysis. For seven of the SSR markers, analyses were performed as described by Micic et al. (2004), while data on the remaining 34 SSR markers were provided by the Department of Biotechnology and Plant Breeding of the Institut National Polytechnique de Toulouse (France).

Statistical analyses

Field data

Lattice analyses of variance were performed with data from each year using plot means calculated from individual plant measurements for each trait. Non-infected plants were excluded from the calculation of plot means. Adjusted-entry means and effective error mean squares were used to compute combined analyses of variance across years. Components of variance were estimated considering all effects in the statistical model as random. Estimates of variance components for the genotypic variance ($\hat{\sigma}^2_g$), genotype-by-environment interaction

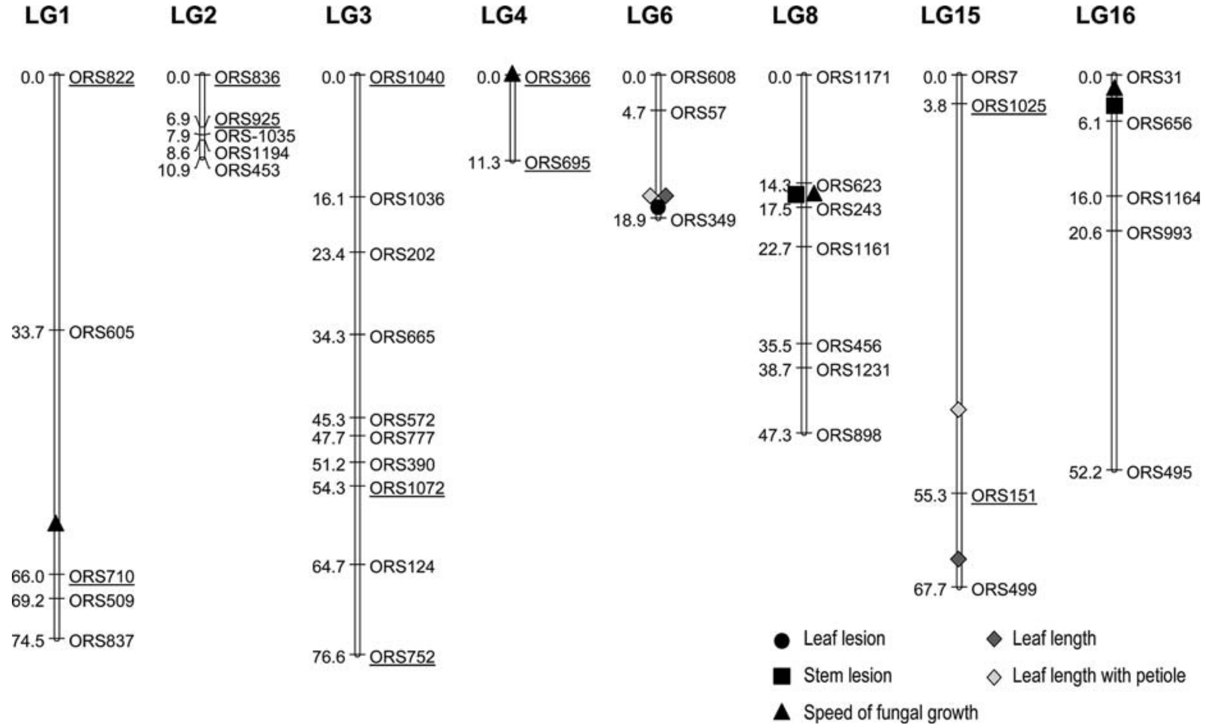

Fig. 1 Common genetic linkage map based on 351 F_2 individuals and 248 RIL derived from cross $P_R \times P_S$ for 41 SSR marker loci. *Numbers* to the *left* of the linkage groups indicate the cumulative distance in centiMorgans (cM) (Haldane 1919). Loci with distorted segregation ratios ($P < 0.01$) are *underlined*. Positions of QTL for scored traits are indicated by *symbols* explained on the figure

