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QTL mapping of *Sclerotinia* midstalk-rot resistance in sunflower

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Abstract In many sunflower-growing regions of the world, *Sclerotinia sclerotiorum* (Lib.) de Bary is the major disease of sunflower (*Helianthus annuus* L.). In this study, we mapped and characterized quantitative trait loci (QTL) involved in resistance to *S. sclerotiorum* midstalk rot and two morphological traits. A total of 351 F_3 families developed from a cross between a resistant inbred line from the germplasm pool NDBLOS and the susceptible line CM625 were assayed for their parental F_2 genotype at 117 codominant simple sequence repeat markers. Disease resistance of the F_3 families was screened under artificial infection in field experiments across two sowing times in 1999. For the three resistance traits (leaf lesion, stem lesion, and speed of fungal growth) and the two morphological traits, genotypic variances were highly significant. Heritabilities were moderate to high ($h^2 = 0.55$–0.89). Genotypic correlations between resistance traits were highly significant ($P < 0.01$) but moderate. QTL were detected for all three resistance traits, but estimated effects at most QTL were small. Simultaneously, they explained between 24.4% and 33.7% of the genotypic variance for resistance against *S. sclerotiorum*. Five of the 15 genomic regions carrying a QTL for either of the three resistance traits also carried a QTL for one of the two morphological traits. The prospects of marker-assisted selection (MAS) for resistance to *S. sclerotiorum* are limited due to the complex genetic architecture of the trait. MAS can be superior to classical phenotypic selection only with low marker costs and fast selection cycles.

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Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is an omnivorous and nonspecific plant pathogen. In all sunflower-growing regions of the world, *S. sclerotiorum* is common and widespread (Gulya et al. 1997). Under severe infection, yield losses in sunflower can reach up to 100% (Sackston 1992), depending on the infected plant parts. The fungus causes three distinct types of disease on sunflower: wilt, midstalk rot, and head rot. Results from the literature are ambiguous concerning the association of susceptibility of sunflower genotypes to *S. sclerotiorum* infection on root, leaf, and head. While Tourvieille and Vear (1984) found no significant associations between the three forms of infection, Castaño et al. (1993) reported relatively high correlations for resistance to mycelial extension in roots, stalk, and head.

In this study, we focused on midstalk rot due to its importance in sunflower growing areas in Germany and the availability of a reliable resistance test that determines the mycelium extension in leaves and stems as a measure of resistance to midstalk rot caused by *S. sclerotiorum* (Degener et al. 1998). Midstalk rot is caused through wind-borne ascospores produced in apothecia (Regnault 1976). The symptoms generally begin as a tan-to-gray lesion that rings the stalk. The stem becomes bleached and shredded, and sclerotia develop in the infected tissue. Such plants usually break at the site of infection, which leads to total yield loss.

Chemical measures to control *S. sclerotiorum* in sunflower are ineffective (Péres and Regnault 1985). Thus, the development of highly resistant sunflower cultivars is desirable under ecological and economical aspects. However, in cultivated sunflower germplasm,

no sources of complete resistance to *S. sclerotiorum* are available, but significant differences in susceptibility exist (Tourvieille et al. 1996; Degener et al. 1998). The genetic mechanisms underlying *S. sclerotiorum* resistance are complex. Genetic studies demonstrated a polygenic inheritance of the resistance for all three forms of infection (root, stalk, and head, Robert et al. 1987; Tourvieille and Vear 1990) and no race specificity (Thuault and Tourvieille 1988). Earlier studies found additive gene action to be more important than dominance (Robert et al. 1987).

The genetic analysis of complex traits has been amended by the application of molecular marker technologies. In the last 9 years, several genetic linkage maps of cultivated sunflower were published based on restriction fragment length polymorphisms (Berry et al. 1995; Gentzbittel et al. 1995; Jan et al. 1998; Gedil et al. 2001), simple sequence repeats (SSRs) (Bert et al. 2002; Tang et al. 2002; Burke et al. 2002; Yu et al. 2003), amplified fragment length polymorphism, and direct amplification of length polymorphisms markers (Langar et al. 2003). Thus, the molecular tools are available in sunflower to efficiently map quantitative trait loci (QTL) for agriculturally important traits such as resistance to midstalk rot caused by *S. sclerotiorum*.

Objectives of our study were to:

1. Estimate the number, genomic positions, and genetic effects of QTL involved in resistance to *S. sclerotiorum* midstalk rot.
2. Determine the proportion of the genotypic variance explained by all detected QTL via cross validation (CV).
3. Investigate associations between midstalk-rot resistance and morphological traits.
4. Draw conclusions about the prospects of marker-assisted selection (MAS) for increasing the level of resistance to *S. sclerotiorum* in sunflower.

Materials and methods

Plant material

Ninety sunflower inbred lines were screened for resistance to *S. sclerotiorum* (Degener et al. 1999). Line $NDBLOS_{sel.}$ (further denoted P_R), an inbred developed from the germplasm pool NDBLOS (Roath et al. 1987), was chosen as parent due to its high resistance to midstalk rot after artificial infection with *S. sclerotiorum*. The source of resistance of parental line P_R is uncertain, as the original germplasm pool NDBLOS was obtained by bulking equal amounts of 49 B lines selected for oil content (Roath et al. 1987). Inbred line CM625 was selected as the susceptible parent (P_S). One F_1 plant derived from the cross $P_R \times P_S$ was self-pollinated to produce F_2 plants. Randomly chosen F_2 plants were selfed to produce 354 F_3 families.

