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QTL MAPPING OF RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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To my parents

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[1] Z. Micic, V. Hahn, E. Bauer, C.C. Schön, S. Tang, S.J. Knapp, A.E. Melchinger (2004) QTL mapping of *Sclerotinia* midstalk rot resistance in sunflower. Theor. Appl. Genet. 109:1474-1484.

[2] Z. Micic, V. Hahn, E. Bauer, C.C. Schön, A.E. Melchinger (2004) QTL mapping of resistance to *Sclerotinia* midstalk rot in RIL of sunflower population $NDBLOS_{sel} \times CM625$. Theor. Appl. Genet. 110:1490-1498.

[3] Z. Micic, V. Hahn, E. Bauer, C.C. Schön, S. Tang, S.J. Knapp, A.E. Melchinger (2004) Identification and validation of QTL for *Sclerotinia* midstalk rot resistance in sunflower by selective genotyping. Theor. Appl. Genet. 110:233-242.

Abbreviations

ANOVA	analysis of variance
cm	centimeter
CV	cross validation
CPS	conventional phenotypic selection
LG	linkage group
LOD	$\log_{10}$ odds ratio
MP	parental mean
MAS	marker-assisted selection
QTL	quantitative trait locus or loci, depending on the context
$\hat{p}$	proportion of genotypic variance explained by QTL
$\hat{p}_{DS}$	proportion of genotypic variance explained by QTL in the data set
$\tilde{p}_{TS}$	proportion of genotypic variance explained by QTL in a test set
RE	relative efficiency of MAS
RIL	recombinant inbred lines
SG	selective genotyping
SSR	simple sequence repeat

1. General Introduction

The cultivated sunflower (*Helianthus annuus* L.) (Figure 1) ranks with soybean [*Glycine max* (L.) Merr.], rapeseed (*Brassica rapa* L., and B. napus L.), and peanut (*Arachis hypogaea* L.) among the four most important annual crops in the world grown for edible oil. In recent years, the sunflower oil has been increasingly used for industrial purposes.

Figure1: Cultivated sunflower (*Helianthus annuus*)

Diseases represent the major limiting factors of sunflower production worldwide. Sunflower is known to be a host for almost 40 pathogenic organisms (Gulya et al., 1997). A major fungal disease that significantly restricts the productivity of sunflower, when grown in humid and temperate environments, is *Sclerotinia sclerotiorum* (Lib.) de Bary.

Sclerotinia sclerotiorum was first described in 1837 and identified as a pathogen of sunflower by Fuckel in 1861 (Purdy, 1979). The fungus is widespread and reported in all sunflower-growing regions of the world. The host range includes 361 plant species belonging to 225 genera in 64 families, including Brassicaceae, Fabaceae, and Solanaceae (Purdy, 1979). *Sclerotinia* species belong to the class Ascomycota and are characterized by producing mycelia and sclerotia in the asexual stage, and apothecia with asci and ascospores in the sexual stage (Figure 2) (Gulya et al., 1997).

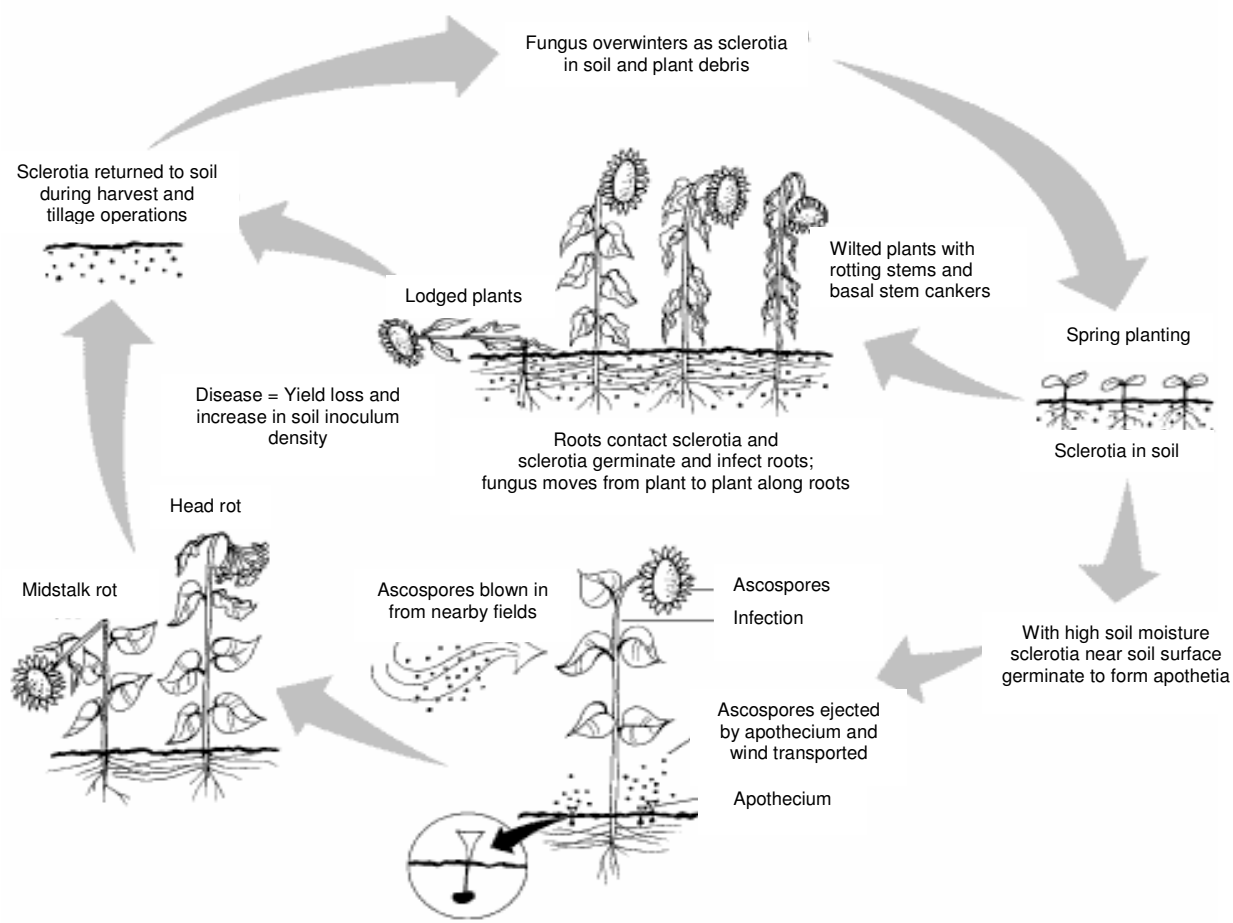

Figure 2: Disease cycle of *Sclerotinia* wilt, midstalk rot and head rot of sunflower (Source: Nelson, 2000)

Mycelia from germinating sclerotia in the soil infect sunflower roots and may result in *Sclerotinia* wilt. *Sclerotinia* wilt may occur anytime from the seedling stage until maturity. Midstalk rot typically originates from a leaf infection of airborne ascospores landing on wounded leaf tissue and colonizing the leaf. The infection progresses down the petiole,

producing a stem lesion (Figure 3) with pith degradation and sclerotia formation inside the stem. The stalks break usually at the point of infection. *S. sclerotiorum* infects the midstalk from the late vegetative stage until maturity. At the end of flowering or later, ascospores may also infect sunflower heads. The ultimate result of head infection is the complete rot (Masirevic and Gulya, 1992).

Figure 3: *Sclerotinia sclerotiorum* midstalk rot

The impact of *S. sclerotiorum* on yield depends on the growth stage at which plants are infected, as well as on subsequent climatic conditions. Commonly, *S. sclerotiorum* infections at the root, midstalk and head result in a total yield loss (Masirevic and Gulya, 1992). Considering the wide host range and longevity of sclerotia, *S. sclerotiorum* is one of the most difficult pathogens to control. Gulya et al. (1997) proposed an integrated control program for combating *S. sclerotiorum* diseases.

(1) Cultural methods to control *S. sclerotiorum* diseases include proper plant density and a 3- to 4- year crop rotation with non-host crops.

(2) Use of fungicides. Fungicide tests of Peres et al. (1992) in sunflower revealed that the most consistent and highest levels of efficacy were obtained by preventive treatments before development of the first symptoms. Curative treatments against *S. sclerotiorum* in sunflower would be cheaper, however, their efficacy is highly dependent on weather conditions and the extent of attack.