variance ($\hat{\sigma}^2_{ge}$), and error variance ($\hat{\sigma}^2$), as well as their standard errors (SE) 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 ($\hat{r}_p$) and genotypic ($\hat{r}_g$) correlation coefficients between traits and between the F_3 family and RIL performance using only the common lines were calculated according to Mode and Robinson (1959). The phenotypic covariance was used as an estimator of the genotypic covariance assuming the covariance of genotype × environment interaction effects to be negligible. All necessary computations for the field trials were performed with software package PLABSTAT (Utz 2000).

Marker data

Observed genotype frequencies at each marker locus were checked for deviations from Mendelian segregation ratios and allele frequency of 0.5 using a χ^2 test. Appropriate type-I error rates were determined by the sequentially rejective Bonferroni test (Holm 1979).

For linkage mapping, 248 RIL were used. Of these, 69 were excluded from the analysis because of non-expected alleles at more than 10% of the loci. A linkage map was constructed by applying software package JOINMAP 3.0 (van Ooijen and Voorrips 2001). Linkage between two markers was declared significant in two-point analyses when the LOD score ($\log_{10}$ of the likelihood odds ratio) exceeded the threshold of 3.0, and recombination did not exceed the threshold of 0.40. Recombination frequencies between marker loci were estimated by multi-point analyses and transformed into centiMorgans (cM) using Haldane's (1919) mapping function. For the QTL analyses, a combined linkage map comprising 41 markers was constructed from the merged data set of 351 F_2 individuals (Micic et al. 2004) and 248 RIL using software package JOINMAP 3.0.

QTL mapping

All necessary computations for QTL mapping and estimation of their effects were performed with software package PLABQTL (Utz and Melchinger 1996). QTL analyses were performed on means across years for the 248 RIL. The method of composite interval mapping (CIM) with cofactors (Jansen and Stam 1994) was used for the detection, mapping, and characterization of QTL. An additive genetic model was chosen for the analysis of the RIL. 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 $P_e < 0.30$ using 1,000 permutation runs (Doerge and Churchill 1996). The QTL positions were determined at local maxima of the LOD-curve plot in the region under consideration. The proportion of the phenotypic ($\hat{\sigma}^2_p$) and the genotypic variance ($\hat{p}$) explained by any QTL was determined as described by Utz et al. (2000).

Standard fivefold cross validation (CV) implemented in PLABQTL with test sets (TS) comprising 20% of the genotypes was used for determining the effect of genotypic sampling (Schön et al. 2004). Two hundred randomizations were generated for assigning genotypes to the respective subsamples, yielding a total of 1,000 replicated CV runs. Estimates of the proportion of the genotypic variance explained by QTL detected simultaneously were calculated for the total data set ($\hat{p}_{\mathrm{DS}}$) and as the median over all TS ($\tilde{p}_{\mathrm{TS}}$). Two QTL were declared as congruent across traits and generations if they had the same sign and were within a 20-cM distance (Melchinger et al. 1998).

The genotypic correlation between the predicted and observed performance of a RIL $\hat{r}_{\mathrm{g}}(\mathrm{M}_{\mathrm{iF3}},\mathrm{Y}_{\mathrm{iRIL}})$ was estimated. Here, $\mathrm{M}_{\mathrm{iF3}}$ is the predicted value of RIL *i* based on the marker genotype at the 41 selected SSR markers and QTL positions and effects estimated for the 351 F_3 families analyzed by Micic et al. (2004) and $\mathrm{Y}_{\mathrm{iRIL}}$is the observed value of RIL *i*. For details, see Utz et al. (2000).

