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Specifying the introgressed regions from *H. argophyllus* in cultivated sunflower (*Helianthus annuus* L.) to mark Phomopsis resistance genes

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Abstract A method based upon targetting of introgressed markers in a Phomopsis-resistant line (R) of cultivated sunflower, issuing from a *H. argophyllus* cross was used to mark the Phomopsis resistance regions. Our study was based upon 203 F_3 families derived from a cross between an inbred line susceptible to Phomopsis (S1) and the introgressed resistant line (R). Families were checked for Phomopsis resistance level in a design with replicated plots and natural infection was re-inforced by pieces of contaminated stems. Thirty four primers were employed for RAPD analysis. Out of 102 polymorphic fragments between (S1) and *H. argophyllus*, seven were still present in (R) suggesting that they marked introgressions of *H. argophyllus* into (R). The F_2 plants were scored for the presence or absence of 19 fragments obtained from five primers, and the relationships between the presence/absence of fragments in F_2 plants and Phomopsis resistance/susceptiblity in the F_3 progenies was determined by using an analysis of variance. We found that at least two introgressed regions, as well as favourable factors from sunflower, contributed to the level of Phomopsis resistance in cultivated sunflower.

Key words *Argophyllus* · *Helianthus* · Sunflower · Introgression · Phomopsis · *Diaporthe helianthi* · RAPD

Introduction

The cultivated sunflower (*Helianthus annuus* L.) is one of the most important annual oil plants in the world. Several pathogens cause yield losses. Among these pathogens, Phomopsis (*Diaporthe helianthi* Munt. - Cvet.), remains one of the main diseases (average losses of 20–30% may occur). The release of tolerant varieties is required to prevent fungal epidemics and to avoid chemical treatment.

Phomopsis resistance most likely involves several genes (Skoric 1985; Vranceanu et al. 1993). Breeders have now obtained commercial hybrids with a good level of resistance to Phomopsis but the transfer of this resistance to elite lines takes a long time and is expensive. Skoric (1985) has shown that tolerant sunflower varieties can be obtained from crosses between sunflower and wild annual species, such as *H. argophyllus* Torr. & Gray (section *Helianthus*), or wild perennial *H. tuberosus* L. (section *Atrorubens* Anashch. Schill. & Heiser). However, it remains unclear from this work whether the resistance originated in the cultivated sunflower or in the wild forms. In particular there is no evidence for the absence of resistance to Phomopsis in sunflower although the most-resistant sources came from wild species. Interspecific hybrids have been obtained from crosses between *H. argophyllus*, *H. debilis debilis*, or *H. praecox* sect. *Helianthus*, and sunflower (Griveau et al. 1992). Tolerant inbred lines to Phomopsis have been obtained and it has been shown that the wild parents were responsible for the introduction of resistance factors.

For some traits, like disease resistance, the genetic variability of sunflower has been increased by crosses with related species or wild types (Leclercq et al. 1970). The identification of such traits may then be performed by searching for markers of the wild-type in interspecific progenies. This method has been widely used previously to mark introgressed traits such as resistance genes or restorer genes. Jena et al. (1992) have shown that only some of the very short introgression segments were linked to resistance to the brown plant hopper. In potato, resistance to nematodes was identified by Krieke et al. (1993) by screening RFLP markers from the resistant parent. Xu and Kasha (1992) searched for foreign DNA in barley with RAPD in order to define the

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introgressed traits. Garcia et al. (1994) demonstrated that RAPDs were efficient for detecting introgression in an interspecific population of peanuts. Additionally, Delourme et al. (1994) using radish-specific RAPD markers, have shown, that the restorer allele for cytoplasmic male fertility of radish was introgressed into rapeseed. In a preceding paper (Quillet et al. 1995) we have constructed a partial map of *H. argophyllus* no. 92 using RAPD fragments. Since this accession has been used to transfer Phomopsis resistance to a sunflower line we looked for these fragments in the introgressed line rather than constructing a genetic map of the resistant line.

Markers of introgressed zones of *H. argophyllus* in the resistant inbred line (R) were assumed to be good candidates to identify the segments carrying Phomopsis-resistant QTLs. The F_2 and F_3 populations issuing from a cross between the (S1) susceptible and the (R) tolerant lines were obtained and resistance to Phomopsis was scored in the F_3 family population. To mark the Phomopsis resitance genes, we first looked for *H. argophyllus* fragments still present in the resistant lines (candidate fragments) because of introgression. We then determined the relationship between the presence of candidate fragments in F_2 plants and the level of Phomopsis attack in their F_3 progenies.

