MAPPING COMPONENTS OF RESISTANCE TO PHOMOPSIS (*DIAPORTHE HELIANTHI*) IN A POPULATION OF SUNFLOWER RECOMBINANT INBRED LINES

Kamel Langar, INRA UMR Diversité et Génome des Plantes Cultivées, 2, place Pierre Viala, F-34060, Montpellier Cedex 1, France

Yves Griveau and **Hervé Serieys,** INRA UMR Diversité et Génome des Plantes Cultivées, Domaine de Melgueil, F-34130, Mauguio, France
E-mail: Yves.Griveau@ensam.inra.fr

François Kaan and **André Bervillé,** INRA UMR Diversité et Génome des Plantes Cultivées, 2, place Pierre Viala, F-34060, Montpellier Cedex 1, France

Abstract

A population of recombinant inbred lines was obtained from a cross between inbred lines HA 89, susceptible to Phomopsis, and LR4, resistant. This population was tested for resistance to Phomopsis in seminatural infection, and artificial infection by mycelium placed on leaves. QTLs for parameters of resistance to Phomopsis were detected with composite interval mapping on a molecular map covering 2169 cM. At least 8 chromosomal regions were found carrying QTLs controlling resistance, some with different segments implied in resistance on leaves and stems. QTL analysis with composite interval mapping led to mapping of three significant QTLs influencing final expansion rate of lesions on leaves. A major one was detected on group 6 (LOD>7, R-square=24%). Several significant QTLs were detected for attack rates at flowering with seminatural infections, the main one explaining 46% of variation (LOD>11). The inheritance of resistance to Phomopsis appeared complex. However, since unlinked segments carried major QTLs for different components of resistance, resistance on leaves and resistance on stems could be pyramided with marker-assisted selection.

Introduction

Phomopsis stem canker (*Diaporthe helianthi* Munt.-Cvet. et al.) is a fungal disease widespread in all the non-arid cultivation areas for sunflower. It causes yield losses up to 1t/ha in France (Carré, 1993). The breeding programs for genetic control of Phomopsis began twenty years ago, and led to the breeding of resistant cultivars (Skoric, 1985). Observations with natural and artificial inoculations allowed differentiation of genotypes with different levels of susceptibility, but no genotype has been found fully resistant (Skoric, 1985; Tourvieille, 1989; Griveau et al., 1992; Vear et al., 1997; Langar et al., 1997). The resistance of sunflower to Phomopsis would be under additive (Vear et al., 1997), oligogenic (Vranceanu et al., 1993), and horizontal control (Viguié et al., 1999). Moreover, resistance of the hybrids is related to that of their parents (Vear et al., 1997).

An inbred line issued from NS-H-45 appeared to cumulate factors of resistance to Phomopsis. In particular, it expressed both early resistance and late resistance to infection (Langar et al., 1997), resistance on leaves and resistance on stem (Langar et al., 2000). Thus, a population of Recombinant Inbred (RI) lines issued from the cross HA 89 x LR4 was developed and used to map resistance to Phomopsis. This population was tested for Phomopsis resistance with artificial infections and seminatural infections in F7 and in the following generations. It was suggested that resistance to Phomopsis would be the consequence of several mechanisms, independently inherited. It was concluded that several parameters should be retained for QTL mapping of Phomopsis resistance, both for resistance on leaves and resistance on stems (Langar et al., 2002). In addition, the RI lines from HA 89 x LR4 have been used to construct a molecular map covering 2169 cM (Langar et al., 2003)

In this paper, we present the main QTLs for the resistance parameters toward Phomopsis detected in this RI line population in the F7 generation.

Materials and Methods

Plant Material. LR4 was fixed in the progenies of the hybrid NS-H-45 (from IFVC Novi Sad). This line may display slow necrosis on leaf blades, petioles and stems, whereas the susceptible line, HA 89, may show rapid lesion expansion on leaves and stems. The inbred sunflower line HA 89 was chemically emasculated with gibberellic acid and was crossed using pollen from the line LR4-17. Two F1 plants were obtained and self-pollinated. These led to 242 F2 descendants. Each generation of RI lines was derived by selfing one plant chosen randomly from each family. The F7 generation of the RI lines was tested in 1997 with semi-natural infection by *D. helianthi* and with artificial inoculation on leaves.

