FULL RESEARCH PAPER

Genetic control of partial resistance to 'collar' and 'root' isolates of *Phoma macdonaldii* in sunflower

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Abstract *Phoma macdonaldii* is one of the most important pathogens of sunflower (*Heliantus annuus*) in France. In order to determine the inheritance of resistance to the disease, five sunflower genotypes with wide genetic variability for resistance to two 'collar' and two 'root' Phoma isolates were crossed in a diallel programme. Four separate experiments were undertaken under controlled conditions. In each one, the response of parental genotypes and their F1 hybrids were evaluated with one of the four Phoma isolates. Analysis of variance was performed to determine the effects of genotype on disease severity score when inoculated with 'collar' or 'root' Phoma isolates and showed significant variability among parents and F1 hybrids for disease severity score. Diallel analysis showed that general combining ability (GCA) and specific combining ability (SCA) effects for resistance to 'collar' and 'root' Phoma isolates were highly significant for each of the four isolates indicating that both kinds of gene effects were important in controlling the resistance. The GCA/SCA ratios were more than one for three out of four isolates showing that additive genetic effects were more important than non-additive effects for resistance to three of the studied Phoma isolates. Hence, conventional breeding methods could be recommended to achieve genetic improvement to such 'collar' and 'root' Phoma isolates.

Keywords Combining abilities · Disease severity · F1 hybrids · Inheritance · *Helianthus annuus*

Introduction

Phoma macdonaldii is one of the most important pathogens of sunflower (*Helianthus annuus*) in France (Debaeke & Pérès, 2003) causing Phoma black stem. Generally, Phoma infects the lower leaves, either by wind or rain-splashed spores (Gulya, Rashid, & Masirevic, 1997). Once the infection reaches the stem, a black round to oval lesion forms, which reaches a maximum diameter of 5 cm. The spot may eventually girdle the stem, although the lesions generally affect only the epidermal layer and do not penetrate into the pith. Yield losses due to Phoma black stem lesions are moderate, ranging from .2 to .7 t ha^{-1} (Penaud, 1996). Phoma also infects the collar and the root system of plants, leading to a girdling lesion at the soil level. This may result in stunted plants with thin stems, smaller heads, lighter and fewer seeds per head, blackened pith, and

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frequently premature death (Donald, Venette, & Gulya, 1987). These symptoms, referred to as 'early death' or 'premature ripening', and yield losses up to 1.3 t ha^{-1} have been recorded in France (Pérès, Poisson-Bammé, & Drolon, 2000). Direct infection by contact with infected shallow or buried residues with stem base or the roots results in collar and root disease symptoms (Poisson-Bammé & Pérès, 2000).

Genetic variability for partial resistance to Phoma black stem in sunflower has been reported in both the field (Pérès, Allard, Deverchère, & Penaud, 1994) and under controlled conditions (Abou Al Fadil, Dechamp-Guillaume, Poormohammad, & Sarrafi, 2004; Bert et al., 2004; Rachid Al-Chaarani et al., 2002; Roustaee, Barrault, Dechamps-Guillaume, Lesigne, & Sarrafi, 2000a). Genetic variability for resistance to Phoma black stem has been observed in F3 families from a cross between a partially resistant mutant with its susceptible original line under controlled conditions (Abou Al Fadil et al., 2004). Roustaee et al. (2000a), using parental genotypes and their F1 hybrids showed that the variation among genotypes studied is due to the general combining ability (GCA) and thus most of the variation is attributed to additive effects. Recombinant inbred lines (RILs) derived from a cross between PAC2 and RHA266 genotypes were inoculated with an aggressive French isolate causing Phoma black stem symptoms and seven quantitative trait loci (QTL) were identified (Rachid Al-Chaarani et al., 2002). The detected QTL together explained 92% of the phenotypic variation of the trait. Bert et al. (2004), using F$_2$–F$_3$ families derived from a cross between PAZ2 and FU genotypes, detected four QTL controlling Phoma black stem resistance. These genetic studies have been carried out using plant material inoculated at the junction of the cotyledon petioles and hypocotyls with an aggressive French isolate of *P. macdonaldii* (MP6). The authors are not aware of any previous reports about the inheritance of partial resistance to isolates exhibiting 'collar' and 'root' symptoms. Understanding the inheritance of disease resistance will help to design an effective breeding programme. The diallel cross analysis is an efficient instrument in the genetic analysis of quantitative characters. This study aimed to determine combining abilities for partial resistance to four 'collar' and 'root' isolates exhibiting large differences in term of susceptibility in five sunflower lines and their F1 hybrids.