Materials and methods

Plant material and crosses

Lines (S1) and (S2) are currently used in our sunflower breeding programs and are both susceptible to Phomopsis. The resistant (R) line was obtained by crossing (S2) with *H. argophyllus* (accession IBPGR code MPHE 92) followed by subsequent intercrosses between 100 F_1 plants. Further selection for Phomopsis resistance in the F_3–F_6 generations has led to the production of a number of resistant lines including the (R) line (Griveau et al. 1992). A cross between (S1) and (R) produced F_1 plants and three of them were selfed to produce 203 F_2 plants. These plants were scored for RAPD markers and their F_3 descendants were evaluated for Phomopsis resistance.

Targetting of introgressions from *H. agrophyllus* in the sunflower resistant line

DNAs from the sunflower lines (R), (S1), (S2), the F_2 plants, and *H. argophyllus* MPHE92, were prepared and analysed with RAPD markers according to the methods described by Quillet et al. (1995). Thirty four primers (Bioprobe) were tested in the (R), (S1), (S2) lines and in *H. argophyllus*. We looked for markers introgressed from *H. argophyllus* in the (R) line. The markers were screened for their presence in both *H. argophyllus* and (R) and their absence in (S1) and (S2). We considered the fragment size first and then verified its molecular homology through hybridization with the *H. argophyllus* fragment used as a probe.

DNAs from RAPD gels were transferred onto Hybond N^+ membranes (Amersham) by capillarity. RAPD fragments were picked up, purified, and then labelled by random priming using 74 mBq of [α^{32}P] dCTP (111 TBq/mmole) according to Peltier et al. (1994). The membranes were hybridized in 0.5% SDS, 6 × SSC and 5 × Denhardt at 65°C for 18 h. They were rinsed twice in 2 × SSC and a 0.1% SDS solution for 30 min, then once 0.2 × SSC and a 0.1% SDS solution for 30 min. A Fuji ≪X Ray≫ film was exposed with the membrane at − 80°C for a sufficient time depending on the labelling intensity.

We analysed the (S1) × (R) F_2 progeny for the absence/presence of each marker. A chi-square test enabled us to confirm whether segregation in the F_2 was in accordance with the 3:1 expected ratio for a dominant marker. The linkage between markers was detected using MAPMAKER version 3.0 b with the mapping function of Kosambi (1994) using a LOD-score threshold of 3 (Lander and Green 1987) and a recombinantion rate threshold of 45 cM.

Evaluation of Phomopsis resistance

From each F_2 plant 40 F_3 plants were obtained by selfing and evaluation for Phomopsis resistance at Auzeville (near Toulouse) during the summer of 1994, with two replicates made of 20 plants in a complete block design. These families were distributed into ten triats, each of them containing the controls Agrisol and Viki, resistant and susceptible to Phomopsis, respectively. The parental lines (S1) and (R) were added to the experiment. In order to check any influence of flowering data on the level of Phomopsis attack, Agrisol and Viki were sown at different dates every 2 weeks. The contamination by Phomopsis spores was performed in conditions of natural infestation reinforced-adding a 20-cm piece of sunflower stem contaminated the previous year at a 5-m interval in the field every two rows. The measurement of resistance was carried out at the beginning of flowering (notation 1) and 2 weeks later (notation 2). The observation of symptoms was carried out on each plant according to the flowering scale: 0 = no attack on stem; 1 = one spot on stem but not encircling the stem; 2 = at least two spots on the stem; 3 = at least one spot encircling the stem; 4 = encircling spots on stem with lodging. The quantitization was computed either by means of the attack notes – PHOMO 1 for the first notation and PHOMO 2 for the second notation – calculated for the F_3 family and representing a genetic evaluation of each F_2 plant, or by STEM 1 = number of plants attacked on stem (plants with symptoms 1, 2, 3, 4)/total number of plants in the first notation, STEM 2 = number of plants attacked on stem (plants with symptoms 1, 2, 3, 4)/total number in the second notation, CIRCLE 1 = number of plants with spots encircling stem (plants with symptoms 3, 4)/number of plants attacked on stem (plant with symptoms 1, 2, 3, 4) in the first notation, and CIRCLE 2 = number of plants with spots encircling stem (plant with symptoms 3, 4)/number of plants attacked on stem (plant with symptoms 1, 2, 3, 4) in the second notation. The flowering date was determined at the R.5.5 stage (Schneiter and Miller 1981).