Pathogenicity Tests and Parameters Measured. Trials with seminatural infections were performed at Auzeville, near Toulouse, France. For seminatural infections of F7 plants, each RI line was represented by two replicate sets of 20 plants. Sections of stems infected by Phomopsis were dispersed in the field as inoculum. The number of plants attacked by *D. helianthi* on stems, and the number of plants with encircling stem lesions, both at the end of flowering (stage R6) and at physiological maturity and the beginning of stage R9 (Schneiter and Miller, 1981) were noted. The following parameters were calculated: frequency (percentage) of attack at flowering with seminatural infections, 1) initial infection frequency, i.e., percentage of plants at flowering with stem lesions, 2) final infection frequency, i.e., percentage of plants at physiological maturity with stem lesions, 3) initial frequency (percentage) of plants with encircling stem lesions at flowering, and 4) final frequency (percentage) of plants at physiological maturity with encircling lesions.

The experiments with artificial inoculation of F7 plants were carried out in a nylon net tunnel, at Mauguio near Montpellier, France. Each RI line was represented in a plot of three plants. The parental lines, divided into 8 blocks randomly distributed throughout the tunnels, were used as controls. Water was supplied by a sprinkler system regulated to maintain the presence of water drops on the canopy (Tourvieille and Vear, 1986). Leaves were inoculated as described by Bertrand and Tourvieille (1987), using mycelium from strain 96001, an aggressive isolate of *D. helianthi* (Viguié et al., 1999). Inoculations were carried out 75 days after sowing, after all the plants reached the R1 stage. The length of lesions on leaf blades, petioles and stems was measured twice a week for 6 weeks from infection date until physiological maturity of the flower heads. For each interval between two measurement dates,

the rate of lesion expansion was calculated as being the ratio of the increase in length of the leaf blade lesion over the time interval in days. Thus, the following parameters were taken into account: 1) initial expansion rate of lesions on leaf blades in mm/day, i.e., expansion rate calculated on the two first records of lesion length, 2) final expansion rate on leaf blades in mm/day, i.e., rate of lesion expansion on leaf blades, calculated before lesions appeared on petioles or at maturity, 3) maximal expansion rate of lesions on leaf blades in mm/day, 4) expansion rate of lesions on petioles in mm/day.

Genetic Map. Specific PCR, AFLP and DALP (Desmarais et al., 1998) were used to construct a genetic map on 171 RI lines from the cross HA 89 x LR4. Procedures and map construction were described by Langar et al. (2003). This led to a map covering 2168.6 cM in 18 linkage groups.

Statistical Analyses. Angular transformations were computed on the frequency of attack at flowering, the initial infection frequency, the final infection frequency, the initial frequency of encircling lesions, and the final frequency of encircling lesions. Statistical analyses for normality tests and broad sense heritabilities were performed with, respectively, the UNIVARIATE and GLM procedures of the SAS software (SAS institute, 1996). QTLs were detected with Composite Interval Mapping, using the PLABQTL software (Utz and Melchinger, 1995). The LOD threshold was determined as equal to 3.17 for a type I risk of 5% with permutations of the quantitative data.

Results

Genetic variation was highly significant for all parameters (Table 1). Heritabilities ranged from 23% for expansion rate of lesions on petiole to 82% for the final expansion rate on leaf blades that were obtained with artificial infections on leaves (Table 1). Seminatural infections were broadly successful and provided reliable estimations of genotypic values.

Table 1. Means and heritabilities of parameters of resistance to *Phomopsis* in the F7 RI lines from HA 89xLR4.

Mode of infection	organ	trait	unit	HA89	LR4	RI lines	heritability
artificial	Leaf blade	IER	mm/day	1.6	0.3	1.6	29.9%***
	Leaf blade	FER	mm/day	7.5	0.8	4.1	82.3%***
	Leaf blade	MER	mm/day	7.5	1.7	4.7	73.8%***
	Petiole	ERP	mm/day	1.1	0.0	0.5	23%**
seminatural	All	FATT	%	100.0	28.3	88.0	59.4%***
	Stem	IIF	%	21.0	0.0	9.8	61.7%***
	Stem	FIF	%	94.0	0.0	64.0	47.7%***
	Stem	IFEL	%	5.0	0.0	1.1	38.7%***
	Stem	FFEL	%	46.0	1.0	16.4	61.0%***

***= $p<0.001$, **= $p<0.01$

IER**:** initial expansion rate of lesions on leaf blades, FER: final expansion rate of lesions on leaf blades, MER: maximal expansion rate of lesion on leaf blades, ERP: expansion rate of lesions on petiole, FATT: frequency of attack at flowering with seminatural infections, IIF: initial infection frequency on stems at flowering, FIF: final infection frequency on stems at maturity, IFEL: initial frequency of encircling stem lesions at flowering, FFEL: final frequency on encircling stem lesions at maturity.