Field experiments

Resistance of F_3 families against midstalk rot caused by *S. sclerotiorum* was evaluated in 1999 in two experiments in Eckartsweier, located in the Upper Rhine Valley (140 m above sea level, 9.9°C mean annual temperature, 726 mm mean annual precipitation) 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 overplanted and later thinned to a final plant density of about 8 plants/m^2. Experiments were sown on 7 May (experiment 1) and 23 June (experiment 2) and inoculated on 7 July and 16 August, respectively. Each experiment was laid out as a 19×19 lattice design, with three replications consisting of 354 F_3 families and parental lines as triplicate (P_R) and quadruple entries (P_S).

Fungal isolate

The *S. sclerotiorum* isolate used in this study was collected in 1995 from naturally infected sunflower plants at Eckartsweier. The inoculum was cultured at 25°C on a 1.5% agar medium containing 2% malt and 0.2% peptone extract. After 2 days, mycelial growth was visible on the agar discs.

Leaf infection method

The leaf test of Degener et al. (1998) was used to determine the midstalk rot of sunflower after 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 to the bag to maintain sufficient air humidity.

Two morphological and three resistance traits were recorded:

1. Leaf length measured in centimeters from the leaf apex to the base of the petiole 1 week after inoculation.
2. Leaf length with petiole measured in centimeters.
3. Leaf lesion measured in centimeters as the length of the brown rotted zone along the leaf vein, beginning around the explant 1 week after inoculation.
4. Speed of fungal growth reflecting fungal progression inside the leaf and petiole tissue, estimated from the ratio between leaf length with petiole in centimeters and the time in days from leaf inoculation until the lesion of the fungus reached the base of the petiole.
5. Stem lesion measured in centimeters as length of the tan-to-gray rotted zone on the stem, 1 month after inoculation.

Marker analyses

Leaf tissue from 352 F_2 plants was collected and dried. The leaf material was ground to a fine powder by using a mixer mill Retsch MM2000. Genomic DNA was extracted as described in detail by Köhler and Friedt (1999). The two parent lines were screened for polymorphism, with a total of 1,109 SSR primer pairs, of which 1,089 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 were published by Paniego et al. (2002). 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), using fluorescence (CY5) labeled primers. The computer program Allele Link (Amersham Pharmacia Biosciences) was used for allele scoring.

Statistical analyses

Field data

Lattice analyses of variance were performed with data from each experiment, 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 experiments. 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 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). 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 calculated according to Mode and Robinson (1959). All necessary computations for the field trials were performed with the software package PLABSTAT (Utz 2000).

Marker data

At each SSR marker locus, deviations of observed frequencies from allele frequency 0.5 and from the expected Mendelian segregation ratio (1:2:1) were tested with χ^2 tests (Weir 1996). Because of multiple tests, appropriate type I error rates were determined by the sequentially rejective Bonferroni procedure (Holm 1979). A linkage map for cross $P_R \times P_S$, based on the 352 F_2 plants and 117 codominant SSR marker loci, was constructed by using the software package JoinMap, version 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 a recombination threshold 0.25. After the determination of linkage groups (LGs) and the corrected linear alignment of marker loci along the LGs, 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 the software package PLABQTL (Utz and Melchinger 1996). QTL analyses were performed with means across experiments of 351 F_3 families for which both high-quality marker and phenotypic data were available. The method of composite interval mapping (CIM) 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 $P_e < 0.38$, 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 ($\hat{\sigma}^2_p$) explained by QTL was determined by the estimator $\hat{R}^2_{adj}$ as described by Utz et al. (2000). The proportion of the genotypic variance explained by all QTL ($\hat{p}$) was determined from the ratio:

$$\hat{p} = \frac{\hat{R}^2_{adj}}{\hat{h}^2}.$$

Standard fivefold 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 detected QTL simultaneously were calculated for the total data set (DS) ($\hat{p}_{DS}$) and as the median over all TS ($\tilde{p}_{TS}$). Two QTL were declared as congruent across traits if they had the same sign and were within a 20-cM distance (Melchinger et al. 1998).

Results

Phenotypic data

After 3 days of 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 93% for the first and 94% for the second experiment. Means of parental inbred lines P_R and P_S differed significantly ($P < 0.01$) for all traits (Table 1). Histograms of 354 F_3 line means across experiments for the three resistance traits are presented in Fig. 1. The values for leaf lesion ranged from 4.1 cm to 11.9 cm, and their distribution was significantly skewed towards higher values. The distribution of stem lesion was significantly skewed towards lower values and varied from 0.1 cm to 64.4 cm. Speed of fungal growth of the F_3 families followed a normal distribution, ranging from 1.1 to 2.1 cm/day. For leaf lesion and speed of fungal growth, F_3 families transgressed the means of the parents. For stem lesion, the parents formed the tails of the distribution. The orthogonal contrast of the mean of the parental lines ($\bar{P}$) and the mean of F_3 families was significant ($P < 0.01$) for the resistance traits but not for the morphological traits. F_3 families were on average more resistant than the mean of the parents. Means across experiments for morphological traits of the F_3 families also followed a normal distribution (data not shown).

Genotypic variances among F_3 families ($\hat{\sigma}^2_g$) were highly significant for all traits (Table 1). Estimates of genotype × environment interaction variances ($\hat{\sigma}^2_{ge}$) were small compared with $\hat{\sigma}^2_g$ and significant ($P < 0.01$) only for leaf length and leaf length with petiole but not for resistance traits. Heritability estimates for resistance traits were intermediate to high.