The attack notes PHOMO 1 and PHOMO 2 were transformed with the $\sqrt{x}$ function and the rates STEM 1, STEM 2, CIRCLE 1, CIRCLE 2 were transformed with the arcsin $\sqrt{x}$ function for the two notations. Analysis of variance (SAS 1992) was performed with three models. For model 1, $Y_{ijk} = \mu + A_i + b_j + t_k + e_{ijk}$ was tested, where Y_{ijk} represents the mean of the attack note by plot, A_i the effect of family i, b_j the effect of block j. t_k the effect of trial k, and e_{ijk} is the error term. With the least-square method, a_i and b_i were determined for each family. The coefficient of determination was calculated as $R^2 = SS_F/SS_T$ with SS being the sum of squares (F) for families and (T) the total SS.

Relations between markers and Phomopsis resistance

Model 2 was $Y_{ijkl} = \mu + m_i + b_j + t_k + A_{1/i} + e_{ijkl}$, where Y_{ijkl} = mean of the attack note by family; m_i = effect of the presence of the marker; b_j = effect of block j; t_k = effect of trial k, A_1 = effect of family 1 belonging to the marker class i. Model 3 was $Y_{ijkln} = \mu + m1_i + m2_j + (m1m2)_{ij} + b_k + t_l + A_{n/ij} + e_{ijkln}$, where $m1_i$ = effect of presence of marker m1; $m2_j$ = effect of presence of marker m2; $(m1m2)_{ij}$ = interaction between the m1 and m2 markers, An/ij = effect of the family n belonging both to the marker classes i and j. We accepted the QTL for the Phomopsis resistance at a significant level of $P < 0.01$.

The explained percentage of Phomopsis resistance variability was given by $R^2 = SS_M/SS_T$ where SS_M = sum of squares of the marker (or of the markers combination) and SS_T = sum of squares of the experiment. The explained genetic variation $EGV = SS_M/SS_M + SS_v$ where SS_v = sum of squares of the family effect.

The explained difference (ED) of the attack level between lines (S1) and (R) was $ED = (N + m) - (N - m)/N_R - N_{S1}$, where $(N + m)$ = mean of the attack note (or attack frequency) in the presence of the marker (or the marker combination), $(N - m)$ = mean of the attack note (or attack frequency) in the absence of the marker (or of the marker combination), N_R = mean of the attack note (or attack frequency) (for the (R) line, and N_{S1} = mean of the attack note (or attack frequency) for the (S1) line. Moreover, effects enhancing or decreasing resistance to Phomopsis were marked by (+) or (−), respectively.

Results

Molecular analysis of the parents and of the F_2 population: targetting of introgressed from *H. argophyllus*

With the 34 primers used, 102 polymorphic fragments were revealed between (S1) and *H. argophyllus*. Seven fragments were equivalent in size between *H. argophyllus* and (R). The fragment of *H. argophyllus*, used as a probe, hybridized to the fragment from (R) (data not shown) confirming that it originated in the wild species. CH1 and EH were also found to be of *H. argophyllus* origin. Consequently, each plant of the F_2 progeny was genotyped with four primers (B, C, D, F) generating potential introgressed fragments (Table 1). Nineteen fragments were segregating. No segregating distorsion was detected for any of these fragments according to a chi-square test ($P < 5\%$). Genetic linkage was found between DH and CH2 ($d = 13$ cM) and between DS1 and BS1-2 ($d = 42$ cM).

Table 1 Origin of markers screened in the F_2 population

Primer code	Fragment code	Fragment present in
B	BH	R, *H. argophyllus*
	BS1.1	S1
	BS1.2	S1
	BS1.3	S1
	BS2	R, S2
C	CS2	R, S2
	CS1.1	S1
	CS1.2	S1
	CS1.3	S1
	CT	S1
	CH1	R, *H. argophyllus*
	CH2	R, *H. argophyllus*
D	DH	R, *H. argophyllus*
	DS2	R, S2
F	FS1	S1
	FS2.1	R, S2
	FS2.2	R, S2
	FS2.3	R, S2
	FH	R, *H. argophyllus*