(3) Biological control agents such as adding bacteria (Expert and Digat, 1995) or soil micoorganisms (Jones et al., 2003) to the seeds or soil reduce the disease incidence and subsequent loss in seed yield and, therefore, represent an alternative method for controlling *S. sclerotiorum* wilt.

(4) Deployment of moderate levels of resistance in the host plants.

The search for resistance to *S. sclerotiorum* in sunflower has been the objective of most sunflower breeding programs worldwide (Gulya et al., 1997). Several wild *Helianthus* species were described as potential sources of genes for resistance to *S. sclerotiorum* (Seiler and Rieseberg, 1997) and have been used to produce interspecific hybrids (Kräuter et al., 1991, Schnabl et al., 2002).

Hitherto, no complete resistance to *S. sclerotiorum* in cultivated sunflower could be achieved, but lines derived from interspecific crosses between wild species and cultivated sunflower showed improved resistance when infected with *S. sclerotiorum* (Degener et al., 1999; Köhler and Friedt, 1999; Rönicke et al., 2004). Inheritance of resistance to *S. sclerotiorum* in sunflower was generally found to be quantitative for all three forms of infection (root, stalk, head) with different genes controlling the resistance in different organs (Robert et al., 1987; Castaño et al., 1993; Bert et al., 2002) and no race specificity (Thuault and Tourvieille de Labrouhe, 1988). Additive gene action prevailed over dominance or epistasis (Robert et al., 1987; Vear and Tourvieille, 1988; Genzbittel et al., 1998; Bert et al., 2002).

Genetic analysis of complex traits has been amended by the application of molecular marker technologies. Molecular markers help to construct high-resolution genetic maps that can be used for the mapping and estimation of genomic positions and genetic effects of quantitative trait loci (QTL) involved in quantitatively inherited traits. During the last decade, several genetic linkage maps of cultivated sunflower were published based on Restriction Fragment Length Polymorphisms (RFLPs)(Berry et al., 1995; Gentzbittel et al., 1995; Jan et al., 1998), Simple Sequence Repeat (SSRs) markers (Bert et al., 2002; Tang et al., 2002; Burke et al., 2002; Yu et al., 2003), Amplified Fragment Length Polymorphism (AFLPs) (Gedil et al., 2001), Direct Amplification of Length Polymorphisms markers (DALPs) (Langar et al., 2003), and Target Region Amplification Polymorphism (TRAPs) (Hu and Vick, 2003). Thus, molecular tools are available in sunflower to efficiently map QTL for agriculturally important traits such as resistance to midstalk rot caused by *S. sclerotiorum.* Selecting for favorable QTL effects based on marker data (marker-assisted selection, MAS) has great potential for improving quantitative traits.

Concerning *S. sclerotiorum* resistance of sunflower, several QTL studies were published up to now (Mestries et al., 1998; Bert et al., 2002, 2004). The authors used different F_3 populations to study the resistance to *S. sclerotiorum* leaf and capitulum attack. QTL reported in these studies were based on the nomenclature defined by Gentzbittel et al. (1995), so that they can be compared. On 14 of the 17 sunflower linkage groups (LG) QTL have been found. In general, each of them explained less than 20% of phenotypic variance. Some of them appeared to be specific only for one cross. One particularly strong QTL was reported on LG1 linked to a protein-kinase gene (Genzbittel et al., 1998), but while it explained 50% of the variation in one cross, in other crosses it explained only 15% or was absent. The most frequent LG that carried QTL for resistance to *S. sclerotiorum* was LG7, probably related to the branching genotype (Bert et al., 2004).

During the last years, attempts have been made to establish resistance against *S. sclerotiorum* by genetic engineering (Lu et al., 2000, Scleonge et al., 2000, Hu et al., 2003). These studies are based on a gene controlling the production of an enzyme oxalate oxidase (OXO). Oxalate is a phytotoxin secreted by *S. sclerotiorum*. It weakens the plant tissue and plays a key role in the pathogenicity of *S. sclerotiorum*. Crops with natural resistance to *S. sclerotiorum* such as wheat, barley, maize, or rice, produce OXO, which breaks down and detoxifies the phytotoxin produced by *S. sclerotiorum*. Contrary to such crops, sunflower has a very low OXO activity. An OXO gene from wheat was isolated and inserted into sunflower plants via *Agrobacterium*–mediated transformation. The *Sclerotinia*-induced lesions in transgenic sunflower were significantly smaller than those in the control leaves (Hu et al., 2003). Compared with the original line, this gene increased resistance but in generally, the level was not better than in lines obtained by conventional breeding. Therefore, it should be possible to combine the transgenic lines with natural resistance to provide a level of resistance higher than in the currently available commercial hybrids (Bazzalo et al., 2000).

In the present study, we focused on midstalk rot due to its importance in sunflower growing areas in Germany, and the availability of a reliable resistance test (Degener et al., 1998). The latter determines the mycelium extension in leaves and stems as a measure for resistance to midstalk rot caused by *S. sclerotiorum.* Three resistance (leaf lesion, stem lesion, speed of fungal growth) and two morphological traits (leaf length, leaf length with petiole) were recorded.

Based on observations of stem lesion, two inbred lines of different genetic origins (NDBLOS and TUB5-3234) with high level of resistance to *S. sclerotiorum* (Degener et al., 1999) were crossed with a highly susceptible line (CM625) to develop two segregating populations for the estimation of QTL in this study.

To obtain information about the prospects of marker-assisted selection (MAS) for increasing the level of resistance to *S. sclerotiorum* in sunflower, the objectives of the present study were to:

(1) estimate the number, genomic positions, and genetic effects of QTL involved in midstalk-rot resistance to *S. sclerotiorum* in two F_3 populations,

(2) verify the QTL for midstalk-rot resistance in recombinant inbreed lines (RIL) of the $NDBLOS_{sel} \times CM625$ population, and

(3) asses the consistency of QTL for midstalk-rot resistance across populations of different genetic origins.

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ORIGINAL PAPER

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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

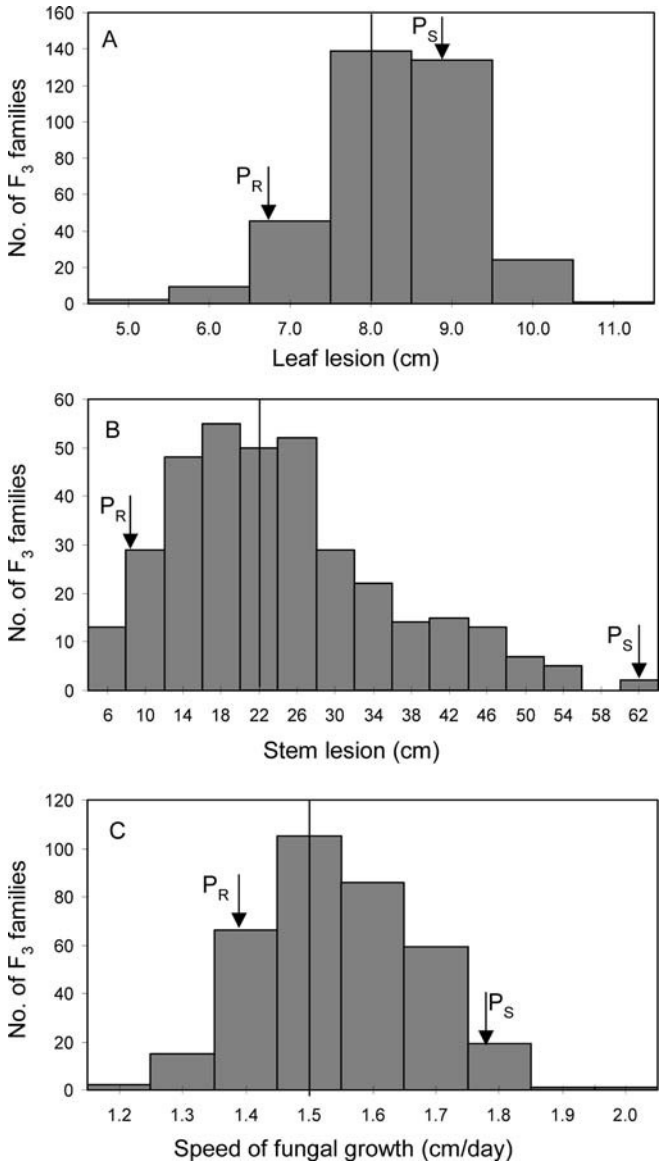

Fig. 1 Histograms for **a** leaf lesion, **b** stem lesion, and **c** speed of fungal growth measured in two experiments in 1999, for means of 354 F_3 families derived from the cross $P_R \times P_S$. A *solid line* indicates the overall mean. *Arrows* indicate the means of parental lines P_R and P_S