Resistance traits were significantly but only moderately correlated with each other (Table 2). Leaf length with petiole was tightly ($P < 0.01$) correlated with leaf length. Correlations of both morphological traits were medium with stem lesion, weak with leaf lesion, and close to zero with speed of fungal growth. Genotypic correlations were generally slightly higher than corresponding phenotypic correlations.

Linkage map

Out of the 1,109 tested primer pairs, 117 high-quality, codominant marker loci were chosen for construction of the genetic linkage map. Dominant markers were not used. Seven out of the 117 loci (5.9%) showed significant ($P < 0.01$) deviations from the expected segregation ratio (1:2:1). Allele frequencies did not deviate significantly from 0.5 at any marker locus. The proportion of the P_R genome among the 352 F_2 individuals followed a normal distribution and ranged from 29.0% to 76.1% with a mean of $\bar{x} = 49.9\%$ (standard deviation = 8.0%). A genetic linkage map of the 352 F_2 individuals was constructed based on 113 of the 117 polymorphic marker loci that coalesced into 16 LGs (Fig. 2). Each LG was numbered according to Tang et al. (2002) and presumably corresponds to one of the 17 chromosomes in the haploid sunflower genome ($x = 17$). Four loci were unlinked (ORS 502, ORS 601, ORS 1086, and ORS 1193). The LGs ranged in length from 8.2 cM to 127.1 cM, covering a total map distance of 961.9 cM, with an average interval length of 9.6 cM. About 97.2% of the mapped genome was located within a 20-cM distance to the nearest marker. For QTL analyses, the four unlinked loci were assigned to an artificial LG, with 50-cM interval lengths between markers.

QTL analyses

For leaf lesion, a total of nine QTL were detected, with resistance alleles originating from the susceptible parent at three QTL (Table 3). The partial R^2 of individual

Table 1 Means of parental inbred lines P_R and P_S, as well as estimates of variance components and heritabilities for 354 F_3 families for resistance and morphological traits measured in two experiments

Parameters	No.	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	3	7.4 ± 0.34	7.6 ± 2.30	1.4 ± 0.05	21.8 ± 0.60	34.7 ± 0.94
P_S	4	9.2 ± 0.30	60.8 ± 2.05	1.8 ± 0.05	15.4 ± 0.58	27.4 ± 0.91
$\bar{P}$	7	8.3 ± 0.22	34.2 ± 1.54	1.6 ± 0.03	18.6 ± 0.41	31.1 ± 0.65
F_3 families	354	7.8 ± 0.04	22.1 ± 0.60	1.5 ± 0.01	18.7 ± 0.08	31.4 ± 0.12
Range of F_3 families		4.1–11.9	0.1–64.4	1.1–2.1	14.4–27.7	23.4–41.0
Variance components (F_3 families)						
$\hat{\sigma}^2_g$		0.40 ± 0.06**	115.50 ± 9.77**	0.011 ± 0.001**	1.83 ± 0.18**	4.10 ± 0.43**
$\hat{\sigma}^2_{ge}$		0.02 ± 0.05	1.88 ± 2.33	0.001 ± 0.001	0.51 ± 0.09**	1.23 ± 0.23**
$\hat{\sigma}^2$		1.88 ± 0.07	78.36 ± 3.04	0.037 ± 0.001	1.97 ± 0.08	5.01 ± 0.19
Heritability (F_3 families)						
$\hat{h}^2$		0.55	0.89	0.62	0.76	0.74
95% CI on $\hat{h}^2$[a]		(0.46; 0.64)	(0.87; 0.91)	(0.54; 0.69)	(0.70; 0.80)	(0.68; 0.79)

[a]Confidence intervals on $\hat{h}^2$ were calculated according to Knapp et al. (1985)
**Variance component was significant at the 0.01 probability level

