QTL ANALYSIS OF RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN SUNFLOWER

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Abstract

Sclerotinia sclerotiorum is an important pathogen of sunflower. We analyzed quantitative trait loci (QTL) affecting resistance to mid-stalk rot in two F3 populations from crosses NDBLOSsel (resistant) x CM625 (susceptible) and TUB-5-3234 (resistant) x CM625. Each F3 line was evaluated in field experiments for the resistance traits leaf lesion, stem lesion, and speed of fungal growth using artificial leaf infections. The methods of composite interval mapping and cross validation were employed for QTL mapping with SSR linkage maps. For the second population a selective genotyping approach was used. In Population 1 six to nine QTLs and in population 2 three to four QTLs were found for the three resistance traits, respectively. The prospects of marker-assisted selection for resistance to *S. sclerotiorum* are limited due to the complex genetic architecture of the trait.

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). The fungus causes three distinct types of disease on sunflower: wilt, midstalk rot and head rot. 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 which 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. Such plants usually break at the site of infection, which leads to total yield loss. The development of highly resistant sunflower cultivars is desirable with respect to an ecologically and economically efficient sunflower production. In cultivated sunflower germplasm no sources of complete resistance to *S. sclerotiorum* are available. Significant differences in susceptibility exist (Tourvieille et al., 1996; Degener et al., 1998), but the genetic mechanisms underlying *S. sclerotiorum* resistance are complex. The analysis of complex traits has been amended by the application of molecular marker technologies. In the last eight years, several genetic linkage maps of cultivated sunflower have been published based on RFLPs, SSRs, AFLPs and DALP markers. 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.*

Materials and Methods

Ninety sunflower inbred lines were screened for their resistance to *S. sclerotiorum* (Degener et al., 1999). The lines NDBLOSsel (PR1) and TUB-5-3234 (PR2), an inbred line developed from an interspecific cross between *Helianthus tuberosus* and HA 89 were chosen as parents due to their high resistance to midstalk rot after artificial infection with *S. sclerotiorum*. Inbred line CM625 (further denoted PS) was selected as the susceptible parent and was crossed with PR1 and PR2. One F1 plant derived from the crosses PR1 x PS (Pop1) and PR2 x PS (Pop2), respectively, was self-pollinated to produce F2 plants. Randomly chosen F2 plants were selfed to produce F3 lines.

Resistance of 354 F3 lines of Pop1 against midstalk rot caused by *S. sclerotiorum* was evaluated in 1999 in two experiments in Eckartsweier, Germany. Experiments were sown on May 7 (Experiment 1) and June 23 (Experiment 2) and inoculated on July 7 and August 7, respectively. Each experiment was laid out as a 19 x 19 lattice design with three replications consisting of F3 lines and parental lines. Resistance of 434 F3 lines of Pop2 was evaluated in 2000 and 2001 in Eckartsweier, Germany. The experiments included F3 and parental lines and were conducted as a 21 x 21 lattice design with three replications.

On five plants per plot the tip of one leaf of the fifth fully grown leaf pair was inoculated with mycelium. The *S. sclerotiorum* explant was placed at the extremity of the main vein. The inoculated leaf was covered with a transparent plastic bag to maintain sufficient humidity. The three resistance traits, leaf lesion, stem lesion and speed of fungal growth, and two morphological traits were recorded as described by Micic et al. (2004a).

Leaf tissue from F2 plants of both populations was collected and dried. For Pop1, the genomic DNA of 352 F2 plants was extracted. For Pop2, 140 F2 plants were selected regarding stem lesion of their corresponding F3 lines. Half of them were selected from the upper tail, and half from the lower tail of the phenotypic distribution of 434 F3 lines. 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).

Lattice analyses of variance were performed with data from each environment using plot means calculated from individual plant measurements for each trait. Components of variance were estimated considering all effects in the statistical model as random. All necessary

computations for the field trials were performed with software package PLABSTAT (Utz, 2000).

Linkage maps for Pop1 based on 352 F2 plants and 117 codominant SSR marker loci and for Pop2 based on 140 F2 plants and 78 SSR marker loci were constructed by using software package JOINMAP 3.0 (Van Ooijen and Voorrips, 2001). Linkage group nomenclature was described by Tang et al. (2002). All necessary computations for QTL mapping and estimation of their effects were performed with software package PLABQTL (Utz and Melchinger, 1996). The method of composite interval mapping (CIM) by the regression approach (Haley and Knott, 1992) 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 proportion of the phenotypic variance explained by all QTLs was determined by the estimator *R2adj* as described by Utz et al. (2000). The proportion of the genotypic variance explained by all QTLs (*p*) was determined from the ratio: R^2adj / *h2***.** Standard five-fold cross validation (CV) implemented in PLABQTL was used for testing the effect of genotypic sampling (Schön et al., 2004). Estimates of the proportion of the genotypic variance explained by detected QTLs simultaneously were calculated for the total data set (*pDS*) and as the median over all test sets ($\widetilde{p}_{TS}$).

Results

Phenotypic Data. For both populations, heritability estimates for resistance traits were intermediate to high and genotypic variances among F3 individuals were highly significant for all traits (Micic et al., 2004a, b). Estimates of genotype x environment interaction variances were not significant for the resistance traits of Population 1. For Population 2 they were not significant for leaf lesion, significant ($P<0.05$) for stem lesion, and highly significant ($P<0.01$) for speed of fungal growth, but relatively small compared with genotypic variances. In both populations resistance traits were significantly but only moderately correlated with each other.