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

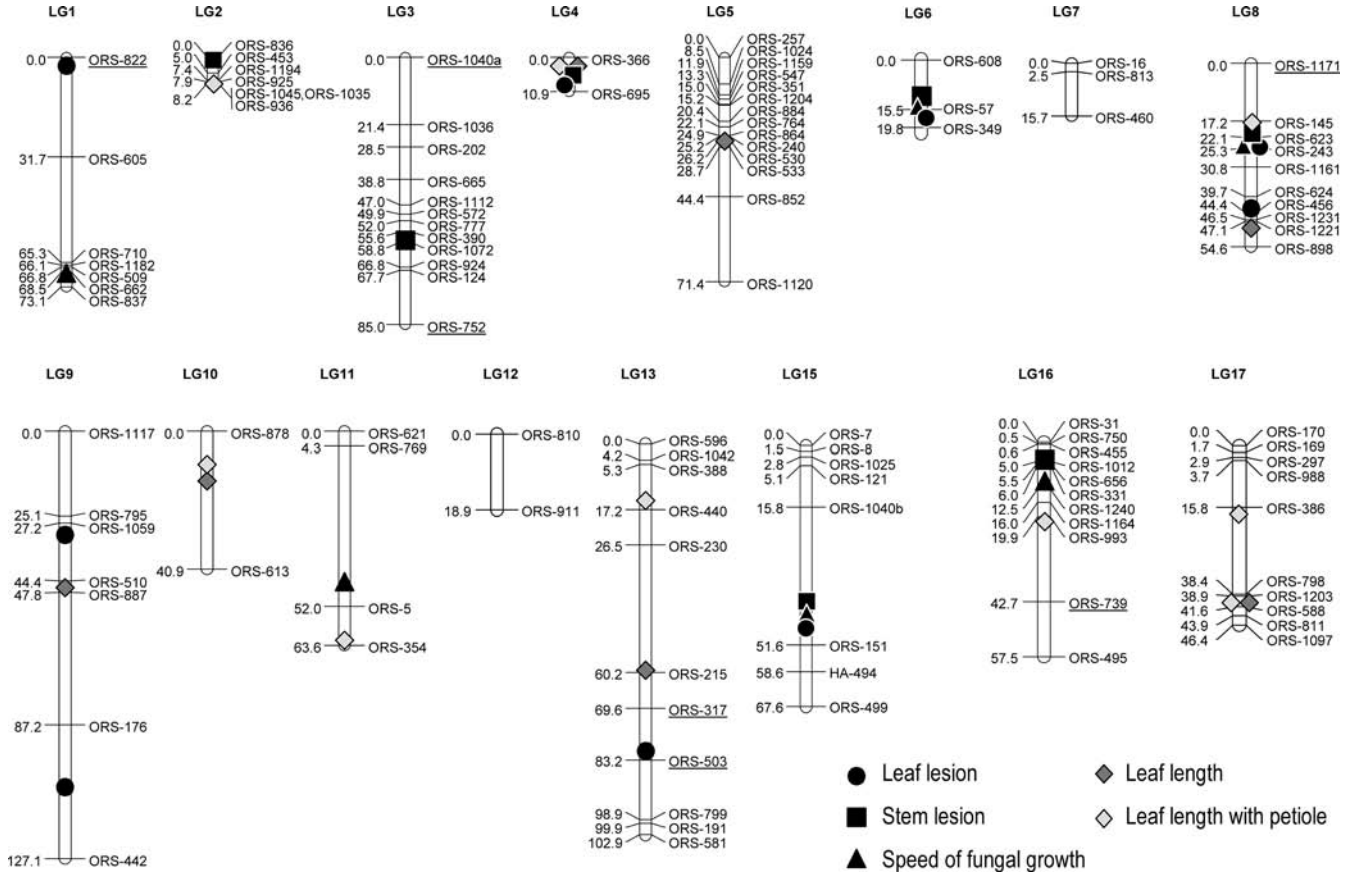

Fig. 2 Genetic linkage map of sunflower based on 352 F_2 individuals derived from cross $P_R \times P_S$ for 113 SSR marker loci. *Numbers to the left* of the linkage group (LG) indicate the cumulative distance in centiMorgans (Haldane). Loci with distorted segregation ratios ($P < 0.01$) are *underlined*.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

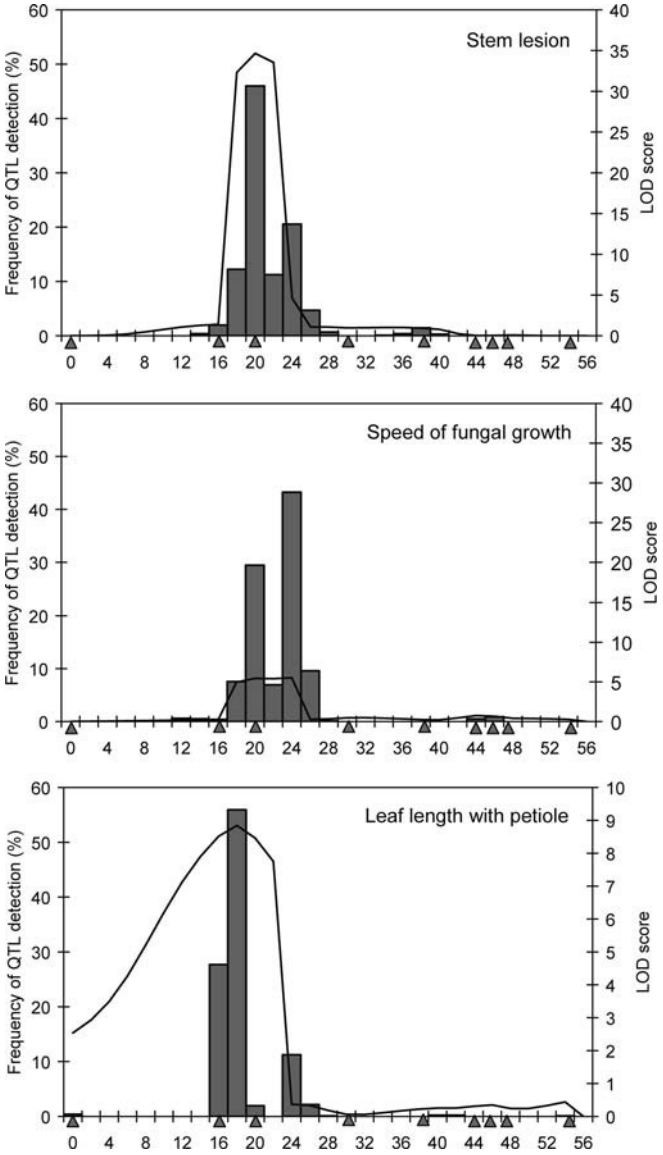

Fig. 3 QTL frequency distributions for stem lesion, speed of fungal growth, and leaf length with petiole on LG8 obtained from 1,000 cross validation runs for 351 F_3 families of the cross $P_R \times P_S$. The *solid line* indicates the LOD curves determined from the entire data set, using composite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

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QTL mapping of resistance to *Sclerotinia* midstalk rot in RIL of sunflower population NDBLOS$_{sel}$ × CM625

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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 NDBLOS$_{sel}$ × 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 ($\hat{r}_g(M_{iF3}, Y_{iRIL}) = 0.53$).The genetic effects and predictive quality of these two QTL are promising for application in marker-assisted selection to *Sclerotinia* midstalk-rot resistance.