Results

Phenotypic data

Means of parental inbred lines P_{R} and P_{S} differed significantly ($P<0.01$) for all traits except speed of fungal growth (Table 1). For the three resistance traits, histograms of 317 RIL means across environments are presented in Fig. 2. For leaf lesion and speed of fungal growth, the mean of the RIL transgressed those of the parents. For stem lesion, the resistant parent formed the tail of the distribution, while the mean of the RIL transgressed the mean of P_{S}. Based on data from 2003, the orthogonal contrast of the mean of the parental lines ($\bar{P}$) and the mean of the RIL was not significantly different for all traits. Means across environments for all traits followed a normal distribution.

Genotypic variances among RIL ($\hat{\sigma}_{\mathrm{g}}^2$) were highly significant for all traits (Table 1). Estimates of genotype × environment interaction variances ($\hat{\sigma}_{\mathrm{ge}}^2$) were significant ($P<0.01$) and, with the exception of leaf length, smaller than $\hat{\sigma}_{\mathrm{g}}^2$.Heritabilities for resistance traits were intermediate to high ($0.51<\hat{h}^2<0.79$).

Resistance traits were significantly correlated with each other ($0.45<\hat{r}_{\mathrm{g}}<0.78$).Phenotypic correlations of both morphological traits were significant ($P<0.01$) but small with stem lesion ($-0.17<\hat{r}_{\mathrm{p}}<-0.15$) and speed of fungal growth ($0.19<\hat{r}_{\mathrm{p}}<0.26$) and close to zero with leaf lesion ($-0.09<\hat{r}_{\mathrm{p}}<-0.01$).Leaf length with petiole was highly ($P<0.01$) correlated with leaf length ($\hat{r}_{\mathrm{p}}=0.79$).Phenotypic correlations between F_3 families and RIL were low, but significant ($P<0.01$) for all traits. Genotypic correlations were highly significant and ranged between 0.50 for leaf length and 0.64 for stem lesion and speed of fungal growth (Table 2).

Linkage map

Of the 41 selected codominant marker loci, 11 showed significant ($P<0.01$) deviations from the expected segregation ratio. Seven marker loci deviated significantly ($P<0.01$) from the expected allele frequencies of 0.5. The proportion of the P_{R} genome among the 248 RIL ranged from 9.7% to 85.4% with a mean of $\bar{x}=49.9\%$ (standard deviation = 15.4%). The average heterozy-

Table 1 Means of parental inbred lines P_{R} and P_{S} and estimates of variance components and heritabilities for 317 RIL for resistance and morphological traits measured in two environments

Parameters	Number	Resistance traits			Morphological traits	
		Leaf lesion (cm)	Stem lesion (cm)	Speed of fungal growth (cm/day)	Leaf length (cm)	Leaf length with petiole (cm)
Means						
P_{R} [a]	4	5.8 ± 0.39[c]	3.9 ± 2.83	0.84 ± 0.05	11.2 ± 0.91	20.6 ± 0.78
P_{S} [a]	4	8.0 ± 0.39	22.5 ± 2.83	0.85 ± 0.05	13.5 ± 0.91	16.1 ± 0.78
$\bar{P}$[a]	8	6.9 ± 0.27	13.2 ± 1.99	0.84 ± 0.03	12.4 ± 0.64	18.2 ± 0.65
RIL_{2003}[a]	317	6.6 ± 0.05	10.2 ± 0.47	0.78 ± 0.006	11.8 ± 0.10	18.5 ± 0.10
RIL	317	5.3 ± 0.04	12.2 ± 0.45	0.82 ± 0.006	13.1 ± 0.06	19.8 ± 0.09
Range of RIL		3.2–8.5	0.2–39.5	0.6–1.1	10.2–17.6	15.5–24.8
Variance components (RIL)						
$\hat{\sigma}_{\mathrm{g}}^2$		0.31 ± 0.05**	50.80 ± 5.18**	0.006 ± 0.001**	0.31 ± 0.13**	1.60 ± 0.21**
$\hat{\sigma}_{\mathrm{ge}}^2$		0.20 ± 0.05**	12.53 ± 2.16**	0.003 ± 0.001**	1.53 ± 0.16**	1.00 ± 0.15**
$\hat{\sigma}^2$		1.21 ± 0.05	41.45 ± 1.69	0.016 ± 0.0006	1.58 ± 0.06	2.67 ± 0.11
Heritability (RIL)						
$\hat{h}^2$		0.51	0.79	0.57	0.23	0.63
95% CI on $\hat{h}^{2\mathrm{b}}$		(0.39, 0.60)	(0.74, 0.83)	(0.47, 0.66)	(0.04, 0.39)	(0.54, 0.70)