Family distribution and genetic variability

There was no significance correlation between attack notes and flowering dates. According to the global attack note (Fig. 1) and attack components (Fig. 2A–D), the distribution of the F_2 families in the first and the second notations were continuous. The values of R^2 for parents and controls range from 0.31 to 0.95 while those from families range from 0.39 to 0.70 (Table 2). This indicates that most of the uncontrolled difference is not explained by the family effect but is due to the environmental hetrogeneity of the Phomopsis attack (Table 2). The means of attack notes for each parental line, as well as for Agrisol and Viki, are indicated on the histograms (Figs. 1, 2, arrows). Some F_3 families displayed a higher resistance than the resistance parent (Fig. 2A). Using the contrast method, a transgression ($F_{1.44} = 16.04$; $P = 0.002$) for the two more-resistant families was revealed.

Simple effects of markers on Phomopsis resistance in the F_2 population

The CH1 marker from *H. argophyllus* and present in (R), explains 2% and 3.75% of the genetic variability of the

Fig. 1A, B Distribution of Phomopsis notes among F_3 families from the cross (S1) × (R) lines. **A** PHOMO 1; **B** PHOMO 2

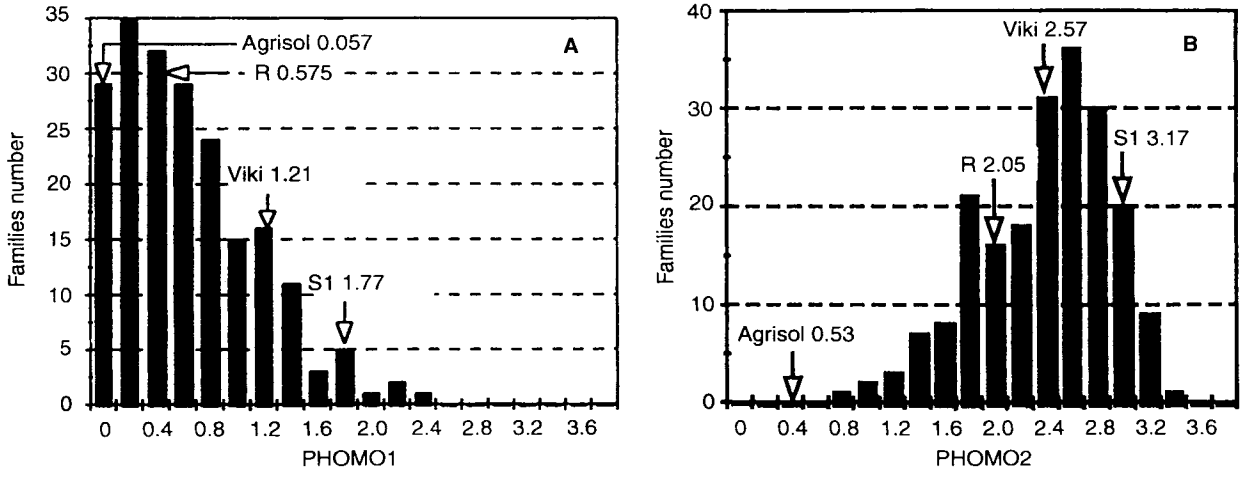

Table 2 Coefficient of determination of the families of the (S1) × (R) line cross and parent plus control effects in the variance analysis for the global attack note and the two components for notations 1 and 2

% Genetic variation	Notation 1			Notation 2		
	PHOMO 1	STEM 1	CIRCLE 1	PHOMO 2	STEM 2	CIRCLE 2
R^2 families	0.70	0.68	0.39	0.65	0.56	0.64
R^2 parents and controls	0.65	0.62	0.31	0.77	0.95	0.63