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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

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 $NDBLOS_{sel} \times$ 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 $NDBLOS_{scl} \times$ 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 $NDBLOS_{sel} \times$ 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

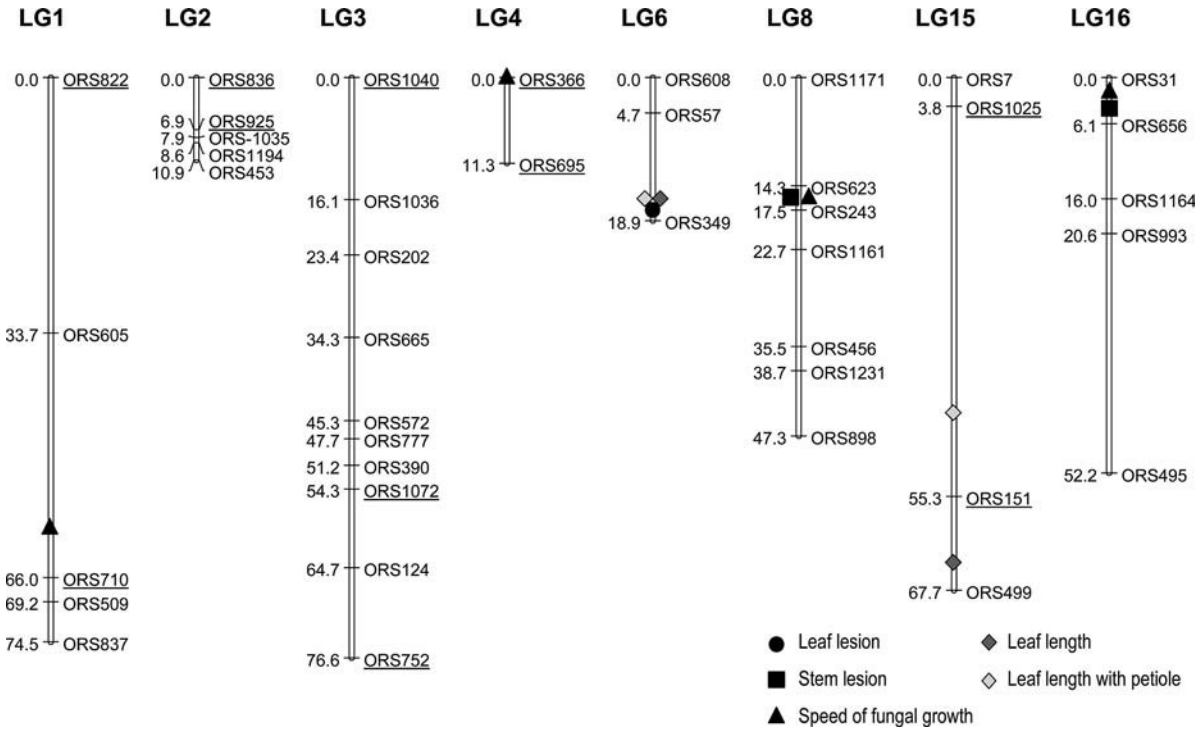

Fig. 1 Common genetic linkage map based on 351 F_2 individuals and 248 RIL derived from cross $P_R \times P_S$ for 41 SSR marker loci. *Numbers* to the *left* of the linkage groups indicate the cumulative distance in centiMorgans (cM) (Haldane 1919). Loci with distorted segregation ratios ($P < 0.01$) 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 ($\hat{p}_{DS}$) and as the median over all TS ($\tilde{p}_{TS}$). 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 $\hat{r}_g(M_{iF3}, Y_{iRIL})$ was estimated. Here, M_{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 $Y_{iRIL}$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 ($\hat{\sigma}^2_g$) were highly significant for all traits (Table 1). Estimates of genotype × environment interaction variances ($\hat{\sigma}^2_{ge}$) were significant ($P < 0.01$) and, with the exception of leaf length, smaller than $\hat{\sigma}^2_g$ .Heritabilities for resistance traits were intermediate to high ($0.51 < \hat{h}^2 < 0.79$).

Resistance traits were significantly correlated with each other ($0.45 < \hat{r}_g < 0.78$).Phenotypic correlations of both morphological traits were significant ($P < 0.01$) but small with stem lesion ($-0.17 < \hat{r}_p < -0.15$) and speed of fungal growth ($0.19 < \hat{r}_p < 0.26$) and close to zero with leaf lesion ($-0.09 < \hat{r}_p < -0.01$).Leaf length with petiole was highly ($P < 0.01$) correlated with leaf length ($\hat{r}_p = 0.79$).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)						
$\hat{\sigma}^2_g$		0.31 ± 0.05**	50.80 ± 5.18**	0.006 ± 0.001**	0.31 ± 0.13**	1.60 ± 0.21**
$\hat{\sigma}^2_{ge}$		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 $\hat{h}^2$[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

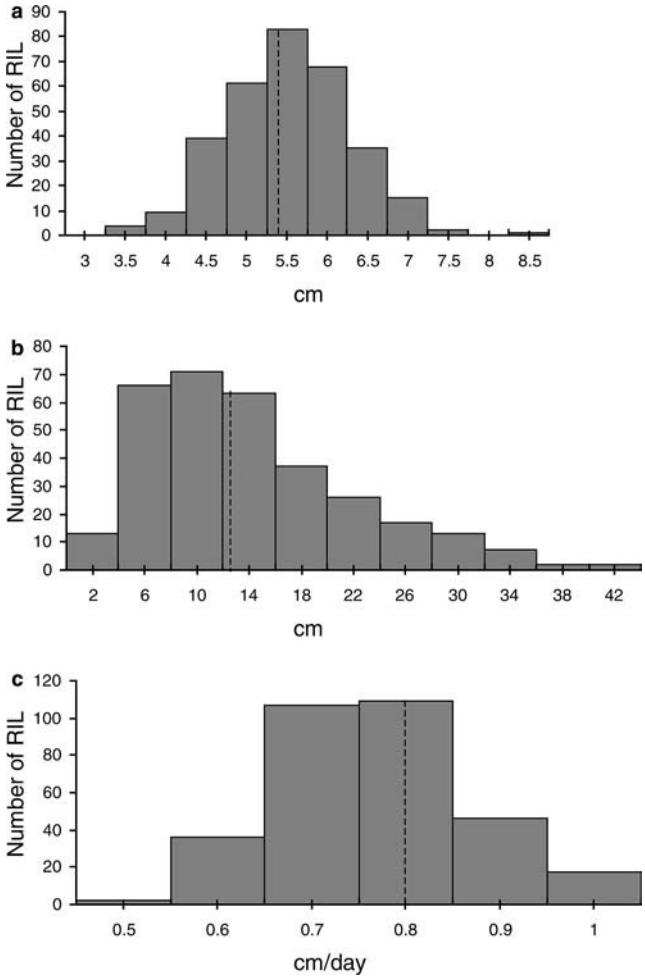

Fig. 2 Histograms for means of leaf lesion (**a**), stem lesion (**b**), and speed of fungal growth (**c**), measured in two environments in 317 RIL derived from cross $P_R \times P_S$. *Dashed lines* indicate the overall means

Table 2 Genetic correlations between F_3 families and RIL ($(\hat{r}_g(F_3, RIL))$) 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
$\hat{r}_g(F_3,RIL)$	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		LG8/ORS623	16	15.51	3.86	25.5
(cm)		LG16/ORS31	4	5.48	2.48	9.8
	$\hat{p}_{DS}$					34.2
	$\tilde{p}_{TS}$					26.5
Speed of		LG1/ORS605	50	2.65	−0.03	5.0
fungal growth		LG4/ORS366	0	2.66	0.02	4.9
(cm/day)		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		LG6/ORS57	16	4.20	−0.34	8.0
(cm)		LG15/ORS151	62	2.71	0.26	5.1
	$\hat{p}_{DS}$					41.1
	$\tilde{p}_{TS}$					13.9
Leaf length		LG6/ORS57	16	4.41	−0.41	8.4
With petiole (cm)		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 $NDBLOS_{sel} \times CM625$, 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 ≤ 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-

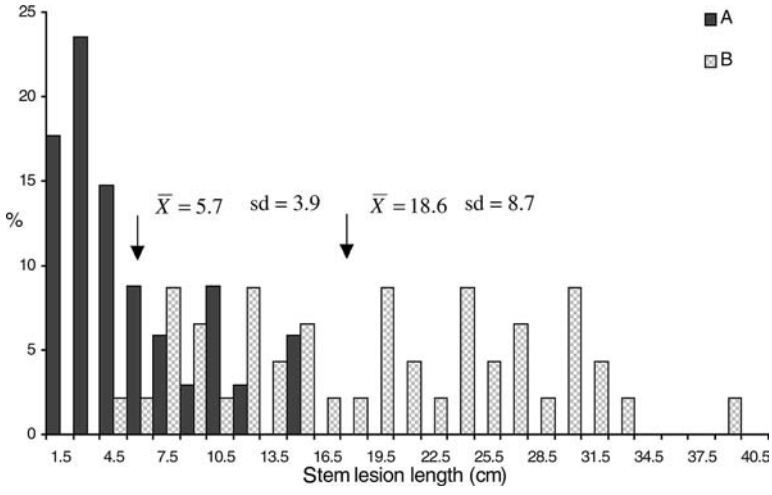

Fig. 3 Frequency distributions of stem lesion length for plants carrying exclusively resistant (**a**) and susceptible (**b**) alleles at four markers flanking two QTL on LG8 and LG16 in RIL. *Arrows* indicate the means of genotype classes homozygous for the A and B allele

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 $NDBLOS_{sel}$ 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 $NDBLOS_{sel}$ and the markers identified in this study can aid in improving resistance against *S. sclerotiorum*.