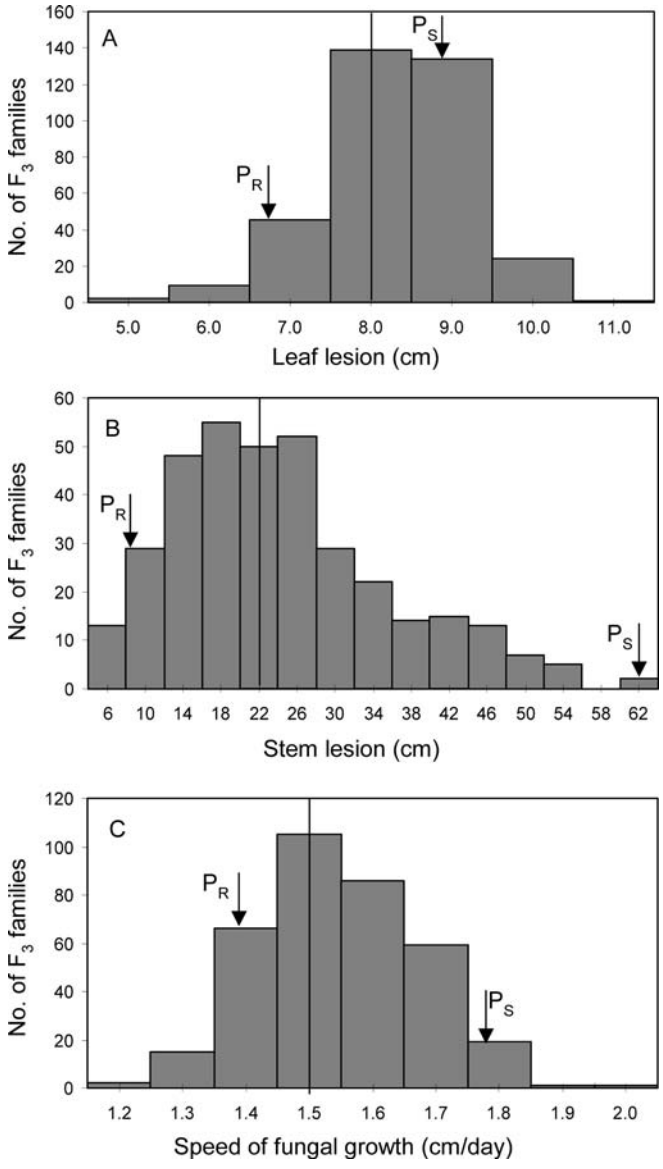

Fig. 1 Histograms for **a** leaf lesion, **b** stem lesion, and **c** speed of fungal growth measured in two experiments in 1999, for means of 354 F_3 families derived from the cross $P_R \times P_S$. A *solid line* indicates the overall mean. *Arrows* indicate the means of parental lines P_R and P_S

QTL ranged from 3.4% to 11.3%. Most of the QTL displayed additive gene action, except the QTL on LG1 and LG9, where significant dominance effects were found. The estimate of the proportion of σ_g^2 explained by all QTL was 45.6% for $\hat{p}_{DS}$, but considerably lower with CV ($\tilde{p}_{TS} = 25.3\%$).

For stem lesion, eight putative QTL were identified. At seven of them, the partial R^2 was 6% or smaller, but the effect of the QTL detected on LG8 was substantial and explained 36.7% of the phenotypic variance. With the exception of the QTL on LG3, which showed a significant partial dominance effect, only significant additive gene effects were found. At three QTL, the resistance allele originated from the susceptible parent P_S. A simultaneous fit of all putative QTL explained 50.5% of σ_g^2 in DS and 33.7% in CV.

For speed of fungal growth, six putative QTL with partial R^2 values up to 10.2% were detected. All alleles showed additive gene action, and the resistance was always contributed by the resistant parent, except on LG1. Estimates of σ_g^2 explained by all detected QTL were $\hat{p}_{DS} = 39.5$ and $\tilde{p}_{TS} = 24.4\%$.

For leaf length and leaf length with petiole, seven and nine putative QTL with partial R^2 values between 3.3% and 11.9% were detected (Table 3). Most QTL showed additive gene action, except the QTL on LG10 exhibiting overdominance. In a simultaneous fit, estimates of $\hat{p}_{DS}$ were 38.4% and 51.2%, respectively, but the corresponding values for $\tilde{p}_{TS}$ were only half as much.

Discussion

Inheritance of resistance to midstalk rot

In elite sunflower material, the inheritance of resistance to *S. sclerotiorum* has been found to be polygenic, with medium heritability (Mestries et al. 1998). The frequency distributions of the three resistance traits and results from the ANOVA confirmed these findings. Consequently, a large population size ($n = 351$) was chosen for the mapping of QTL to increase the power of QTL detection. QTL were detected for all three resistance traits,

Table 2 Phenotypic (above diagonal) and genotypic (below diagonal) correlations of morphological and resistance traits estimated in a population of 354 F_3 families derived from the cross $P_R \times P_S$ evaluated in two experiments

	Resistance traits			Morphological traits	
	Leaf lesion	Stem lesion	Speed of fungal growth	Leaf length	Leaf length with petiole
Leaf lesion		0.45^{**}	0.52^{**}	-0.25^{**}	-0.28^{**}
Stem lesion	0.55^{++}		0.66^{**}	-0.48^{**}	-0.37^{**}
Speed of fungal growth	0.68^{++}	0.75^{++}		0.04	−0.02
Leaf length	-0.31^{++}	-0.56^{++}	−0.08		0.81^{**}
Leaf length with petiole	-0.35^{++}	-0.41^{++}	-0.10^{+}	0.80^{++}	

**Phenotypic correlation was significant at the 0.01 probability level
$^{+,++}$Genotypic correlation exceeded once or twice its standard error, respectively