The part of variation explained by QTLs ranged from 4.8% for initial expansion rate of lesions on leaf blades to 51.1% for frequency of attack at flowering with seminatural infections (Table 2). The number of QTLs detected varied from 1 for expansion rate of lesions

Table 2. Part of variations explained by QTLs and number of QTLs detected in R1 lines from HA 89XLR4.

Mode of infection	organ	trait	overall R-square	number of QTLs	Number of linkage groups
Artificial infection	Leaf blade	IER	4.8	2	2
	Leaf blade	MER	25.9	5	5
	Leaf blade	FER	11.5	4	4
	Leaf blade	ERP	12.7	1	1
Seminatural infection	All	FATT	51.1	10	10
	stem	IIF	25.8	4	4
	stem	FIF	38.7	5	5
	stem	FFEL	34.4	5	4

IER**:** initial expansion rate of lesions on leaf blades, FER: final expansion rate of lesions on leaf blades, MER: maximal expansion rate of lesion on leaf blades, ERP: expansion rate of lesions on petiole, FATT: frequency of attack at flowering with seminatural infections, IIF: initial infection frequency on stems at flowering, FIF: final infection frequency on stems at maturity, FFEL: final frequency on encircling stem lesions at maturity.

Table 3. Main QTLs detected for parameters of resistance to *Diaporthe helianthi* in the F7 RI lines from HA 89xLR4.

LG	organ	trait	Position of the peak cM	Flanking markers	Confidence interval cM	LOD	R-square	effect.
4	All	FATT	32	CAH16TG-ACH8AG	8-44	3.38	17.1	-0.130
4	All	FATT	78	M235H6-CCL5AG	76-80	7.52	27.7	0.207
4	stem	FIF	78	M235H6-CCL5AG	72-84	3.26	13.1	0.077
4	petiole	ERP	112	M242L3-M242L6	106-112	4.30	19	-0.510
6	Leaf blade	IER	14	ACH20TT-ACH14TT	10-16	4.02	15.5	-0.712
6	All	FATT	84	ACL4TT-ACH9AA	78-88	3.07	15.7	0.081
6	Leaf blade	FER	106	ACH9AA-ACH7TC	100-112	6.37	24	1.130
6	Leaf blade	MER	110	ACH9AA-ACH7TC	100-112	3.10	12.5	0.709
6	All	FATT	112	ACH7TC-CAH2TG	110-118	4.36	23.5	-0.091
12	Leaf blade	FER	74	ACL15TC-CCL25AA	72-84	3.04	7.9	-0.508
12	Leaf blade	MER	74	ACL15TC-CCL25AA	72-84	3.34	8.7	-0.554
12	All	FATT	86	ACH15AT-M233H5	82-88	3.98	13.1	-0.083
12	stem	IIF	86	ACH15AT-M233H5	82-88	5.28	16.9	-0.084
12	stem	FFEL	86	ACH15AT-M233H5	82-88	3.71	12.2	-0.081
13	stem	FFEL	42	CAL7AG-M233H3	34-54	3.97	19.8	-0.121
13	stem	IIF	60	M233H3-ACH18TC	58-62	5.28	25.6	-0.119
15	stem	FFEL	12	CAL12AC-CCL6TA	4-20	3.10	15.8	-0.096
15	all	FATT	44	CCL6TA-CCH5AC	36-48	11.11	46	-0.154
15	stem	IIF	46	CCH5AC-M233L11	32-50	4.98	24.4	-0.087
15	stem	FIF	48	CCH15AC-M233L11	36-54	5.77	27.7	-0.108
15	stem	FFEL	68	M233L11-CCH6AT	58-74	3.73	22	-0.123
16	all	FATT	42	ACH7AT-ACH6AG	36-50	6.65	30.9	-0.129
16	Leaf blade	IER	86	ACH4AG-CAH8TG	76-90	3.08	13.1	-0.543
18	stem	FIF	30	CAL23AC-M233L6	26-34	4.68	22.9	0.091
18	stem	FFEL	32	M233L6-M233H1	30-36	7.93	24.3	0.131
18	all	FATT	38	M233L6-M233H1	34-40	5.81	18.5	0.101
18	Leaf blade	MER	42	M233H1-CCH24AA	38-50	3.66	14.2	0.768
18	stem	FER	44	CCH24AA-CAL21TG	38-48	3.65	14.2	0.714

IER**:** initial expansion rate of lesions on leaf blades, FER: final expansion rate of lesions on leaf blades, MER: maximal expansion rate of lesion on leaf blades, ERP: expansion rate of lesions on petiole, FATT: frequency of attack at flowering with seminatural infections, IIF: initial infection frequency on stems at flowering, FIF: final infection frequency on stems at maturity, FFEL: final frequency on encircling stem lesions at maturity, LG: linkage group.

on petiole to 10 for frequency of attack at flowering with seminatural infections, and not QTL was detected for initial frequency of plants with encircling stem lesions at flowering.