Materials and methods

Genotypes and Phoma isolates

Genotypes used in this study exhibit a high genetic variability in their susceptibility to Phoma collar and root isolates. M6-54-1 is one of the mutant lines developed in our department by seed irradiation of 'AS 613' genotype with gamma rays (Sarrafi et al., 2000). ENSAT-B5 and ENSAT-R5 are inbred lines selected in our crossing programmes. B454/03 is an inbred line introduced from Hungary. These five genotypes were crossed in a diallel mating system without reciprocals to produce 10 F1 hybrid combinations.

Collar fragments collected from field grown sunflower plants affected with the disease in different regions, where sunflower is cultivated in France were used to obtain *P. macdonaldii* isolates by single pycnidiospore cultures according to Roustaee, Costes, Dechamp-Guillaume, and Barrault (2000b). Single pycnidiospore isolate cultures were placed on V8 medium (Agar 20 g l^{-1}, V8 200 ml l^{-1}, pH 6) and incubated for 7 days at 25°C in the dark. Conservation of isolates was done using the method described by Roustaee et al. (2000b). In previous studies we investigated the host reaction of 10 commercial sunflower genotypes after inoculation with 10 single pycnidiospore isolates in different plant tissues (root, collar and petiole). Results showed that there are differences among these isolates in terms of the ability to cause disease on different plant parts (Abou Al Fadil, 2006). Considering these results, four isolates (two 'collar' and two 'root') were selected for our diallel programme. These four selected isolates were derived from samples collected from central (Loir and Cher) and western (Vendée) regions of France.

Collar inoculation

The response of parental genotypes and their F1 hybrids were evaluated in two separate experi-

ments, which were done at the same time and under the same conditions. In each experiment, the plants were inoculated by one of the two selected isolates (TA4 and TA8). The experimental design was a randomised complete block with three replications. Each replication consisted of 10 plants. Seeds of sunflower genotypes were sterilized for 5 min in a sodium hypochlorite solution (6 chlorometric degrees) and washed in sterile distilled water. Two rows of five seeds per genotype per replication were sown in plastic containers. The experiments were carried out in a controlled growth chamber ($25 \pm 1°C$ day, $18 \pm 1°C$ night and a relative humidity of 75–80%). Light intensity was $200~\mu Em^{-2}s^{-1}$ with a 14 h photoperiod. A disc of mycelium (6 mm diam.) was placed beside the collar of 12 day-old sunflower plants (first pair of developed leaves). After inoculation, each container was enclosed for 48 h using a special transparent cover (plexiglass) to maintain a near-saturated humidity favourable for fungal inoculation. Disease severity was evaluated 7 days after inoculation, on a 1–9 scale for the percentage of necrotic area on 1 cm of the stem base and all around it, where: 1 = 0–5%, 2 = 6–10%, 3 = 11–20%, 4 = 21–30%, 5 = 31–40%, 6 = 41–60%, 7 = 61–80%; 8 = 81–99% and 9 = necrosis all around the stem base and spreading up more than 1 cm.

Root inoculation

Two other experiments were undertaken with each of the Phoma 'root' isolates (TA6 and TA9) at the same time and under the same conditions. The experimental design and seed sterilization were the same as explained for inoculation with 'collar' isolates. Seeds were sown in Magenta boxes (one plantlet per box) containing Murashige and Skoog medium (4.4 g l^{-1}), solidified with 2.8 g l^{-1} Phytagel (Sigma). Experiments were performed in a growth chamber ($25 \pm 1°C$ days, $18 \pm 1°C$ night) with 75–80% relative humidity and 14 h photoperiod with $200~\mu Em^{-2}s^{-1}$ light intensity provided by NAV-T 600 W lamps Osram-Vialox, Molsheim, France. Twelve-day-old plants were inoculated by 20 μl of a pycnidiospore suspension (10^6 pycnidiospores ml^{-1} of water containing .25% gelatin) added to the

medium near the root using a syringe. Disease severity was scored on a 1–9 scale for the length of necrotic area 7 days after inoculation, where: 1 = 0–.5 cm, 2 = .6–1 cm, 3 = 1.1–1.6 cm, 4 = 1.7–2.2 cm, 5 = 2.3–2.8 cm, 6 = 2.9–3.4 cm, 7 = 3.5–4 cm, 8 = 4.1 –4.6 cm, 9 = 4.7–6 cm necrosis area on the roots.

Statistical analysis

Disease severity score of 'collar' isolates were transformed by square root ($\sqrt{x}$) to normalize the data distribution. In the case of disease severity of 'root' isolates the distribution of the data was normal and did not need any transformation. To determine the effects of genotype on disease severity score for 'collar' and 'root' isolates, analysis of variance was performed separately for each experiment. Diallel analyses were then conduced according to Griffing's Method 2 and Model 1 (Griffing, 1956) using the SAS programme for Griffing's diallel analysis (Zhang & Kang, 1997). The statistical model is the following:

$$Y_{ij} = \mu + \lambda_i + \lambda_j + S_{ij} + e_{ij}$$

where: μ = general mean effect; λ_i (λ_j) = general combining ability (GCA) of the ith (jth) parent; S_{ij} = specific combining ability (SCA) of the cross between the ith and jth parent; and e_{ij} = residual. The Newman–Keuls test was used for comparing mean performance of parents and F1s. However, the mean values for disease severity score in the collar experiments are presented in the tables as non-transformed data of disease severity.