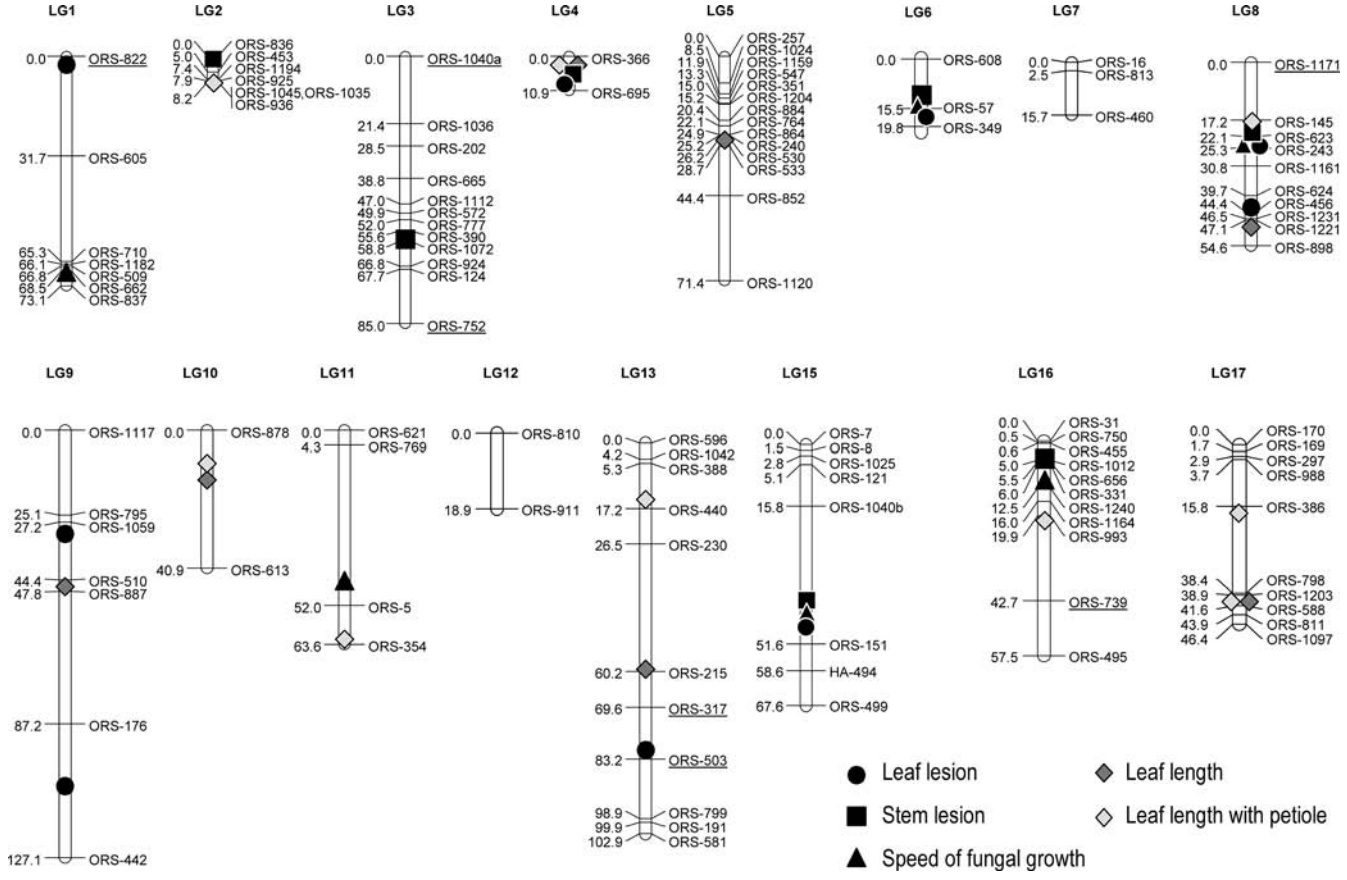

Fig. 2 Genetic linkage map of sunflower based on 352 F_2 individuals derived from cross $P_R \times P_S$ for 113 SSR marker loci. *Numbers to the left* of the linkage group (LG) indicate the cumulative distance in centiMorgans (Haldane). Loci with distorted segregation ratios ($P < 0.01$) are *underlined*. Positions of quantitative trait loci (QTL) for scored traits are indicated by *symbols* explained in the legend

but estimated effects at most QTL were small and severely inflated despite the large population size, as indicated by the large difference between $\hat{p}_{DS}$ and $\tilde{p}_{TS}$. In total, only between 24.4% and 33.7% of the genotypic variance for resistance against *S. sclerotiorum* could be accounted for by QTL. Thus, the data confirm the hypothesis that a large number of genes with small effects are involved in resistance to midstalk rot.

The superior resistance of parental line P_R was confirmed in this study. At most QTL, alleles conferring increased resistance against *S. sclerotiorum* originated from P_R. Line CM625 was chosen as parent, because it had shown high susceptibility to *S. sclerotiorum* in artificial leaf infections (V. Hahn, unpublished data). However, as reported for other resistance traits (Schön et al. 1993; Bohn et al. 2000), the susceptible parent P_S also carried resistance alleles. For leaf lesion, significant transgression towards higher resistance of F_3 families was observed, suggesting that the susceptible parent CM625 contributed favorable alleles for resistance. The results from QTL analyses confirmed this hypothesis with three out of nine favorable QTL alleles for leaf lesion resistance originating from the susceptible parent P_S (Table 3). It was also apparent that the susceptible parent carried some resistance alleles for the other two resistance traits, but the sum of partial R^2 values for QTL with favorable alleles from P_R for stem lesion and speed of fungal growth were considerably larger than those from P_S (Table 3).

To compare the chromosomal positions of QTL detected in our study with those of previous studies, the LGs of Tang et al. (2002) were cross-referenced to the nomenclature of the SSR maps of Mestries et al. (1998) and Bert et al. (2002) (A. Leon, personal communication). Bert et al. (2002) found three QTL explaining about 56% of the phenotypic variance for the trait mycelium on leaves on LGs 6, 8, and 13, which coincided with LGs 13, 9, and 1 in our study, all three carrying significant QTL for leaf lesion. An integrated genetic map with data from all available SSR markers is currently being established (L. Gentzbittel, personal communication) and will provide further insight if the genomic regions identified in the two studies overlap. Bert et al. (2002) found no common QTL between their results and those of Mestries et al. (1998), who detected five different QTL for lesion length on leaves in different selfing generations. On three of the four LGs reported by Mestries et al. (1998), we detected significant QTL for stem lesion but not for leaf lesion. In all three studies, a similar resistance test for mycelial extension on leaves was used but with different genetic materials. Bert et al.