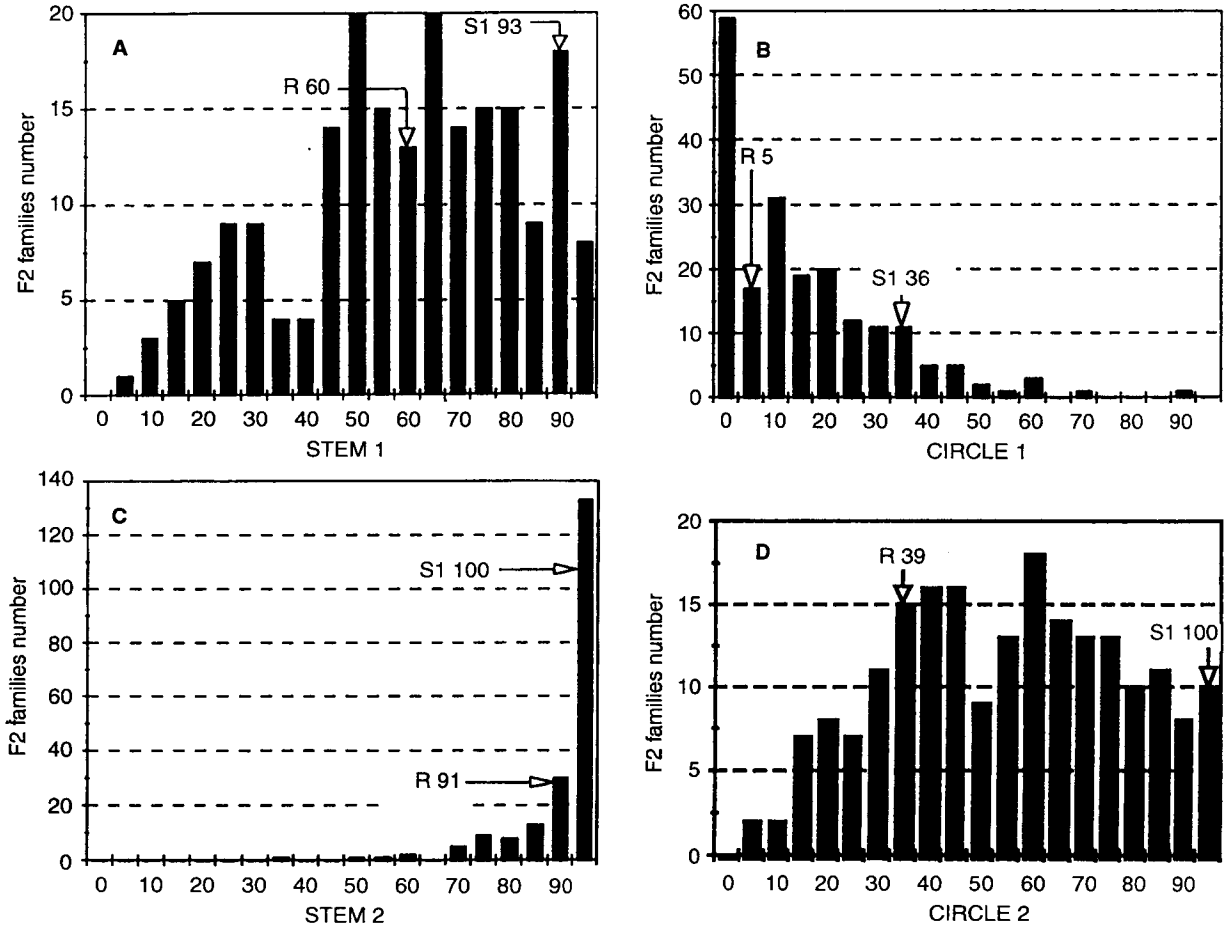

Fig. 2A–D Distribution of attack among F_3 families from the cross between (S1) × (R) lines. **A** STEM 1; **B** CIRCLE 1; **C** STEM 2; **D** CIRCLE 2

Phomopsis attack notations 1 and 2, respectively. The DH marker has no detectable effect. The CT marker from sunflower and present in (S1) explains 5.03% of the genetic variability for the Phomopsis attack notation 1. The BS1-2 marker explains 4.24% of the genetic variability for Phomopsis attack notation 1, and was associated with susceptibility to Phomopsis. CS2 present in (S2) explains 7.09% of the genetic variability for Phomopsis attack notation 2 (Table 3).

Crossed effects of markers and interactions between markers

We analysed the population with model 3, testing all combinations including CT, CH1, CS2, and BS1.2 which have an effect with model 2, to see whether markers also have an effect on Phomopsis attack through model 3 (Table 4). With this model, and using the CH1*BH combination in PHOMO 2, a significant effect at $P = 0.009$ ($F_{1.183} = 6.91$) was found for BH ($R^2 = 3.05\%$ and EGV = 4.96%) while the CH1 effect was significant at $P = 0.0009$ ($F_{1.183} = 11.40$) and the interaction was not significant. No other simple effect was found ($P < 0.01$) but interactions between markers were revealed. The CT*DH interaction calculated on PHOMO 1 led to $R^2 = 2.18\%$ ($F_{1.375} = 9.70$; $P = 0.002$) although DH had no single effect. In the absence of CT, DH displayed a significant effect ($F_{1.62} = 6.11$; $P = 0.018$) on resistance with an R^2 of 6.87% (EVG = 14.2%). CT in the absence of DH also had a significant effect on PHOMO 1 ($F_{1.109} = 16.53$;