**Variance component was significant at the 0.01 probability level

[a] Data from 2003 only

[b] Confidence intervals on $\hat{h}^2$ were calculated according to Knapp et al. (1985)

[c] Standard errors are attached

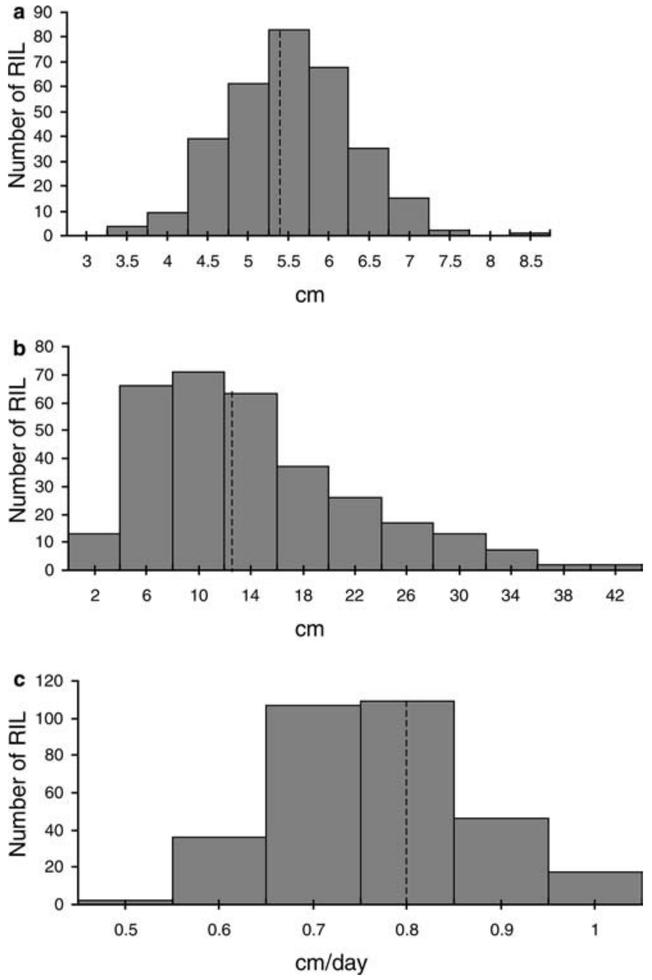

Fig. 2 Histograms for means of leaf lesion (**a**), stem lesion (**b**), and speed of fungal growth (**c**), measured in two environments in 317 RIL derived from cross $P_R \times P_S$. *Dashed lines* indicate the overall means

Table 2 Genetic correlations between F_3 families and RIL $((\hat{r}_g(F_3, \mathrm{RIL})))$ as well as of predicted and observed performance $((\hat{r}_g(M_{iF3}, Y_{iRIL})))$ for three resistance and two morphological traits. Parameters were estimated from the genotypic and phenotypic data of 248 RIL from the cross $P_R \times P_S$ evaluated in two environments

	Resistance traits			Morphological traits	
	Leaf lesion	Stem lesion	Speed of fungal growth	Leaf length	Leaf length with petiole
$\hat{r}_g(F_3,\mathrm{RIL})$	0.55	0.64	0.64	0.53	0.50
$\hat{r}_g(M_{iF3},Y_{iRIL})$	0.21	0.53	0.37	0.17	0.04

gosity at codominant markers (3.5%) was in close agreement with the theoretically expected proportion of 3.1% for F_6 plants calculated as 0.5^n, where n is the number of selfing generations. Forty-one marker loci coalesced in eight LGs (Fig. 1). With respect to both the linear order of the marker loci and the estimates of the genetic distances, the linkage map of the RIL was in good agreement with other published maps (Tang et al. 2002; Micic et al. 2004).