Table 3 Parameters associated with putative quantitative trait loci (*QTL*) for three resistance and two morphological traits estimated from genotypic and phenotypic data of 351 F_3 families from the cross $P_R \times P_S$, evaluated in two experiments

Traits	Linkage group (LG)	Marker	Position on LG (cM)	LOD at QTL position	Genetic effect[a]		Variance explained[b]
					Additive	Dominance	
Leaf lesion (cm)	LG1	ORS 822	2	7.70	−0.38	0.14	10.0
	LG4	ORS 366	10	4.46	−0.26	NS[c]	6.3
	LG6	ORS 57	18	3.63	0.25	NS	4.9
	LG8	ORS 623	24	5.56	0.37	NS	7.1
	LG8	ORS 624	44	2.64	−0.23	NS	3.4
	LG9	ORS 795	30	2.79	0.19	NS	3.6
	LG9	ORS 176	94	8.71	0.35	0.34	11.3
	LG13	ORS 317	82	3.55	0.30	NS	4.6
	LG15	ORS 1040b	48	3.59	0.24	NS	4.6
$\hat{p}_{DS}$							45.6
$\tilde{p}_{TS}$							25.3
Stem lesion (cm)	LG2	ORS 836	2	4.38	−3.01	NS	5.7
	LG3	ORS 390	58	4.67	−2.78	−1.82	6.0
	LG4	ORS 366	8	2.63	−2.34	NS	3.7
	LG6	ORS 608	10	3.30	2.54	NS	4.5
	LG8	ORS 145	20	34.67	8.74	NS	36.7
	LG15	ORS 1040b	42	2.51	3.43	NS	3.3
	LG16	ORS 455	4	4.69	2.71	NS	6.0
		ORS 502[d]	84	4.44	4.16	NS	5.8
$\hat{p}_{DS}$							50.5
$\tilde{p}_{TS}$							33.7
Speed of fungal growth (cm/day)	LG1	ORS 509	68	4.70	−0.03	NS	6.0
	LG6	ORS 608	14	4.88	0.04	NS	6.6
	LG8	ORS 623	24	5.52	0.04	NS	7.0
	LG11	ORS 769	40	3.53	0.04	NS	4.6
	LG15	ORS 1040b	44	8.18	0.06	NS	10.2
	LG16	ORS 331	10	7.34	0.05	NS	9.2
$\hat{p}_{DS}$							39.5
$\tilde{p}_{TS}$							24.4
Leaf length (cm)	LG4	ORS 366	4	8.75	0.63	NS	11.9
	LG5	ORS 240	26	2.54	0.97	NS	3.3
	LG8	ORS 1221	48	3.66	−0.41	NS	4.9
	LG9	ORS 510	46	4.50	−0.44	NS	5.8
	LG10	ORS 878	16	3.97	−0.23	0.85	5.5
	LG13	ORS 230	60	3.82	−0.41	NS	4.9
	LG17	ORS 1203	40	2.94	−0.46	NS	3.8
$\hat{p}_{DS}$							38.4
$\tilde{p}_{TS}$							21.6
Leaf length with petiole (cm)	LG2	ORS 925	8	3.29	0.52	NS	4.3
	LG4	ORS 366	4	6.89	0.85	NS	9.5
	LG8	ORS 145	18	8.84	−0.89	NS	11.0
	LG10	ORS 878	12	3.14	−0.31	NS	4.4
	LG11	ORS 5	62	2.51	0.46	NS	3.4
	LG13	ORS 388	16	2.87	−0.56	NS	3.7
	LG16	ORS 993	20	2.63	0.49	NS	3.4
	LG17	ORS 386	16	4.54	−0.79	NS	5.8
	LG17	ORS 1203	40	4.73	–0.86	NS	6.1
$\hat{p}_{DS}$							51.2
$\tilde{p}_{TS}$							31.0

[a]Genetic effects were estimated in a simultaneous fit with composite interval mapping using multiple regression

[b]For individual QTL, the proportion of the phenotypic variance (R_{adj}^2) 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 the median over 1,000 test sets ($\tilde{p}_{TS}$)using fivefold standard cross validation was estimated

[c]*NS* Not significant

[d]Marker not assigned to linkage map

(2002) attributed the lack of congruency of their results with those of Mestries et al. (1998) to the polygenic nature of *S. sclerotiorum* resistance in sunflower with different QTL being involved, depending on the source of resistance. Furthermore, the poor congruency could be explained by the different environmental conditions under which the resistance tests were conducted.