Table 3 Markers segregating on the F_2 population issuing from the (S1) × (R) line cross explaining the variability of the global attack note and their two components in notations 1(A) and 2(B)

A

Marker	Explained percentage of PHOMO 1			N-M[a]	N + M[b]	Explained percentage of STEM 1			Explained percentage of CIRCLE 1.			ED
	R^2[c]	EGV[d]	$P < F$[e]			R^2	EGV	$P < F$	R^2	EGV	$P < F$	
CT	3.52	5.03	●●●●*	0.88	0.59	3.42	5.02	●●●●	1.28	2.40	●	24.27
BS1.2	3.00	4.24	●●●	0.45	0.69	2.45	3.55	●●	–	–	ns	−20.08
CS1.1	1.71	2.45	●	0.46	0.67	1.78	2.65	●	–	–	ns	−17.57
CH1	1.41	2.00	●●	0.77	0.61	1.01	1.48	●	–	–	ns	+13.39

B

Marker	Explained percentage of PHOMO 2			N − M	N + M	Explained percentage of STEM 2			Explained percentage of CIRCLE 2.			ED
	R^2	EGV	$P < F$			R^2	EGV	$P < F$	R^2	EGV	$P < F$	
CS2	4.63	7.09	●●●●	2.73	2.43	2.85	5.11	●●●	2.48	3.69	●●●	26.79
CH1	2.49	3.75	●●	2.67	2.47	1.20	2.13	●	1.78	2.63	●	17.86
CS1.2	2.06	3.03	●	2.66	2.47	1.83	3.33	●	–	–	ns	16.96
CT	–	–	ns	2.61	2.49	0.96	1.71	●	1.27	1.88	●	10.71

* ●●●●, ●●●, ●●, ●: significance level at 0.01%, 0.1%, 1% and 5%, respectively; ns: not significant
[a] N − M = attack note in the absence of the marker
[b] N + M = attack note in the presence of the marker
[c] R^2 = coefficient of determination of marker
[d] EGV = explained genetic variation
[e] $P < F$ = probability level for the independence of marker/resistance

Table 5 Cumulative effects of markers on the Phomopsis attack notes. A: PHOMO 1; B: PHOMO 2

A

Marker combinations	N + Ms[a]	N − Ms[b]	$P < F$[c]	R^2Mg[d]	EGV	ED
BS1.2/CS1.1	0.79	0.19	●●*	4.53	6.90	−50.00
CT/CS1.2	0.53	1.14	●●●	8.38	13.25	50.83
CT/CS1.2/FS1	0.5	2.69	●●●	5.50	8.09	182.50
BH/CH1	0.53	1.17	●●	6.18	9.60	53.33
FH/CH1	0.55	1.06	●●●	5.85	8.43	42.50
BH/CH1/DH	0.52	2.43	●●	8.05	12.50	159.17
CT/CH1	0.55	1.06	●●●	5.23	7.34	42.50
CT/CH1/DH	0.48	3.24	●●●	9.48	13.04	230.00
CT/DH	0.59	1.28	●●●●	7.29	10.19	57.50

B

Marker combinations	N + Ms[a]	N − Ms[b]	$P < F$[c]	R^2Mg[d]	EGV	ED
CT/CS1.2	2.44	2.73	ns	7.25	13.23	25.89
BH/CH1	2.34	3.05	●●●	8.99	14.39	63.39
FH/CH1	2.41	2.85	●●	4.26	6.11	39.29
BH/CH1/DH	2.28	3.39	●	5.18	8.33	99.12
CS2/CH1	2.39	2.79	●●●	5.11	7.99	35.71
CT/DH	2.48	2.88	●●●	3.72	5.93	35.71

*●●●●, ●●●, ●●, ●: Significance level at 0.01%, 0.1%, 1% and 5%, respectively
[a] N + Ms = global attack note in the presence of the marker combination
[b] N − Ms = global attack note in the absence of the marker combination
[c] $P < F$ = probability level for the independence of marker combination/resistance
[d] R^2M = coefficient of determination of the marker combination

$P = 0.0002$) with an R^2 of 7.17% (EGV = 14.7%). The CH1*CS1-1 interaction gave an $R^2 = 3.95\%$ in PHOMO 1 ($F_{1,187} = 9.84$; $P = 0.002$). The CH1 marker almost had a significant effect ($F_{1,140} = 5.83$, $P = 0.017$) but only in the absence of the CS1-1 marker. The R^2 of CH1 in the absence of CS1-1 is 2.01% (EGV = 5.64%), but the simultaneous absence occurred in only two families.