QTL analyses

For stem lesion, two putative QTL on LG8 and LG16 were identified (Table 3). The effect of the QTL detected on LG8 was substantial and explained 25.5% of the phenotypic variance. A simultaneous fit of both putative QTL explained 34.2% of σ_g^2 in the data set (DS) and 26.5% in CV. The resistant parent contributed the resistance-increasing allele at both QTL. For the other two resistance traits, five genomic regions with significant association between marker and phenotypic data were detected. The single QTL for leaf lesion could not be confirmed with CV. For speed of fungal growth, the estimated proportion of σ_g^2 explained by all four QTL was 41.7% but considerably lower with CV $(\tilde{p}_{TS} = 20.5\%)$.For the QTL identified for speed of fungal growth on LG1, the resistant allele was contributed by the susceptible parent.

For leaf length and leaf length with petiole, two QTL on LG6 and LG15 were identified. Partial $R^2{}_{adj}$ values ranged between 5.1% and 8.4% (Table 3). With CV, only a small proportion of the genotypic variance could be explained for the two morphological traits. Genotypic correlations between predicted and observed performance $\hat{r}_g(M_{iF3}, Y_{iRIL})$ were moderate to low $(0.53 > \hat{r}_g > 0.21)$ for the resistance traits and weak for the morphological traits $\hat{r}_g = 0.71$.

Discussion

Quantitative genetic parameters estimated for resistance against *S. sclerotiorum* and morphological traits were similar for RIL and F_3 families (Micic et al. 2004). The continuous distribution for disease ratings of the RIL corroborated the quantitative inheritance of the resistance. Estimates of the genotypic variance, heritability, and correlations among traits are not directly comparable for the two experiments. Environmental conditions in 1999 were extremely favorable for *Sclerotinia* infection of the F_3 families, and the genetic differentiation of resistance was more pronounced than in 2002 and 2003 for the RIL. This was reflected in lower means and smaller estimates of the genotypic variance $(\hat{\sigma}_g^2)$ for the RIL than for F_3 families. Heritability estimates and phenotypic correlations between resistance traits were somewhat lower for the RIL than for the F_3 families but generally of the same order except for leaf length with substantial genotype × environment interactions. The highest heritability estimate was found for stem lesion, corroborating our earlier findings (Micic et al. 2004). The good agreement between heritability estimates from the F_3 families and their RIL indicated a consistent expression of resistance alleles under varying environmental conditions.

Table 3 Parameters associated with putative QTL for three resistance and two morphological traits. The parameters were estimated from genotypic and phenotypic data of 248 RIL from the cross $P_R \times P_S$ evaluated in two environments

Resistance traits	Parameters	Linkage group/marker	Position on LG (cM)	LOD at QTL position	Genetic effect[a]	Variance[b] explained (%)
Leaf lesion (cm)		LG6/ORS57	18	2.89	0.15	5.6
	$\hat{p}_{DS}$					6.1
	$\tilde{p}_{TS}$					−0.3
Stem lesion (cm)		LG8/ORS623	16	15.51	3.86	25.5
		LG16/ORS31	4	5.48	2.48	9.8
	$\hat{p}_{DS}$					34.2
	$\tilde{p}_{TS}$					26.5
Speed of fungal growth (cm/day)		LG1/ORS605	50	2.65	−0.03	5.0
		LG4/ORS366	0	2.66	0.02	4.9
		LG8/ORS623	16	4.45	0.02	8.1
		LG16/ORS31	2	5.29	0.03	9.5
	$\hat{p}_{DS}$					41.7
	$\tilde{p}_{TS}$					20.5
Leaf length (cm)		LG6/ORS57	16	4.20	−0.34	8.0
		LG15/ORS151	62	2.71	0.26	5.1
	$\hat{p}_{DS}$					41.1
	$\tilde{p}_{TS}$					13.9
Leaf length With petiole (cm)		LG6/ORS57	16	4.41	−0.41	8.4
		LG15/ORS1025	42	3.08	0.70	5.7
	$\hat{p}_{DS}$					13.5
	$\tilde{p}_{TS}$					4.3