Further evidence for the complex inheritance of resistance against *S. sclerotiorum* stems from the limited congruency of QTL for different resistance traits. As expected from the intermediate genotypic correlations, only two genomic regions (LG8, LG15) showed common QTL for all three traits measuring resistance to midstalk rot. In a third genomic region on LG6, QTL for leaf lesion and stem lesion were located 22 cM apart. However, the LOD curve for stem lesion did not have a well-defined maximum. In the vast majority of the 1,000 CV runs, the QTL was located at position 82 instead of 74 as in the DS, indicating that the same QTL could affect leaf lesion, stem lesion, and speed of fungal growth on LG6 (data not shown). For the two resistance traits leaf lesion and stem lesion, only half of the detected QTL were in common. This could be a result of the limited power of QTL detection, but it is also possible that different genetic factors are responsible for expression of resistance to mycelial extension in leaves and

stems as described for resistance of sunflower to *Phomopsis* (Langar et al. 2002). Consequently, a large number of markers associated with QTL for different resistance traits will have to be considered in MAS for obtaining maximum resistance against *S. sclerotiorum*. The challenge is even greater when attempting to combine resistance to *S. sclerotiorum* in stem, head, and root. Depending on the genetic material analyzed, most authors found different genetic factors to control resistance against the three types of disease (Tourvieille and Vear 1984). This was confirmed by the QTL mapping studies of Mestries et al. (1998) and Bert et al. (2002), who identified different genomic regions for resistance against stem and head rot. In a recent study, P_R was also highly resistant against head-rot resistance (Hahn 2002). Further research is warranted to test, if common genomic regions can be identified that regulate both, midstalk and head rot, or if different QTL are responsible for resistance against the two traits.

Correlation between resistance and agronomic traits

The resistance mechanisms of parental line P_R are unknown. Previous studies indicated that morphological traits, such as branching (Jouan et al. 2000) or leaf length (Degener et al. 1998), can affect sunflower resistance against *S. sclerotiorum*. Leaf length of P_R is large and therefore, the association of morphological characters with resistance traits was investigated. Only 5 of the 15 genomic regions carrying a QTL for either of the three resistance traits also carried a QTL for one of the two morphological traits. Two of the genomic regions carrying QTL for all three resistance traits had no effect on morphological traits. This was encouraging with respect to making progress in selection based on true resistance genes, but could also be attributed to sampling and the fact that for all traits a large proportion of the genotypic variance could not be accounted for by QTL. However, the low genotypic correlations of the morphological and resistance traits do not support this hypothesis but rather corroborate findings of Degener et al. (1999), who selected an inbred line, with high levels of resistance to midstalk rot and short leaf length, out of a cross between P_R and a susceptible line with short leaf length.

An example for a genomic region, which affected both resistance as well as morphology, was found on LG8. A major QTL for stem lesion explaining more than 36% of σ_p^2 was located between markers ORS 145 and ORS 243. The same interval also harbored QTL for the other two resistance traits and the largest QTL for leaf length with petiole, explaining 11.0% of σ_p^2. The QTL for stem lesion and leaf length with petiole were mapped at a 2-cM distance. To validate the most likely QTL position for these traits, QTL frequency distributions based on 1,000 CV runs were analyzed. The vast majority of runs clearly separated the two QTL at the positions determined by CIM in the DS (Fig. 3). The presence of a QTL for speed of fungal growth a trait that

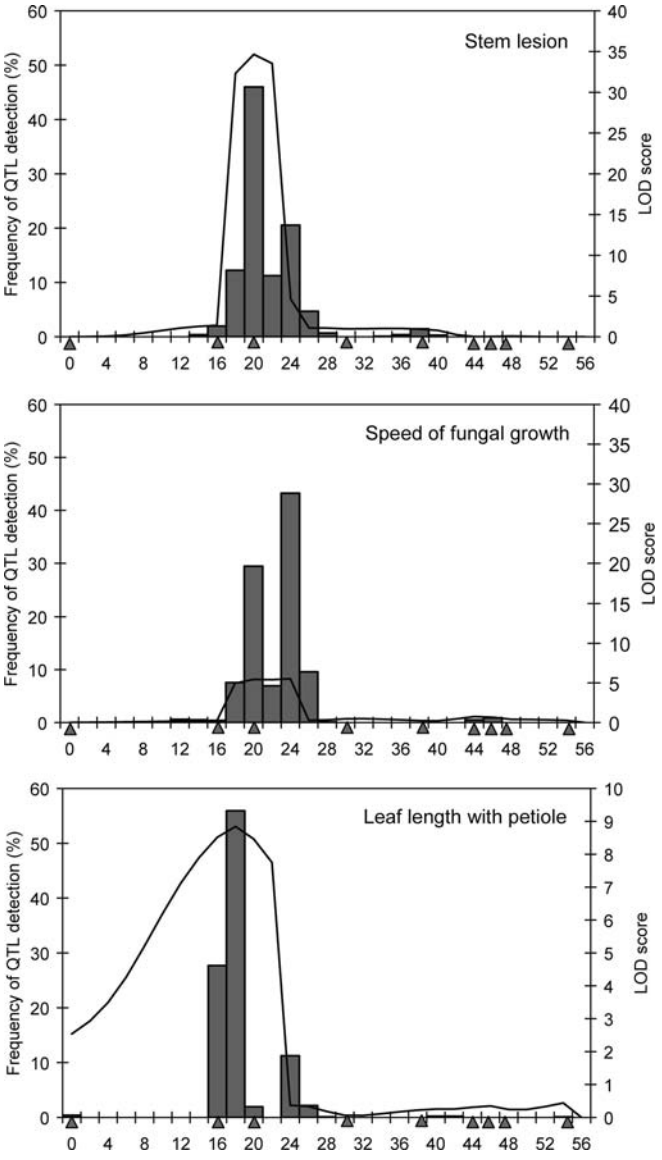

Fig. 3 QTL frequency distributions for stem lesion, speed of fungal growth, and leaf length with petiole on LG8 obtained from 1,000 cross validation runs for 351 F_3 families of the cross $P_R \times P_S$. The *solid line* indicates the LOD curves determined from the entire data set, using composite interval mapping. Marker positions are denoted by *triangles*

is independent of leaf morphology in the same interval supports the hypothesis of tightly linked QTL rather than one QTL with pleiotropic effects in this genomic region on LG8. The LOD curve for speed of fungal growth was very flat in the respective marker interval, but frequency distributions corroborated the most likely position of the QTL at position 24 cM, i.e., closer to stem lesion than to leaf length with petiole.