Results and discussion

Analyses of variance for each of four isolates separately presented in Table 1 showed significant variability among parents and F1 hybrids for disease severity score, indicating that partial resistance to 'collar' and 'root' Phoma isolates is genetically controlled in the genotypes tested. Means of disease severity (Table 2) show that parental lines ENSAT-B5 and B454/03 were partially resistant to three out of the four isolates

Table 1 Analysis of variance and combining abilities for disease severity in sunflower genotypes infected by two 'collar' and two 'root' isolates of *Phoma macdonaldii*

Source of variation	df[a]	MS[b]			
		Collar		Root	
		TA6	TA9	TA4	TA8
Variance analysis					
Total	44	.034	.04	3.21	1.75
Block	2	.003[ns]	.002[ns]	.07[ns]	.14[*]
Genotype	14	.093[***]	.122[***]	1.01[***]	5.43[***]
Residual	28	.006	.002	.03	.03
Diallel analysis					
GCA[c]	4	.255[***]	.213[***]	5.53[***]	13.69[***]
SCA[d]	10	.029[***]	.085[***]	11.80[***]	2.13[***]
Error	28	.002	.001	.01	.01
GCA/SCA		8.80	2.51	.47	6.43
Coefficient of variation		10.78	5.55	3.03	5.16

[a] df, Degrees of freedom

[b] MS, Mean of squares

[c] GCA, General combining ability

[d] SCA, Specific combining ability

* and ***, significant at .05 and .001 probability level respectively; ns, non significant

studied. ENSAT-R5 has partial resistance to 'collar' isolates whereas it was susceptible to 'root' isolates. AS613 and its mutant line M6-54-1 were susceptible to both 'collar' isolates and also to one of the 'root' isolates and showed partial resistance to the TA8 'root' isolate. These results confirm the genetic variability for partial resistance to *P. macdonaldii* reported previously for Phoma black stem in both field and controlled conditions (Abou Al Fadil et al., 2004; Bert et al., 2004; Pérès et al., 1994; Rachid Al-Chaarani et al., 2002; Roustaee et al., 2000a). F1 hybrids presented a continuous range of disease severity from partially resistant to very susceptible (Table 2). ENSAT-B5 × ENSAT-R5 exhibited partial resistance to 'collar' isolates whereas it was susceptible to 'root' isolates. Conversely, AS613 × M6-54-1 was susceptible to 'collar' isolates whereas it exhibited partial resistance to 'root' isolates. B454/03 × M6-54-1 showed partial resistance to one of the 'collar' and one of the 'root' isolates and was susceptible to the two other isolates. ENSAT-R5 × AS613 exhibited partial resistance to three out of four isolates and ENSAT-R5 × M6-54-1 was susceptible to 'collar' isolates.

Mean square values of GCA and SCA revealed that the variance due to GCA and SCA were highly significant for each of the four isolates studied (Table 1). Thus, both kinds of gene effects were important in controlling the inheritance of resistance to 'collar' and 'root' Phoma isolates. However, the GCA/SCA ratios are more than one for three out of four isolates. This indicates that the additive gene effects are more important than non-additive ones in controlling partial resistance to 'collar' and 'root' isolates. Assessment of the contribution of individual lines to hybrid resistance was accomplished by comparing the GCA effect among the parents (Table 2). A parent with a significant negative GCA value would contribute with a high level of resistance whereas a parent with a positive value would contribute with a high level of susceptibility. ENSAT-B5 showed highly significant negative GCA values for three out of four isolates. AS613 and its mutant line M6-54-1 exhibited highly significant positive GCA values for both of the 'collar' isolates whereas GCA had negative values for both 'root' isolates in AS613 and only for TA8 'root' isolate in the mutant line. ENSAT-R5 exhibited a highly significant negative GCA value for 'collar' isolates whereas the GCA value was positive for the TA4 'root' isolate. In order to accumulate favourable genes in new germplasm it

Table 2 Estimated general and specific combining ability effects and means for disease severity of five sunflower genotypes and their F1 hybrids infected by two 'collar' and two 'root' necrosis isolates of *Phoma macdonaldii*