Effects of simultaneous absence or presence of markers

The simultaneous presence or absence of DH, CH1 and FH enabled us to define two groups of individuals for which the means for Phomopsis attack levels corresponded to the parental lines (R) and (S1) (Table 5). Except for BS1/CS1.1 in the PHOMO 1 notation, the simulta-

Table 4 Mean of Phomopsis notations according to the combinations of CT and DH markers

DH	CT	*n*	PHOMO 1	PHOMO 2	STEM 1	STEM 2	CIRCLE 1	CIRCLE 2
0[a]	0	13	1.28	2.87	86[c]	100	18	75
0	1[b]	41	0.58	2.46	61	98	5	50
1	0	29	0.69	2.50	67	98	7	58
1	1	112	0.59	2.51	62	97	5	56
CH1	CS1-1	*n*	PHOMO 1	PHOMO 2	STEM 1	STEM 2	CIRCLE 1	CIRCLE 2
0	0	2	2.16	3.28	99	100	14	93
0	1	21	0.86	2.73	73.6	98	6	65
1	0	16	0.35	2.27	47	94	6	46
1	1	62	0.64	2.50	64	98	8	55
CH1	BH	*n*	PHOMO 1	PHOMO 2	STEM 1	STEM 2	CIRCLE 1	CIRCLE 2
0	0	5	1.30	3.11	86	100	12	75
0	1	23	0.55	2.63	65	98	4	64
1	0	17	0.41	2.45	49	95	5	55
1	1	54	0.58	2.33	58	95	5	48

[a] 0 = absence of marker
[b] 1 = presence of marker
[c] Percentage

Table 6 Cumulative effects of markers on the components of resistance. A: effect on rate of attack STEM 1; B: effect on rate of encircling spots CIRCLE 1; C: effect on rate of encircling spots CIRCLE 2

A

Marker combinations	% + Ms[a]	% − Ms[a]	$P < F$[c]	R^2M[d]	EGV	ED
BH/CH1	58	81	●●*	5.57	9.17	69.70
FH/CH1	59	79	●●	4.61	6.79	60.60
BH/CH1/DH	57	98	●	5.67	8.94	124.24
CT/DH	62	86	●●●	6.85	9.75	72.7
CT/CH1/DH	61	99	●●●	10.1	14.04	97
CT/CS1.2	59	85	●●●	5.36	8.51	78.8

B

Marker combinations	% + Ms[a]	% − Ms[b]	$P < F$[c]	R^2M[d]	EGV	ED
CT/CS1.2	3	11	●	5.44	10.93	25.8
CT/DH	5	18	●●●	5.26	10.35	41.94
BH/CH1/DH	4	52	●●	8.23	15.40	154.84

C

Markers combinations	% + Ms[a]	% − Ms[b]	$P < F$[c]	R^2M[d]	EGV	ED
CT/CS1.2	53	65	ns	6.99	10.45	19.7
BH/CH1	49	77	●●	4.25	6.68	45.90
FH/CH1	52	75	●●	3.51	5.04	37.70
CT/DH	58	80	●●●	4.28	6.40	36.07

*●●●, ●●, ●: significance at 0.01%, 0.1%, 1% and 5%, respectively; ns: not significant
[a] % + Ms = attack rate in the presence of the marker combination
[b] % − Ms = attack rate in the absence of the marker combination
[c] $P < F$ = probability level for the independence of marker combination/resistance
[d] R^2M = coefficient of determination of the marker combination

neous presence of markers is linked to resistance. The cumulative effects of two markers explained more than 50% ED between parents, both for PHOMO 1 and PHOMO 2 (Table 5) and for STEM 1, CIRCLE 1 and CIRCLE 2 (Table 6). The effects of the simultaneous presence of three markers was equal to, or greater than, the difference between the parents. However, the number of plants carrying such a simultaneous absence was 1–3 depending on the marker.