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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 ($\tilde{p}_{TS} = 72.7\%$).

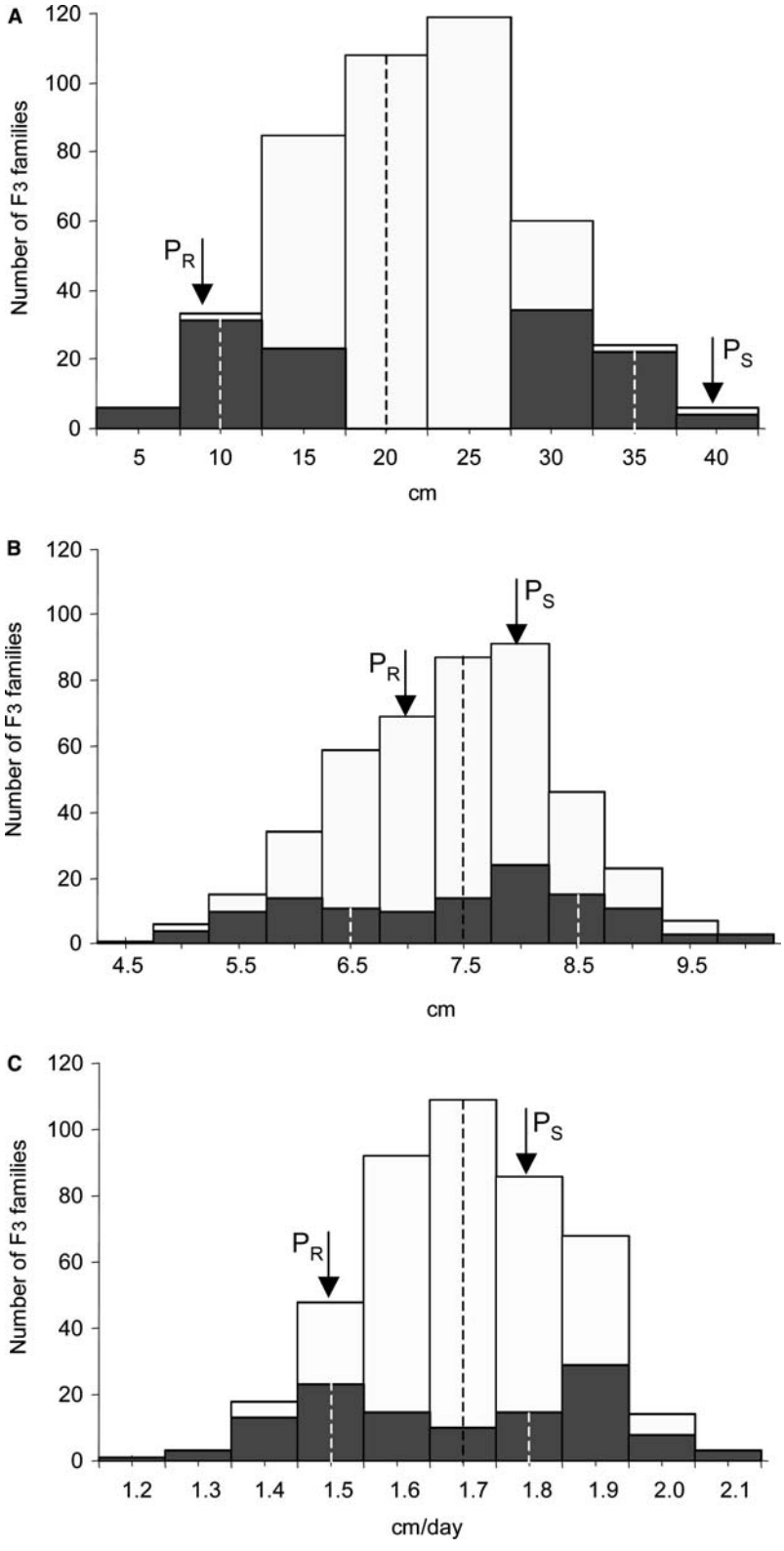

Fig. 1 Histograms for means of **a** stem lesion, **b** leaf lesion and **c** speed of fungal growth, measured in two environments in 434 F_3 families and 120 selected F_3 families derived from cross CM625 [susceptible parent (P_S)] ×TUB-5-3234 [resistant parent (P_R)]. *Dashed lines* indicate the overall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

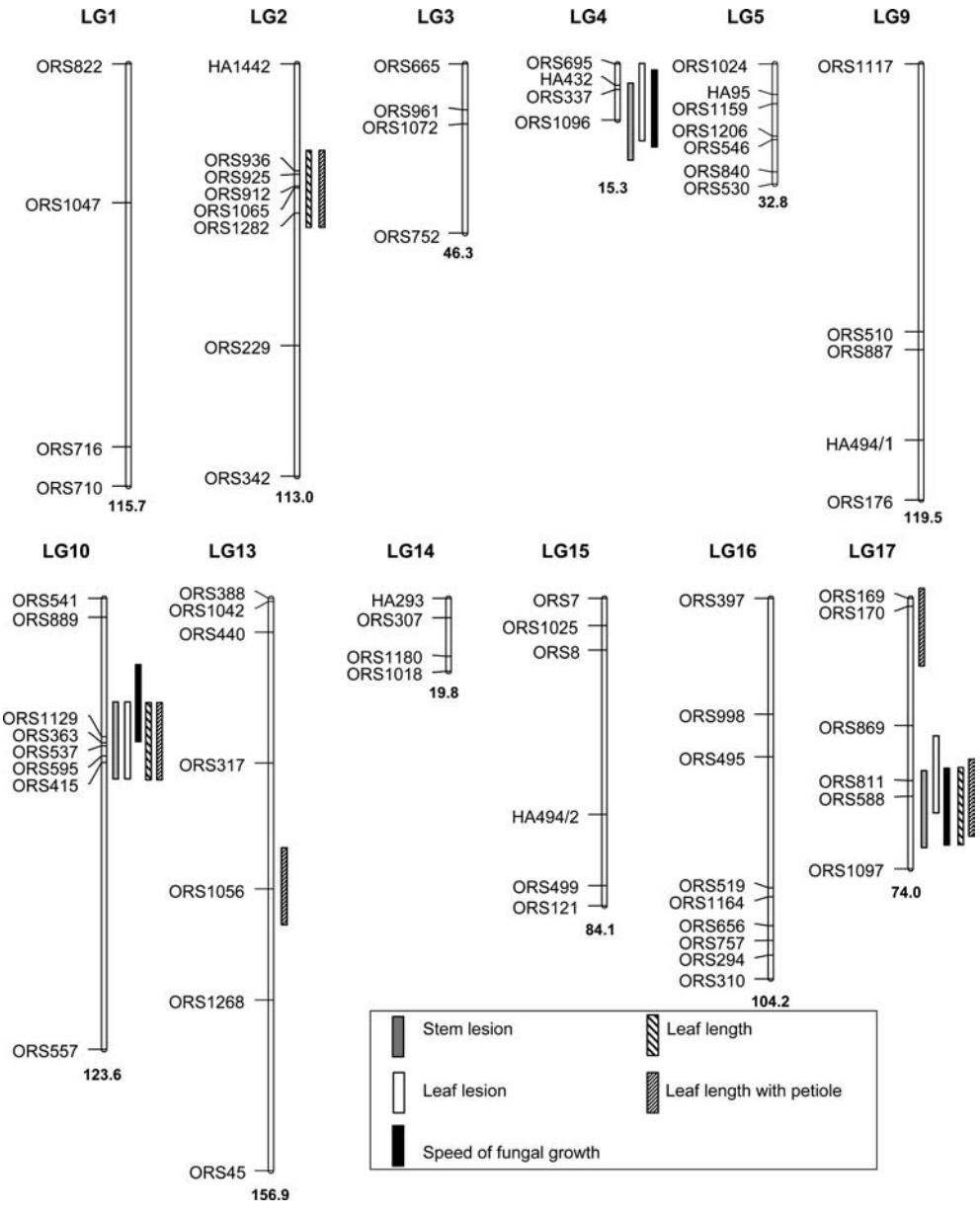

Fig. 2 Genetic linkage map of cross CM625 (P_S) × TUB-5-3234 (P_R) based on 72 simple sequence repeat marker loci analysed in 120 F_2 plants. Marker names are listed to the *left* of each linkage group. At the *bottom* of each linkage group the total length in centiMorgans (Haldane) is given. Chromosomal 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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5. General Discussion

Genetic foundation of midstalk rot resistance

In elite sunflower material, the inheritance of resistance to *S. sclerotiorum* has been found to be polygenic with medium heritability (Mestries et al., 1998). Consequently, two large sunflower F_3 populations from the crosses $NDBLOS_{sel} \times CM625$ (N = 354) and $CM625 \times TUB\text{-}5\text{-}3234$ (N = 434) as well as 317 RIL derived from population $NDBLOS_{sel} \times CM625$ were evaluated in field trials. Quantitative genetic parameters (variances and heritabilities) and the frequency distributions of entry means were similar for all experiments and confirmed the polygenic nature of the resistance.