Prospects of MAS for *S. sclerotiorum* resistance

The key parameters for evaluation of the efficiency of MAS compared to classical phenotypic selection (CPS) are the heritability of the trait under study and the

Table 4 Relative efficiency (*RE*) of marker-assisted selection (MAS) based on pure MAS (RE_{MAS}) or combined MAS (RE_{cMAS}), using both phenotypic and marker data

Underlying estimate of $\hat{p}$	RE_{MAS}[a]	RE_{cMAS}[b]
Leaf lesion		
$\hat{p}_{DS}$	0.91	1.11
$\tilde{p}_{TS}$	0.68	1.05
Stem lesion		
$\hat{p}_{DS}$	0.75	1.00
$\tilde{p}_{TS}$	0.62	1.00
Speed of fungal growth		
$\hat{p}_{DS}$	0.80	1.07
$\tilde{p}_{TS}$	0.62	1.06

[a]For MAS based on marker data, RE was calculated as $RE_{MAS} = \sqrt{\hat{p}/\hat{h}^2}$

[b]For phenotypic and marker data, RE was calculated as $RE_{cMAS} = \sqrt{\left(\hat{p}/\hat{h}^2\right) + \left((1-\hat{p})^2/(1-\hat{h}^2\hat{p})\right)}$

proportion of the genotypic variance explained by QTL ($\hat{p}$). The relative efficiency (RE) of MAS compared to CPS was calculated with formulas of Lande and Thompson (1990) and estimates of $\hat{h}^2$ and $\hat{p}_{DS}$ or $\tilde{p}_{TS}$. Both pure MAS based on marker data and combined MAS (cMAS), with optimum weights for phenotypic and marker data, were considered. We assumed (1) the same selection intensity for MAS, cMAS and CPS, implying equal costs for genotyping and phenotyping, and (2) marker data points to be recorded without error.

Values of RE_{MAS} were notably below 1.0 for all three traits (Table 4). This was expected from theory (Lande and Thompson 1990) and simulation studies (Moreau et al. 1998), showing that MAS was not superior over CPS for traits with medium to high heritability ($\hat{h}^2 > 0.5$) and less than half of ${\sigma_g}^2$ explained by markers. Similarly, RE_{cMAS} barely exceeded 1.0, due to the small proportion of the genotypic variance explained by markers and, consequently, a high weight assigned to the phenotypic score. While $\tilde{p}_{TS}$ tends to slightly underestimate the true parameter $\hat{p}$ (Schön et al. 2004), the relative efficiency of cMAS hardly increased, even when inserting the inflated estimates ($\hat{p}_{DS}$).

Conventional phenotypic selection for resistance to *S. sclerotiorum* is tedious and costly. So far, progress in breeding resistant cultivars has been slow due to the complex inheritance of the trait. Considering the results obtained in different QTL mapping studies, MAS for resistance seems no simple task. A high number of different genomic regions have been identified to affect resistance to *S. sclerotiorum*, depending on the germplasm, the generation, the plant part, and the test environments. Despite large population sizes, generally less than 50% of the phenotypic variance was explained by the detected QTL, and when validated with CV, only a third of the genotypic variance for resistance to *S. sclerotiorum* was accounted for by markers. However, estimates of $\hat{h}^2$ and $\hat{p}$ are calculated separately for different components of resistance to midstalk rot. With the artificial screening test, different resistance mechanisms in different stages of the progression of the fungus are accounted for. Thus, it might well be that if markers for both leaf lesion and stem lesion are used simultaneously as predictors for resistance against *S. sclerotiorum*, a higher proportion of the genotypic variance can be accounted for than expected from estimates of $\hat{p}$ for each trait separately, thus improving the prospects of MAS. In addition, we assumed identical selection intensities and length of selection cycles for different selection schemes. This is not always the case. Phenotypic evaluation of *S. sclerotiorum* resistance must be performed with adult plants and, therefore, it is only possible to complete one selection cycle per year. Recurrent selection is hampered, because the infected plant usually breaks at the site of infection and dies. Hospital et al. (1997) showed that the efficiency of cMAS could be increased when combined with MAS based on markers only in off-season programs. Furthermore, application of markers in a breeding program to improve resistance against *S. sclerotiorum* must take into account economic aspects. The relative superiority of MAS and cMAS over CPS therefore strongly depends on the costs of marker assays. If the latter decrease considerably, selection intensities used in MAS as compared to CPS might be high enough to compensate for the low proportion of genotypic variance explained by markers. A further advantage of MAS is its potential to separate genetic factors for resistance from morphological components of resistance. If linkage between genes regulating resistance and morphology is not too tight, markers are helpful in breaking associations between morphology and resistance. If morphological factors have a pleiotropic effect on resistance, they could be assigned a lower weight in the molecular score than those affecting only resistance. In conclusion, the decision whether molecular markers can efficiently assist breeding for resistance against *S. sclerotiorum* must take all these factors into account and must be made case by case for individual breeding programs.

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