Linkage Maps. Pop1: A genetic linkage map of the 352 F2 individuals was constructed based on 114 of the 117 polymorphic marker loci that coalesced into 16 linkage groups. The linkage groups ranged in length from 8.2 to 127.1 cM covering a total map distance of 941.4 cM with an average interval length of 8.3 cM (Micic et al., 2004a). About 90.5 % of the mapped genome was located within a 20 cM distance to the nearest marker.

Pop2: A genetic linkage map of the selected 140 F2 individuals was constructed based on 74 of the 78 polymorphic marker loci that coalesced into 13 linkage groups. Only one polymorphic locus was found for LGs 7, 8, 11, and 12. The linkage groups ranged in length from 13.1 to 157.9 cM (Micic et al., 2004b). The total map distance covered 1039.7 cM, with an average interval length of 10.4 cM. About 77.0 % of the mapped genome was located within a 20 cM distance to the nearest marker.

QTL Analysis. The parameters associated with putative QTLs for three resistance traits for Pop1 and Pop2 are given in Table 1.

Table 1. Parameters associated with putative QTLs for three resistance traits for Pop1 and Pop2 (Micic et al., 2004a, b).

Resistance traits	Linkage group	Marker	Position on LG (cM)	LOD at QTL position	Variance [a] explained	Direction [b]
Pop. 1						
Leaf lesion	LG 1	ORS 822	2	7.70	10.0	PS
(cm)	LG 4	ORS 366	10	4.44	6.2	PS
	LG 6	ORS 57	96	4.11	5.5	PR1
	LG 8	ORS 623	24	5.57	4.1	PR1
	LG 8	ORS 624	44	2.63	3.4	PS
	LG 9	ORS 795	30	2.76	3.6	PR1
	LG 9	ORS 176	94	8.70	11.3	PR1
	LG 13	ORS 317	82	3.50	4.5	PR1
	LG 15	ORS 1040b	48	3.58	4.6	PR1
	p_{DS}				45.4	
	$\tilde{p}_{TS}$				25.4	
Stem lesion	LG 2	ORS 836	2	4.39	5.7	PS
(cm)	LG 3	ORS 390	58	4.67	6.0	PS
	LG 4	ORS 366	8	2.62	3.7	PS
	LG 6	ORS 608	74	3.56	4.6	PR1
	LG 8	ORS 145	20	34.65	36.7	PR1
	LG 15	ORS 1040b	42	2.50	3.2	PR1
	LG 16	ORS 455	4	4.68	6.0	PR1
		ORS 502[c]	84	4.43	5.8	PR1
	p_{DS}				50.4	
	$\tilde{p}_{TS}$				33.7	
Speed of	LG 1	ORS 509	68	4.70	6.0	PS
fungal growth	LG 6	ORS 57	88	4.82	6.4	PR1
(cm/day)	LG 8	ORS 623	24	5.51	7.0	PR1
	LG 11	ORS 769	40	3.52	4.5	PR1
	LG 15	ORS 1040b	44	8.17	10.2	PR1
	LG 16	ORS 331	10	7.34	9.2	PR1
	p_{DS}				40.1	
	$\tilde{p}_{TS}$				24.1	
Pop. 2						
Leaf lesion	LG 4	ORS 337	12	7.81	23.2	PS
(cm)	LG 9	ORS 887	90	4.58	13.5	PR2
	LG 10	ORS 889	24	2.66	8.4	PR2
	p_{DS}				75.7	
	$\tilde{p}_{TS}$				49.5	
Stem lesion	LG 4	ORS 337	8	8.59	25.2	PS
(cm)	LG 10	ORS 889	38	7.36	21.7	PR2
	LG 17	ORS 588	56	6.64	19.6	PR2
		ORS 456[c]	0	4.29	13.3	PR2
	p_{DS}				80.4	
	$\tilde{p}_{TS}$				69.7	
Speed of	LG 9	HA 494/1	104	2.99	9.7	PR2
fungal growth	LG 10	ORS 889	26	2.78	8.8	PR2
(cm/day)	LG 17	ORS 811	52	3.52	10.9	PR2
	p_{DS}				47.8	
	$\tilde{p}_{TS}$				48.2	

[a] For individual QTL the proportion of the phenotypic variance (R sq.) explained was estimated, for the simultaneous fit the proportion of the genotypic variance explained by putative QTLs in the data set p_{DS} and the median over 1000 test

Discussion

In elite sunflower material the inheritance of resistance to *S. sclerotiorum* has been found to be polygenic with intermediate heritability (Mestries et al., 1998; Bert et al., 2002). The frequency distributions of the three resistance traits and results from the ANOVA of our study confirmed these findings. As a consequence, large population sizes and selective genotyping were chosen for the mapping of QTLs to maximize power of QTL detection. QTLs were detected for all three resistance traits in both populations. Nevertheless, especially in Pop1 estimated effects at QTL were small and severely biased despite the large population size as indicated by the large difference between *pDS* and $\tilde{p}_{TS}$. Additionally, few QTLs were common to the two populations. Thus, the data confirm the hypothesis that a large number of genes with small effects regulate resistance to midstalk rot.

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