[a] Genetic effects were estimated in a simultaneous fit using multiple regression. Positive effects for resistance traits indicate that the QTL allele for resistance was contributed by P_R, while positive effects for morphological traits indicate that the leaf length increasing allele was contributed by P_S

[b] For any individual QTL, the proportion of the phenotypic variance ($R^2{}_{adj}$) explained was estimated; for the simultaneous fit, the proportion of the genotypic variance explained by putative QTL in the data set ($\hat{p}_{DS}$) and test sets ($\tilde{p}_{TS}$) using five-fold standard cross validation (CV) was estimated

The genotypic correlations between F_3 families and RIL were close to expectations (Table 2). Assuming that dominance is negligible, the maximum expected genotypic correlation between F_3 families and derived lines at homozygosity is $\hat{r}_g = 0.71$ (Bernardo 2003). For stem lesion, the estimated genotypic correlation between F_3 families and RIL was close to this maximum ($\hat{r}_g = 0.64$) indicating that early generation selection for resistance against midstalk rot should be effective. As earlier studies have shown that dominance plays only a minor role in *Sclerotinia* resistance (Mestries et al. 1998; Bert et al. 2002), this should also apply for early testing strategies of testcrosses in a hybrid breeding program.

QTL detection in F_3 and RIL generations

In the cross $\text{NDBLOS}_{\text{sel}} \times \text{CM625}$, seven LGs were identified that significantly affect the resistance trait stem lesion in F_3 families (Micic et al. 2004). Estimated effects at most QTL were small except at the QTL on LG8 ($R^2{}_{adj}$=36.7%). In total, 33.7% of the genotypic variance for resistance against *S. sclerotiorum* could be accounted for by those QTL. In the present study, the same seven LGs were covered by 36 of the 41 selected markers. The remaining five markers covered LG1, which was included in the analysis because it carried two QTL affecting leaf lesion with no association with morphological traits. The two largest QTL for stem lesion identified in F_3 families on LG8 and LG16 were also detected in the RIL with LOD $\le$ 2.5. The same two QTL, and only those, were also identified with a selective genotyping approach in the F_3 data set (Micic et al. 2005). The QTL on LG8 could also be confirmed in a second cross with a different resistance source derived from *H. tuberosus* (Micic et al. 2005). Increasing the power of QTL detection by lowering the LOD threshold to 1.5 yielded one additional QTL for stem lesion on LG15. Owing to the flat LOD profile, precise localization of the QTL peak was difficult on this LG, but it can be assumed that it is the same QTL identified in the F_3 data close to marker ORS151. The QTL detected in the F_3 families on LG6 as well as the QTL on LG2, LG3, and LG4 with the resistant allele originating from the susceptible parent could not be verified with the RIL.