Evidence for the complexity of inheritance to *S. sclerotiorum* resistance was clearly obtained from population $NDBLOS_{sel} \times CM625$, where a large population size (N = 351) was employed for QTL mapping. QTL were detected for all three resistance traits 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 all detected QTL. Thus, the data confirm the hypotheses that a large number of genes with small effects are involved in resistance to midstalk rot.

The limited congruency of QTL for different resistance traits is a further evidence of the complex inheritance of midstalk-rot resistance to *S. sclerotiorum*. As expected from the medium genotypic correlations between the resistance traits, only two genomic regions (LG8, LG15) showed common QTL for all three traits giving resistance to midstalk rot. In a third genomic region on LG6, QTL for leaf lesion and stem lesion were located 22 cM apart. However, in this region the LOD curve for stem lesion did not have a well defined maximum. In the vast majority of the 1000 cross-validation runs, the QTL was located at position 82 instead of 74 as in the data set (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 the expression of resistance to mycelial extension in leaves and stems as described for sunflower resistance to *Phomopsis* (Langar et al., 2002). Depending on the genetic material analyzed, most authors found different genetic factors controlling *S. sclerotiorum* resistance to three types of disease: *Sclerotinia* wilt, midstalk rot and head rot (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.

Comparison between generations from cross NDBLOS$_{sel}$ × CM625

Phenotypic data. Quantitative genetic parameters estimated for midstalk-rot resistance and morphological traits were similar for RIL and F_3 families. Estimates of the genotypic variance, heritability and correlations among traits are not directly comparable for the two experiments. Environmental conditions in 1999 were extremely favourable for *Sclerotinia* infection of F_3 families and 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 RIL than for F_3 families. Heritability estimates and phenotypic correlations between resistance traits were somewhat lower for RIL than F_3 families but generally of the same order, except for leaf length with substantial G × E interactions. 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.

Genotypic correlations between F_3 families and RIL were close to expectations. 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. This should also apply to early testing strategies of testcrosses in a hybrid breeding program, because dominance was found to play only a minor role in *Sclerotinia* resistance (Mestries et al., 1998; Bert et al., 2002),

QTL validation. To validate detected QTL from F_3 families, 248 F_6 RIL were used. On the basis of the QTL results from F_3 families, a total of 41 markers were chosen. Thirty-six markers covered seven LG showing QTL for stem lesion. Five additional markers on LG1 were included in the analysis because it carried two QTL affecting leaf lesion without association to morphological traits in F_3 families.

The limited marker coverage of RIL has to be taken into account when interpreting QTL mapping results between F_3 families and RIL for all traits except stem lesion. The two largest QTL for stem lesion detected in the F_3 generation on LG8 and LG16 were confirmed in the RIL. A further QTL for stem lesion on LG15 could be detected after reducing the LOD threshold to 1.5, but precise localization of this QTL was difficult due to a flat LOD profile. Of the nine marker intervals with significant effects on leaf lesion in F_3 families, only six were analyzed in RIL. It was surprising, however, that only one of these genomic regions (LG6) harbored a significant QTL for leaf lesion in the RIL. For speed of fungal growth, slightly better results were obtained. Two QTL on LG8 and LG16 also detected for stem lesion were in common to both generations. An additional QTL on LG1 was located 22 cM apart. Like in the F_3 generation, the favorable allele for this genomic region originated from the susceptible parent. Thus, we assumed that it reflected the same genomic region.

Selective genotyping

Several studies have demonstrated that selective genotyping (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. Two large populations originating from different resistant sources crossed to the same susceptible parent were evaluated for their resistance against *S. sclerotiorum* to determine whether the same QTL were responsible for the 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 analyze QTL for *S. sclerotiorum* resistance in a large reference population $NDBLOS_{sel} \times CM625$ (N = 351) and to use SG in the second population rather than to perform QTL analyses in two medium-sized populations.

To validate the usefulness of SG for detecting QTL for *Sclerotinia* resistance, SG was performed *a posteriori* for population $NDBLOS_{sel} \times CM625$. Based on the phenotypic data for stem lesion, the 60 most resistant and 60 most susceptible F_3 families from cross $NDBLOS_{sel} \times CM625$ were selected and a QTL analysis was performed to investigate the power of QTL mapping under SG. With LOD $\geq$ 2.5, half the number of QTL detected for stem lesion in the reference population were detected using SG. The two largest QTL in the reference population affecting resistance to *S. sclerotiorum* (LG8, 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, a precise localization 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 test set (TS) based on the allelic state at detected QTL has a correlated response at non-detected QTL, leading to an overestimation of $\tilde{p}_{TS}$. As expected, the overestimation of $\tilde{p}_{TS}$ was highest and the difference between $\hat{p}_{DS}$ and $\tilde{p}_{TS}$ estimates smallest for stem lesion compared with leaf lesion and speed of fungal growth.

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. 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 the analysis of congruency of resistance genes in an independent sample.

Consistency of QTL across segregating populations

The congruency of resistance genes identified in the phenotypically selected fractions of population CM625 × TUB-5-3234 was assessed with the results of population $NDBLOS_{sel}$ × CM625, to determine whether the same QTL were responsible for the expression of resistance to *S. sclerotiorum*. For stem lesion, a smaller number of QTL was detected in population CM625 × TUB-5-3234 compared with the reference population $NDBLOS_{sel}$ × CM625, but with SG four significant QTL were detected in each population. One of the four QTL located 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. Contrary to the reference population, no QTL for leaf morphology was detected in this genomic region. A second QTL was identified linked to marker ORS456, assigned to LG8 by Tang et al. (2002) 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 × TUB-5-3234 due to a large genetic distance from the marker.

The two additional genomic regions (LG10, LG17) with significant effects on stem lesion in population CM625 × TUB-5-3234 also had a significant effect on morphological traits and coincided precisely with two genomic regions detected in cross $NDBLOS_{sel}$ × CM625 for leaf morphology. The five QTL detected for stem lesion in population $NDBLOS_{sel}$ × CM625 on LG2, 3, 6, 15, and 16 were not detected in population CM625 × TUB-5-3234.

Mycelial extension on leaves was also observed 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 linkage groups LG1, 9, and 13. Mestries et al. (1998) detected five QTL for this trait in different selfing generations, which coincided with our linkage groups LG 3, 8, 10, and 16. We found QTL for leaf lesion length on LG1, 4, 6, 8, 9, 13, and 15 for population $NDBLOS_{sel} \times CM625$ and on LG1, 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 9 had a significant effect in three of the four populations.

In addition to LG1, 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 population CM625 × TUB-5-3234, 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.

Resistance mechanisms

The resistance mechanisms of parental lines $NDBLOS_{sel}$ and TUB-5-3234 are unknown, but superior resistance was confirmed in both lines. At most QTL, alleles conferring increased resistance against *S. sclerotiorum* originated from resistant parents. Line CM625 was chosen as parent because it had shown high susceptibility to *S. sclerotiorum* in artificial leaf infections (Hahn, unpublished data). However, as reported for other resistance

traits (Schön et al., 1993; Bohn et al., 2000), the susceptible parent also carried resistance alleles.

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 $NDBLOS_{sel}$ is large and therefore the association of morphological characters with resistance traits in the cross $NDBLOS_{sel} \times CM625$ was investigated. Only 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. Two of the genomic regions carrying QTL for all three resistance traits had no effect on morphological traits. The low genotypic correlations of the morphological and resistance traits also do not support the hypothesis of tight correlation between resistance and morphology. In addition, Degener et al. (1999) selected an inbred line with high level of resistance to midstalk rot and short leaf length out of a cross between $NDBLOS_{sel}$ and a susceptible line. Furthermore, the results of RIL showed that resistance could be improved without a significant change of plant 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*.