The main QTLs of resistance to Phomopsis, those with LOD>4, were carried by six linkage groups (Table 3). Moreover, two disjoined segments carried QTLs on linkage group 4 and 16. Thus, 8 main segments at least controlled resistance to Phomopsis. QTLs showing negative effect correspond to resistance brought by the LR4 resistant parent, and are called QTLs of resistance. QTLs with positive effect correspond to resistance brought by HA 89, i.e., susceptibility brought by LR4, and are called QTLs of susceptibility. QTLs of resistance were located in the linkage groups 12, 15 and 16. The group 18 especially carried QTLs of susceptibility for the rates of attack in seminatural infection, in particular frequency of attack at flowering with seminatural infections, final infection frequency on stems at maturity and, to a lesser degree, initial and final expansion rate on leaf blades. Group 12 gathered QTLs related to resistance, in particular those of the initial infection frequency on stems at flowering, initial frequency of plants with encircling stem lesions at flowering and final frequency of plants with encircling lesions at physiological maturity. They were close to a QTL of resistance detected for expansion rates of lesions on leaves, initial and final expansion rate on leaf blades. Group 15 contained the major QTL related to resistance for frequency of attack at flowering with seminatural infections, on the same segment with other QTLs of resistance for initial final infection frequency on stems. Group 16 carried the second major QTL for frequency of attack at flowering with seminatural infections. Another QTL of resistance on group 16 controlled initial expansion rate of lesions on leaf blades, but it was largely distant from the one QTL for frequency of attack at flowering with seminatural infections. In the groups 4 and 6 we located QTLs in the opposite way. Group 4 preferentially controlled the frequencies of symptoms in seminatural conditions and carried a major QTL of susceptibility for frequency of attack at flowering with seminatural infections. Moreover, group 4 carried the one QTL for expansion rate of lesions on petiole, disjoined from other QTLs.

Discussion

The number of QTLs of resistance to Phomopsis varied from 1 for expansion rate of lesions on petiole to 10 for frequency of attack at flowering with seminatural infections. Individual R-squares of QTLs varied from 8% to 28%, little less than those found by Bert et al. (2002). These results highlight the polygenic nature of resistance to Phomopsis in sunflower. Nevertheless, we observed a very high individual effect (> 45%) for frequency of attack at flowering with seminatural infections. This last is located on an interval higher than 20cM, which implies an overestimate of explained variation.

Eight main linkage groups were implied in the inheritance of resistance to Phomopsis. The most significant QTLs detected for the parameters evaluated with seminatural infections (frequency of attack at flowering) and those obtained with artificial infection on leaves (initial, final and maximal expansion rate of lesions on leaf blades) were not located in common areas. No colocalisation was observed between QTLs related to final expansion rate of lesions on leaf blades, and those related to frequencies of symptoms on stems. Moreover, the one QTL detected for expansion rate of lesions on petiole was disjoined from all others. These results are in accordance with the hypothesis that different mechanisms of defence exist on leaf blades, petioles and stems as suggested by Langar et al. (2002). In comparison, Bert et

al. (2002) detected five main QTLs for length of lesions of leaves and three for frequency of attack on stems. One segment carried common QTLs for the two criteria. However, the source of resistance studied by Bert et al. was different, mainly less resistant on leaves than LR4.

A major QTL for frequency of attack at flowering with seminatural infections was located on the group 16, but disjoined from any QTLs for other resistance parameters. This QTL would possibly mark resistance to germination of the spores on leaves. This assumption would require confirmation by infection with ascospores. Indeed, seminatural infection is random and the infection of leaf blades with mycelium bypasses germination of ascospores.

Favorable alleles are brought in inbred lines by the susceptible parent HA 89; this is indicated by QTLs with a positive effect, mainly in group 18 for parameters measured for stems (Table 3). They may explain the occurrence of RI lines more susceptible than HA 89, as observed by Langar et al. (2002). Bert et al. (2002) detected also factors of resistance to Phomopsis issued from the susceptible parent.

The study of this cross showed the interest of a population of RI lines since the same genotypes could be evaluated by different modes of infection. The molecular mapping highlighted the polygenic character of resistance to Phomopsis. However, since unlinked segments carry major QTLs for different components of resistance, resistance on leaves and resistance on stems could be pyramided with marker-assisted selection.

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