To quantify the predictive value of the QTL identified in the F_3 families for the RIL derived from them, we calculated the genotypic correlation between the predicted and the observed performance $\hat{r}_g(M_{iF3}, Y_{iRIL})$.For stem lesion, the correlation was surprisingly high with $\hat{r}_g(M_{iF3}, Y_{iRIL}) = 0.53$ considering that only two QTL explaining 26.5% of σ^2_g could be verified in the analysis of RIL. When the prediction was based on QTL effects identified on LG8 and LG16 only, the correlation was reduced to 0.46, indicating that the other genomic regions had at least partly an effect on prediction but were too small to be detected in the RIL. The marker-based genotypic correlation between F_3 and the RIL ($\hat{r}_g(M_{iF3}, Y_{iRIL})$) and, thus, the potential selection gain was only slightly smaller than what could be expected from the phenotypic evaluation of F_3 families as a measure of the performance of homozygous lines ($\hat{r}_g = 0.64$).Consequently, we conclude that the two genomic regions identified in F_3 can be of use in a marker-assisted breeding program using $NDBLOS_{sel}$ as a resistance donor. The two marker intervals harboring the QTL are fairly small (3.2 cM on LG8 and 6.1 cM on LG16), thus allowing reliable characterization of RIL with respect to their QTL alleles based on flanking marker genotypes. Grouping RIL according to their genotype at markers ORS623, ORS243, ORS656, and ORS31 into resistant and susceptible lines resulted in a mean difference of 12.9% in resistance rating between the two distributions (Fig. 3). A clear separation could not be achieved, but marker-assisted pre-selection and the discarding of highly susceptible lines looks promising.

Micic et al. (2004) identified QTL for morphological traits closely linked to QTL for stem lesion on LG8 and LG16. This association between resistance and leaf morphology was not corroborated in the RIL. Only two QTL located on LG6 and LG15 were identified for each of the two leaf-length traits. It needs to be kept in mind, however, that only markers selected for their association with stem lesion were tested in this study. Therefore, additional QTL for morphological traits might have been identified with more extended marker coverage. However, no QTL for morphological characters were identified on LGs with significant effects on stem lesion. Along with the relatively low genotypic correlations for stem lesion and morphological characters in the RIL ($-0.36 < \hat{r}_g < -0.19$) this indicates that enhanced resistance against midstalk rot can be transmitted to progeny without an undesirable change in morphology.

When we interpret our QTL mapping results and genotypic correlations between F_3 families and RIL, for all traits except for stem lesion, the limited marker coverage of this study has to be taken into account. Of the nine marker intervals with significant effects on leaf lesion in the F_3 families, only six were analyzed in the RIL. It was surprising, however, that only one of these genomic regions (LG6) exhibited a significant QTL for leaf lesion in this study. This was also reflected in the relatively low predictive quality of results from F_3 families for the RIL. Slightly better results were obtained for speed of fungal growth, with three corroborated QTL regions and $\hat{r}_g(M_{iF3}, Y_{iRIL}) = 0.37$.The two QTL on LG8 and LG16 also detected for stem lesion and another QTL on LG1 were common to both generations. An additional QTL was detected in the marker interval ORS366-ORS695 on LG4 where QTL had been detected for leaf and stem lesion in F_3 but not for speed of fungal growth. However, deviating from the results of Micic et al. (2004), the resistance allele originated from P_R and not from P_S as in the F_3 families. On the eight LGs covered by markers in this study, a total of ten genomic regions affected resistance against S. *sclerotiorum* in the F_3 families. At six of these QTL, the resistance increasing allele originated from the susceptible parent CM625. Only one of these for speed of fungal growth on LG1 was confirmed in the RIL. However, with respect to a marker-assisted introgression program of resistance alleles from a donor, it was encouraging that the remaining four QTL, where resistance originated from $NDBLOS_{sel}$, could be confirmed.

According to theory, RIL should be more efficient and powerful for QTL detection because of their increased homozygosity and homogeneity, resulting in increased additive genetic variance and heritability estimates. The separation of linked QTL should be improved due to more recombination. A clear advantage of RIL over F_3 families for the number and resolution of QTL for stem lesion could not be confirmed in this study. Different reasons can account for these findings. First, three of the QTL detected by Micic et al. (2004) in F_3 families exhibited significant dominance effects, which cannot be detected in RIL and therefore might have failed to become significant. Second, F_3 families and RIL were not tested in the same environments. This mimics the situation of a marker-assisted breeding program in which the prediction of performance from early generations will have to be valid for future generations and years. Infection rates and heritability estimates were higher for the F_3 fami-