Gentzbittel et al. (1998) detected one particularly strong QTL on LG1 linked to a protein kinase-like fragment. This is related to the Serine/Threonine protein kinase family, showing significant homologies with a protein kinase gene conferring resistance in tomato and rice (Gentzbittel et al., 1998). The receptor of the kinase-like gene may confer resistance to *S. sclerotiorum* for different plant parts (Bert et al., 2004). According to A. Leon (personal communication), LG1 of Gentzbittel et al. (1998) is equivalent to LG8 in our studies. One

QTL on LG8 was consistently detected across both populations investigated in our study. However, due to paucity of common markers for comparison between other studies, we could not confirm the identity of this QTL. We therefore conclude that the protein kinase-like gene could be involved in general defense mechanisms to *S. sclerotiorum* resistance in different genetic backgrounds and plant parts. Nevertheless, the genomic region on LG8 merits further analyses to corroborate this hypothesis.

Perspectives for MAS

In presented study, we could confirm four common QTL between generations on LG 1, 6, 8, 16 and two for stem lesion on LG8 and LG4 between populations of different genetic backgrounds affecting resistance to *S. sclerotiorum*. 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. Two genomic regions detected in the $NDBLOS_{sel} \times CM625$ population, on LG8 and LG16, carrying QTL for stem lesion and speed of fungal growth are large enough to form a starting point for MAS. These two QTL were stable in all investigated environments as well as in *a priori* SG. In addition, no significant change of plant morphology was carried over in RIL, which is encouraging with respect to making progress with MAS, based on true resistance genes.

Regarding mapping population $CM625 \times$ TUB-5-3234, it could not be answered 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. The genomic region on LG10 should be analyzed 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.

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 $NDBLOS_{sel}$ and the markers identified in this study can aid in improving resistance against *Sclerotinia sclerotiorum.* However, only approximately a quarter of the genotypic variance for midstalk-rot resistance can be explained by the QTL suggested for MAS. Therefore, the identification of new resistance genes from different sources and their pyramiding in elite lines warrants further research.

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6. Summary

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most important pathogens of sunflower. The fungus induces serious yield losses that can reach up to 100%. Three different disease symptoms can be caused by *S. sclerotiorum*: *Sclerotinia* wilt, midstalk rot and head rot. An improvement of the resistance against *S. sclerotiorum* would contribute to yield security and thus increase the profitability of sunflower cultivation. Previous studies have shown that resistance to *S. sclerotiorum* is polygenically inherited.

In the present study, we investigated resistance to midstalk rot with respect to the prospects of marker-assisted selection (MAS). The objectives of the study were to (1) identify quantitative trait loci (QTL) involved in resistance against *Sclerotinia sclerotiorum*, (2) map their position in the genome, (3) characterize their gene effects, and (4) estimate their consistency across generations of the cross $NDBLOS_{sel}$ x CM625.

Based on the results of previous investigations, two sunflower lines with high resistance level to *S. sclerotiorum* and different genetic origins ($NDBLOS_{sel}$ and TUB-5-3234) were used as parents. They were crossed with a highly susceptible line CM625 to develop two mapping populations. A modified leaf test, based on infecting the leaves with mycelium and covering them with plastic bags, was used for the evaluation of midstalk-rot resistance. Three resistance traits (leaf lesion, stem lesion, speed of fungal growth) and two morphological traits (leaf length, leaf length with petiole) were measured.

Disease resistance of 354 F_3 families of the population $NDBLOS_{sel}$ x CM625 was screened in field trials with two different sowing times in 1999. A total 317 recombinant inbred lines (RIL) derived from the F_3 families were tested in 2002 and 2003. The 434 F_3 families of cross CM625 x TUB-5-3234 were screened in 2000 and 2001. The field trials were conducted at the research station Eckartsweier using generalized lattice designs with three replications and five infected plants per replication.

Highly significant genetic variation between the F_3 families and RIL was observed for the resistance traits in all field trials. Heritabilities ($\hat{h}^2$) were highest for stem lesion (0.79 to 0.89) and lowest for leaf lesion (0.51 to 0.55) for all three experiments. The resistance traits were moderately correlated with each other.

For the construction of the genetic map of population $NDBLOS_{sel}$ x CM625, 352 F_2 individuals were analyzed with 117 SSR marker loci. On the basis of results from the QTL mapping in F_3 families, 41 markers were selected and genotyped in 248 RIL. A "selective genotyping" approach was used for population CM625 x TUB-5-3234. For the 60 most resistant and 60 most susceptible F_2 individuals, the SSR genotype at 72 marker loci was

determined. Selection of F_2 individuals was performed for stem lesion measured in F_3 families.

For population $NDBLOS_{sel}$ x CM625, the 117 SSR spanned a map distance of 961.9 centi Morgan (cM) with an average interval length of 9.6 cM. The 78 SSR marker loci of population CM625 x TUB-5-3234 spanned a map distance of 1005.2 cM with an average interval length of 14.0 cM. The genetic distances between the SSR marker loci and their linear order in the genome were in good agreement with previously published sunflower maps.

For QTL mapping and estimation, the method of the "composite interval of mapping" was used. For stem lesion in the population $NDBLOS_{sel}$ x CM625, eight QTL were detected explaining 33.7% of the genetic variance ($\tilde{p}_{TS}$). The QTL on LG8 explained 36.7% of the phenotypic variance (R^2_{adj}). All other QTL for this trait explained between 3.3 and 6.0% of R^2_{adj}. Nine QTL were detected for leaf lesion. The proportion of the phenotypic variance explained by individual QTL ranged from 3.4 to 11.3%. All detected QTL for leaf lesion explained 25.3% of the genetic variance in cross validation. For speed of fungal growth, 6 QTL were detected, which explained from 4.6 to 10.2% R^2_{adj}. Cross validation explained 24.4% of $\tilde{p}_{TS}$. Most QTL showed additive gene action.

QTL occurring consistently across generations can be recommended for MAS and therefore, the QTL results between RIL and F_3 families of population $NDBLOS_{sel}$ x CM625 were compared. One common QTL was identified for leaf lesion, two for stem lesion and three for speed of fungal growth. After reducing the LOD score to 1.5, an additional QTL for stem lesion was found to be common for both generations.

In population CM625 x TUB-5-3234, four QTL for stem lesion, three QTL for leaf lesion and three QTL for speed of fungal growth were identified. Owing to the "selective genotyping" approach used, we conjecture that not all QTL were found. The comparison of QTL results between two F_3 populations showed two common QTL for stem lesion on LG4 and LG8. The QTL on LG4 originated from the susceptible parent CM625. The QTL on LG8 probably corresponds to the QTL with the largest effect determined in the population $NDBLOS_{sel}$ x CM625. Therefore, this genomic region should be analyzed in more detail in future investigations.

Regarding marker-assisted selection, our results indicate that two QTL show great promise. They were detected for stem lesion and speed of fungal growth in population $NDBLOS_{sel}$ x CM625, were consistent across environments, and showed no adverse correlation to leaf morphology in trials with the RIL.

In mapping population CM625 × TUB-5-3234, it remained unclear whether TUB-5-3234 can contribute new alleles with sufficiently large effects for resistance, which have not been identified in line $NDBLOS_{sel}$ and would be useful in marker-assisted introgression. The genomic region on LG10 should be analyzed 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.

Resistance breeding of sunflower against *S. sclerotiorum* is difficult due to the complex inheritance of the trait. This study showed that both the resistance source $NDBLOS_{sel}$ and the markers identified in this study are promising in improving resistance against *S. sclerotiorum* by marker-assisted selection. For a broader resistance against *S. sclerotiorum*, it is necessary to detect new resistance genes from different sources of resistance and pyramide them in elite lines.

7. Zusammenfassung

Sclerotinia sclerotiorum (Lib.) de Bary ist einer der bedeutendsten Schaderreger der Sonnenblume, der Ertragsverluste bis zu 100% verursachen kann. Durch *S. sclerotiorum* können drei verschiedene Krankheitssymptome hervorgerufen werden: Wurzelwelke, Stängelfäule und Korbfäule. Eine Verbesserung der Resistenz gegenüber *S. sclerotiorum* würde die Ertragssicherheit und damit die Wirtschaftlichkeit des Sonnenblumenanbaus erhöhen. Frühere Arbeiten lieferten Hinweise, dass die Resistenz polygen vererbt wird.