Genotype	Collar				Root			
	TA6		TA9		TA4		TA8	
Parents	$\bar{X}$	GCA	$\bar{X}$	GCA	$\bar{X}$	GCA	$\bar{X}$	GCA
ENSAT-B5	2.9[bcd]	–.039[*]	1.87[fg]	–.104[***]	8.60[a]	.76[***]	2.65[g]	–.09[*]
ENSAT-R5	1.6[ef]	–.150[***]	1.60[g]	–.100[***]	5.80[d]	–.04[ns]	6.45[a]	1.34[***]
B454/03	3.5[bcd]	.001[ns]	2.70[cde]	–.057[***]	5.94[d]	–.17[***]	1.35[i]	–.65[***]
AS613	6.8[a]	.037[*]	7.40[a]	.096[***]	4.56[f]	–.66[***]	2.20[h]	–.59[***]
M6-54-1	7.3[a]	.152[***]	6.70[a]	.119[***]	7.25[c]	.09[*]	2.85[fg]	–.19[***]
LSD$_{.01}$	.18	.02	.10	.01	.40	.05	.40	.05
F1 hybrids	$\bar{X}$	SCA	$\bar{X}$	SCA	$\bar{X}$	SCA	$\bar{X}$	SCA
ENSAT-B5 × ENSAT-R5	2.37[def]	.033[ns]	2.47[def]	.052[*]	7.85[b]	1.55[***]	4.15[c]	–.47[***]
ENSAT-B5 × B454/03	2.6[cde]	–.077[ns]	1.27[h]	–.232[***]	3.4[i]	–2.77[***]	3.15[ef]	.52[***]
ENSAT-B5 × AS613	2.7[cd]	–.060[ns]	2.67[cde]	–.056[**]	3.05[i]	–2.62[***]	3.85[d]	1.16[***]
ENSAT-B5 × M6-54-1	5.38[ab]	.025[ns]	7.5[a]	.208[***]	7.35[c]	–.93[***]	3.30[e]	.21[*]
ENSAT-R5 × B454/03	2.1[f]	–.020[ns]	3.47[c]	.136[***]	4.25[g]	–1.12[***]	5.00[b]	1.12[***]
ENSAT-R5 × AS613	1.47[f]	–.200[***]	2.97[cd]	–.068[**]	5.7[d]	.83[***]	3.10[ef]	–.84[***]
ENSAT-R5 × M6-54-1	5.2[ab]	.125[**]	8.87[a]	.196[***]	3.75[h]	–1.87[***]	3.38[e]	–.96[***]
B454/03 × AS613	4[abc]	.020[ns]	7.5[a]	.185[***]	7.6[bc]	2.86[***]	1.00[j]	–.95[***]
B454/03 × M6-54-1	6.7[a]	.038[ns]	2.1[ef]	–.205[***]	5.1[e]	–.39[***]	2.75[fg]	.40[***]
AS613 × M6-54-1	6.67[a]	.099[ns]	4.6[b]	–.273[***]	3.35[i]	–4.21[***]	2.88[fg]	1.41[***]
LSD$_{.01}$	.18	.11	.10	.06	.40	.24	.40	.24

$\bar{X}$, Mean disease severity score

[a] GCA, General combining ability

[b] SCA, Specific combining ability

Means followed by the same letter are not significantly different ($P = .05$) according to Student-Newman–Keuls (SNK) test

*, ** and ***, significant at 0.05, 0.01 and 0.001 probability level respectively; ns, non significant

will be pertinent to cross the lines, which exhibited different reactions depending on the isolates. F1 hybrid 'ENSAT-R5 × AS613' showed significant negative SCA values for three out of four isolates (Table 2). The F1 hybrid coming from the cross between ENSAT-R5 and M6-54-1, which is a mutant line of AS613, has negative and significant SCA values for 'root' isolates but positive and significant ones for 'collar' isolates.

Combining ability analysis estimates, the average additive and dominance effects of all the genes involved in expression of a trait, is based on progeny performance (Dabholkar, 1992). The significant GCA and SCA effects indicated the importance of both additive and non-additive genetic components in controlling partial resistance to four 'collar' and 'root' Phoma isolates. However, for three out of four isolates the GCA/SCA ratios were more than one supporting the preponderance of additive gene effects involved in the genetic control of collar and root necrosis

severity. This is in agreement with a previous report from factorial crosses that also indicated GCA is more important than SCA for Phoma black stem response (Roustaee et al., 2000a). Rachid Al-Chaarani et al. (2002), using RILs and Bert et al. (2004) using F_2–F_3 families found seven and four QTL controlling partial resistance to Phoma black stem respectively. They also reported an additive gene effect for partial resistance to Phoma black stem, which shows the polygenic nature of resistance to the disease. The GCA/SCA effects imply that the parents were highly influential on the performance of the progenies in determining resistance to the disease. Hence, conventional breeding methods can be recommended to achieve genetic improvement to such 'collar' and 'root' Phoma isolates.

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