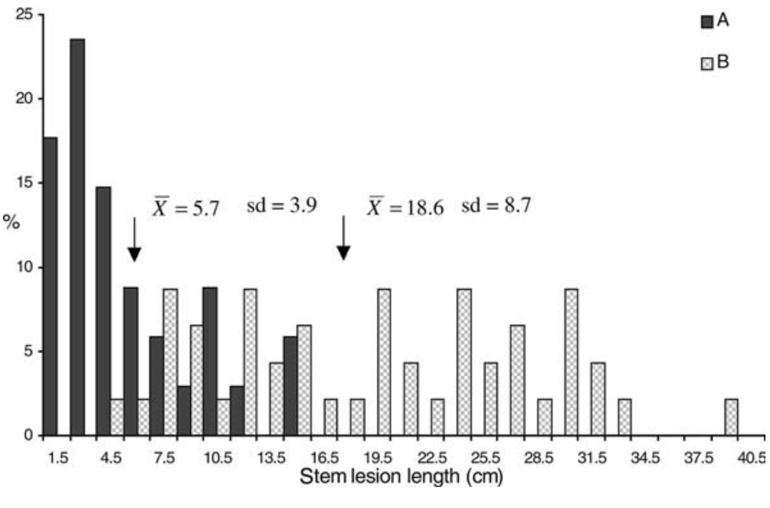

Fig. 3 Frequency distributions of stem lesion length for plants carrying exclusively resistant (**a**) and susceptible (**b**) alleles at four markers flanking two QTL on LG8 and LG16 in RIL. *Arrows* indicate the means of genotype classes homozygous for the A and B allele

lies tested in 1999 than in 2002 and 2003 due to more favorable environmental conditions for fungus development. Those alleles contributed by CM625, the susceptible parent, were particularly not stable across environments and could only be detected under optimum conditions. Third, the size of the RIL population was approximately 30% smaller than that of the F_3. These unequal sample sizes might have led to an imbalance in the power of QTL detection. Similar findings have also been reported in previous studies in maize showing only partial recovery of QTL detected in earlier generations of the RIL (Groh et al. 1998; Austin et al. 2000; Krakowsky et al. 2004).

Consequences for marker-assisted selection (MAS)

QTL identified in early generations can only be used efficiently for MAS if they are recovered in later generations. Consequently a high precision of QTL localization and tight linkage to the selected markers is important for MAS because of repeated recombination during the selfing process. In the present study, we were able to identify two genomic regions having a major effect on resistance to *S. sclerotiorum* consistent across generations. In addition, no significant change of plant morphology was carried over, which is encouraging with respect to making progress with MAS based on true resistance genes. QTL could be assigned consistently to the respective marker intervals, and markers flanking the QTL were tightly linked. Because resistance against midstalk rot in sunflower is difficult to evaluate phenotypically, we believe that increasing the selection intensity by marker-assisted pre-selection of genotypes and subsequent phenotypic selection will lead to improved selection gain.

Enhanced resistance against *S. sclerotiorum* should be achieved through the identification of new QTL and their pyramiding in resistance donor lines. First efforts have been made, and the QTL on LG8 could be confirmed in a resistance source different from $\text{NDBLOS}_{\text{sel}}$ originating from *Helianthus tuberosus* (Micic et al. 2005). Resistance breeding of sunflower against *S. sclerotiorum* is no simple task due to the complex inheritance of the trait, but we believe that both the resistance source $\text{NDBLOS}_{\text{sel}}$ and the markers identified in this study can aid in improving resistance against *S. sclerotiorum*.

Acknowledgements The Deutsche Forschungsgemeinschaft (DFG) (Sp292/7-1, Ha2253/3-1) supported this work. The authors thank S. Schillinger, T. Mellin, S. Kaiser, and A. Harmsen for their technical assistance and the staff at the Experimental Station, Eckartsweier, for conducting the field experiments.

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