Im Rahmen der vorliegenden Studie wurde die Resistenz der Sonnenblume gegenüber der Stängelfäule untersucht. Im Vordergrund stand hierbei die Frage nach den Aussichten für eine markergestützte Selektion. Die Ziele der Arbeit waren: (1) die an der Ausprägung der Resistenz gegen *Sclerotinia* beteiligten QTL (quantitative trait loci) zu identifizieren, (2) deren Lage im Genom zu kartieren, (3) deren Genwirkungweise zu charakterisieren, und (4) deren Stabilität über Generationen zu prüfen.

Zur Ermittlung der Resistenz gegen die Stängelfäule wurde ein modifizierter Blatt-Test benutzt, bei dem die Blätter mit Myzel infiziert und mit befeuchteten Plastiktüten umschlossen wurden. Die drei Resistenzmerkmale Blattläsion, Stängelläsion und Wachstumsgeschwindigkeit des Pilzes sowie die beiden morphologischen Merkmale Blattlänge und Gesamtblattlänge mit Stiel wurden erfasst. Als Ausgangsmaterial dienten zwei weitgehend resistente Sonnenblumenlinien ($NDBLOS_{sel}$ und TUB-5-3234), die mit einer hochanfälligen Linie (CM625) gekreuzt wurden, um zwei spaltende Kartierungspopulationen zu erzeugen. Die Krankheitsresistenz wurde 1999 in Feldversuchen mit unterschiedlichen Aussaatzeiten für 354 F_3-Familien der Population $NDBLOS_{sel} \times CM625$ geprüft. In den Jahren 2002 und 2003 wurden 317 rekombinante Inzuchtlinien (RIL) dieser Population untersucht. Die 434 F_3-Familien der Population $CM625 \times TUB\text{-}5\text{-}3234$ wurden 2000 und 2001 geprüft. Die Versuche wurden auf Flächen der Versuchstation Eckartsweier als generalisierte Gitteranlagen mit 3 Wiederholungen und 5 inokulierten Pflanzen je Wiederholung angelegt.

Bei allen Feldversuchen wurden hochsignifikante genetische Unterschiede zwischen den Familien bzw. Linien für die Resistenzmerkmale ermittelt. Die höchste Heritabilität ($\hat{h}^2$) wurde für das Merkmal Stängelläsion (0,79 bis 0,89), die niedrigste für die Blattläsion (0,51 bis 0,55) geschätzt. Die Resistenzmerkmale waren signifikant miteinander korreliert, die Korrelationen lagen jedoch in einem mittleren Bereich.

Zur Erstellung der genetischen Karte in der Population $NDBLOS_{sel} \times CM625$ wurden 352 F_2-Individuen mit 117 SSR (Simple Sequence Repeat) Markerloci analysiert. Anhand der Ergebnisse aus der QTL-Kartierung der F_3 Familien wurden 41 Markerloci selektiert. Mit diesen wurde der Genotyp für 248 RIL bestimmt. In der Population $CM625 \times TUB\text{-}5\text{-}3234$ wurde die Methode des „Selektive Genotyping“ (SG) angewendet. Die Selektion der F_2-Individuen wurde aufgrund der Ergebnisse für die Stängelläsion der F_3-Familien durchgeführt. Für die 60 resistentesten und die 60 anfälligsten F_2-Individuen wurde der SSR-Genotyp an 72 Markerloci bestimmt. Die Länge der SSR-Kopplungskarte für die Kreuzung $NDBLOS_{sel} \times CM625$ betrug 961,9 centiMorgan (cM) bei einer durchschnittlichen Intervalllänge von 9,6 cM. Die SSR-Kopplungskarte der Kreuzung $CM625 \times TUB\text{-}5\text{-}3234$ hatte eine Gesamtlänge von 1005,2 cM mit einer durchschnittlichen Intervalllänge von 14,0 cM. Die genetischen Distanzen zwischen den Markerloci stimmten gut mit den bisher veröffentlichten SSR-Kopplungskarten der Sonnenblume überein.

Zur Kartierung der QTL und Schätzung ihrer Einzeleffekte wurde die Methode des „Composite Interval Mapping“ angewendet. Für Stängelläsion wurden in der Kreuzung $NDBLOS_{sel} \times CM625$ acht QTL entdeckt, die in einer Kreuzvalidierung insgesamt 33,7% der genetischen Varianz ($\tilde{p}_{TS}$) erklärten. Ein QTL auf Kopplungsgruppe (LG) 8 erklärte 36,7% der phänotypischen Varianz (R^2_{adj}), alle anderen QTL erklärten zwischen 3,3 und 6,0% von R^2_{adj}. Für Blattläsion wurden insgesamt 9 QTL detektiert. Der Anteil der erklärten phänotypischen Varianz einzelner QTL reichte von 3,4 bis 11,3%. Alle detektierten QTL für die Blattläsion erklärten in einer Kreuzvalidierung 25,3% der genetischen Varianz. Für das Merkmal Geschwindigkeit des Pilzwachstums wurden 6 QTL detektiert, die von 4,6 bis 10,2% R^2_{adj} erklärten. Die erklärte genotypische Varianz in einer Kreuzvalidierung betrug bei diesem Merkmal 24,4%. Die meisten QTL zeigten eine additive Genwirkungsweise.

Für markergestützte Selektion können nur solche QTL empfohlen werden, die sich stabil über Generationen erweisen. Deshalb wurden die QTL-Ergebnisse zwischen den RIL und F_3-Familien der Population $NDBLOS_{sel} \times CM625$ verglichen. Die Anzahl der gemeinsamen QTL betrug eins für Blattläsion, zwei für Stengelläsion und drei für Wachstumsgeschwindigkeit des Pilzes. Nach einer Senkung des LOD-Wertes auf 1,5 wurde für Stängelläsion ein weiterer gemeinsamer QTL für beide Generationen identifiziert.

In der Kreuzung $CM625 \times TUB\text{-}5\text{-}3234$ wurden vier QTL für Stängelläsion, drei QTL für Blattläsion und drei QTL für Wachstumsgeschwindigkeit des Pilzes gefunden. Da die Methode des „Selective Genotyping“ benutzt wurde, kann davon ausgegangen werden, dass nur die QTL mit den größten Effekten identifiziert wurden. Der Vergleich der QTL-

Ergebnisse zwischen den Kreuzungen $NDBLOS_{sel} \times CM625$ und $CM625 \times TUB\text{-}5\text{-}3234$ ergab zwei gemeinsame QTL für Stängelläsion auf LG 8 und 4. Der gemeinsame QTL auf LG 4 stammte vom anfälligen Elter CM625. Der zweite gemeinsame QTL auf LG 8 entspricht wahrscheinlich dem in der Population $NDBLOS_{sel} \times CM625$ ermittelten QTL mit dem größten Effekt. In weitere Untersuchungen sollte dieser Genombereich detaillierter analysiert werden.

Im Hinblick auf eine marker-gestützte Selektion zeigen unsere Ergebnisse, dass zwei QTL aussichtsreich sind. Diese wurden in der Population $NDBLOS_{sel} \times CM625$ auf LG 8 und 16 für die Merkmale Stengelläsion und Wachstumsgeschwindigkeit des Pilzes identifiziert. Diese zwei QTL erwiesen sich als stabil über alle Umwelten und zeigten in der Untersuchung der RIL keine störenden Korrelationen zu morphologischen Merkmalen.

In der Population $CM625 \times TUB\text{-}5\text{-}3234$ konnte nicht eindeutig geklärt werden, ob der resistente Elter TUB-5-3234 neue Resistenzallele mit hinreichend großen genetischen Effekten beisteuert, die nicht bereits in $NDBLOS_{sel}$ identifiziert wurden. Aufgrund seiner Assoziation mit der Blattlänge müssen weitere Untersuchungen klären, ob der QTL auf LG 10 für marker-gestützte Selektion genutzt werden kann.

Die Resistenzzüchtung bei der Sonnenblume gegen *S. sclerotiorum* ist aufgrund der komplexen Vererbung dieses Merkmals schwierig. Diese Studie zeigt, dass die gefundenen Marker zusammen mit der Resistenzquelle $NDBLOS_{sel}$ für eine marker-gestützte Selektion eingesetzt werden können, um eine Verbesserung der Resistenz gegen die Stängelfäule zu erzielen. Um die Resistenz gegenüber *S. sclerotiorum* weiter zu erhöhen, ist es erforderlich, zusätzliche Resistenzgene zu finden und diese über eine Pyramidisierung in Elitelinien anzureichern.

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