Discussion

The plant stage seems to have no effect on the attack rate in this experiment. Our experience with Phomopsis clearly showed that this is not common and can be explained by the exceptional weather of 1994 as a result of which the attack occurred late and at a high rate. A correlation between plant stage and attack rate is found

only when the attack occurs early and at a low rate. Because we observed a significant difference and a range of responses for the susceptible and resistant controls we considered further the 1994 experiment. Theoretically the R^2 for lines or the F_1 hybrid is expected to be higher than for segregating F_3 families for all criteria. However, because we computed R^2 from both susceptible and resistant lines, the extremes led to higher R^2 values for controls than for families.

Only CH1 introgressed from *H. argophyllus* into (R) has an effect (Table 3). The other markers correspond to different introgressed regions which did not interfere with Phomopsis resistance. The low effect may result either from a low efficiency of a tightly linked resistant QTL, to poor linkage with QTL for resistance, or to an interaction between QTLs favourable or unfavorable for Phomopsis. Another reason explaining the low R^2 is that the expression of the resistant genes from *H. argophyllus* should be different according to the genetic background and epistatic effects increase the variance of the resistance. Thus, the R^2 of the markers taken individually are lower than if there was no epistatic effect. In potato, where no map has been constructed, Kreike et al. (1993) have looked for QTLs for nematode resistance using ANOVA. They obtained an R^2 of less than 7%, i.e. as low as those we obtained. In both cases, the markers are most probably not closely linked to resistant QTLs. The cumulated presence of BH, CH1 and DH improved the resistance level of the (S1) line compared to that of (R) by 159% on PHOMO 1. The ED can be greater than 100% because of transgressions and epistatic effects. The introgressed markers from *H. argophyllus* were shown to be linked to the Phomopsis resistance trait. This supports Skoric's (1985) observations. The effects of markers from *H. argophyllus* have led to a level of resistance similar to the difference of resistance between lines (R) and (S1); it is therefore likely that the major resistance factors issuing from *H. argophyllus* are all identified in this cross. However (R) was less resistant than Agrisol. Line (R) was chosen because wild and cultivated parents were genotyped for RAPD markers, so that introgression could be easily determined in the inbred line (R). It is likely that (R) does not contain all the favourable factors which could be present in *H. argophyllus*. The line HA74, derived from another accession of *H. argophyllus*, provided a higher level of resistance to *Diaporthe helianthi* (Skoric 1985).

Three markers transmitted through the susceptible parents contributed to an enhancement of the resistance level. CT, coming from line (S1), explains 3.52% of the variability of the resistance (PHOMO 1). This is a transgression factor for PHOMO 1 with less effect on PHOMO 2. The CS2 marker, found in (S2) and (R) but not in *H. argophyllus*, contributed to the resistance. This is consistent with the presence of resistance genes in sunflower. Moreover CT is positively related with the Phomopsis resistance factors issuing from *H. argophyllus*. It has a significant effect in STEM 2 (R^2 2.85%). BS1-2, found in line (S1), is linked to Phomopsis susceptibility. Its absence enhances resistance (R^2 2.45%). We showed that cumulative effects of pairwise or triplet combinations are significant, but only CS1.2 significantly increases the resistance when CT is present.

When CT and DH fragments are absent, the attack is more important. These two markers appear to be linked to genes intervening in the same mechanism of resistance for pathogen receptivity. CT is not the transgression factor which explains the difference between lines (R) and (S) for STEM 1 because it does not provide any effect in the presence of the factor introgressed from *H. argophyllus* close to DH.

It is likely that different genes are implicated in different cellular responses (Lamb 1994). We expected therefore to identify different genes involved in several functions preventing, decreasing or inhibiting Phomopsis. Here STEM 1 and STEM 2 are related to resistance on the leaves, while CIRCLE 1 and CIRCLE 2 deal with resistance on the stem. BS1-2 and CT act on STEM 1, while CS2 acts on STEM 2. BH, CH1 and FH have an effect on both notations. QTL mapping of *Phytophora* resistance in red pepper (Lefebvre and Palloix 1996) has revealed different QTLs according to the stage of the plants or the organs infected. Vranceanu et al. (1993) have studied different F_2s from crosses between Phomopsis-tolerant and -susceptible sunflower lines, and they calculated that at least three major genes are involved in Phomopsis resistance. In the crosses between lines (S1) and (R) our results provide evidence for a few loci interacting to determine the level of Phomopsis resistance with four loci for increased resistance and one responsible for susceptibility.

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