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QTL mapping of partial resistance to Phoma basal stem and root necrosis in sunflower (*Helianthus annuus* L.)

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Abstract

Phoma macdonaldii infects different tissues of sunflower and causes reduction in yield and oil content. The aim of present research was to identify genomic regions involved in partial resistance of sunflower to four *Phoma macdonaldii* basal stem and root necrosis isolates using our improved map constructed with 191 SSR and 304 AFLP markers. The experiment was conducted using F9 recombinant inbred lines (RILs) from a cross between 'PAC2' and 'RHA266'. Results showed that 'PAC2' was more resistant than 'RHA266' to basal stem necrosis isolate 'TA6' and root necrosis isolate 'TA4'. By contrast 'RHA266' was more resistant than 'PAC2' to basal stem necrosis isolate 'TA9' and root necrosis isolate 'TA2'. Transgressive segregation was observed for partial resistance to all four isolates. Some recombinant lines presented partial resistance or susceptibility to all isolates. Twenty seven QTL with phenotypic variance ranging from 7 to 29% were detected. Among them 13 were 'isolate specific' and others were common for partial resistance to different isolates (isolate non specific). Most of the QTLs in common have major effects for resistance to each isolate. The 'isolate non specific' QTLs were located on linkage groups (LG) 5, 6, 8, 12, 13 and 15. The markers 'HA3555' on LG12 and 'E33M48 26' on LG6 as well as 'E33M48 20' on LG13, which are each linked to QTLs of different basal stem and root necrosis isolates, could be used in marker assisted selection to introduce tolerance to four *Phoma macdonaldii* isolates into elite sunflower breeding lines.

Keywords: Phoma macdonaldii; Basal stem; Root; Isolate specific QTL; *Helianthus annuus* L.

1. Introduction

Phoma macdonaldii [1], the causal agent of black stem of sunflower (*Helianthus annuus* L.), is reported from different countries across the world. The disease has been spreading steadily since 1990 in France, and the fungus is now a major component of the pathogenic complex [2]. It causes premature ripening associated with yield losses of 10 30% [3], and also reduction in oil content and thousand seed weight [4]. The disease can also cause premature plant death [5].

Phoma macdonaldii can infect different tissues of sunflower. Generally it infects the lower leaves of the plant and the most notable symptom of infection is a dark lesion at the base of the petiole [6]. Phoma may also infect the basal stem and the root system of plants, resulting in a girdling lesion occurring at the soil level. This may result in stunted plants with thin stems, smaller heads, lighter and fewer seeds per head, blackened pith and premature death [5]. Direct infection by contact with infected shallow or buried residues on the roots or at the stem base results in basal stem and root necrosis symptoms [7]. Phoma infects the plants through direct penetration or indirectly through wounds or plant structural openings such as lenticels and stomata [8]. To date, sunflower genotypes with different levels of resistance to this disease have been identified, but no fully resistant genotypes are available [9].

Estimation of genetic variation is useful to determine the breeding value of lines and choosing the appropriate procedure in breeding programmes for resistance to the disease. Genetic variability for partial resistance to black stem in sunflower has been reported in both field and controlled conditions [2,9 11]. Roustaee et al. [11] showed that additive genetic effects, which are important in breeding programmes to predict breeding value and progeny performance of crosses, are significant for some F1 hybrids used in their research work. Additive genetic effects for partial resistance to Phoma black stem have been also reported by Rachid Al-Chaarni et al. [10] and Bert et al. [12].

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Quantitative trait loci (QTL) associated with disease resistance in sunflowers have been identified for resistance to *Diaporthe helianthi* [13], *Sclerotinia sclerotiorum* [12 14], Phoma black stem [10,12], and downy mildew (*Plasmopara halstedii*) [10]. QTLs for partial resistance to Phoma black stem have been identified by infection of cotyledon petioles of sunflower RILs or F_2-F_3 families with spores of an aggressive French isolate [10,12]. As far as we know, partial resistance to basal stem and root necrosis has not been reported in the literature. The aim of the present study was to study the genetic control of partial resistance to Phoma basal stem and root necrosis isolates and to identify genomic regions involved in resistance.

2. Materials and methods

2.1. Plant material and Phoma macdonaldii isolates

The experiment was conducted using 99 F9 recombinant inbred lines (RILs) and their two parental lines coming from a cross between ‘PAC2’ and ‘RHA266’. Sunflower basal stem fragments from 10 different French geographical origins showing necrosis symptoms were surface-sterilized for 5 min in a sodium hypochlorite solution (6 chlorometric degrees), washed three times (5 min) in sterile distilled water, and transferred to Petri dishes containing potato dextrose agar (PDA) (39 g l ^{1}, pH 6) and incubated for 10 days at 25 °C under alternating periods of illumination (12 h, 37 μE m ^{2} s ^{1}; Philips TLD 15 W 33 lamps) and darkness to induce sporulation. Monopycniospore isolates were prepared by serial dilutions [9] and cultured on PDA for further subculture. To allow mycelial growth, monopycniospore cultures were placed on V8 medium (Campbell Grocery Products Ltd.; Agar 20 g l ^{1}, V8 200 ml l ^{1}, pH 6) and incubated for 7 days at 25 °C in the dark. The pathogenicity of isolates for fulfilling Koch’s postulates was confirmed [15]. Conservation of isolates was done using the method described by Roustaee et al. [9]. Through 10 monopycniospore isolates coming from sunflower basal stem fragments of different regions in France, 4 isolates were selected on the basis of our preliminary study. These isolates (two for basal stem and the two others for root) show contrasted Phoma-sunflower pathosystem behaviors between parental lines. ‘TA4’ and ‘TA6’ isolates were derived from samples collected from central France and ‘TA9’ and ‘TA2’ were derived from samples collected from the west and southwest of France in 1997. These isolates are representative of most French isolates in terms of phenotypic characters studied (growth, morphology and aggressivity). Two inoculation methods for evaluation of partial resistance to basal stem necrosis and root necrosis isolates were developed in our department.

2.2. Basal stem inoculation

The responses of 99 RILs and parental lines were evaluated in two experiments. In each experiment the plant materials were inoculated by one of the two isolates (‘TA6’ and ‘TA9’). The experimental design was a randomized complete block with three replications. Each replication consisted of 12 plantlets. Seeds 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 conducted in a controlled environment chamber (25 ± 1 °C day, 18 ± 1 °C night and a relative humidity of 75 80%). Light intensity was 200 μEm ^{2} s ^{1} with a 14 h photoperiod. A disc of agar medium with mycelium (6 mm diameter) was placed beside the basal stem of 12-day-old sunflower plantlets (first pair of leaves were developed). After inoculation, each container was enclosed for 48 h using a transparent cover to maintain a near-saturated humidity favorable for fungal inoculation. The development of lesions on the basal stem was evaluated on a 1 9 scale for the percentage of necrotic area 7 days after inoculation, 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 = 100%, with necrosis spreading up the basal stem.

2.3. Root inoculation

Two other experiments were undertaken each with one of the two Phoma root necrosis isolates (‘TA2’ and ‘TA4’). Eighty-three RILs and their parents were used with the same experimental design as explained for basal stem inoculation. Seeds were sown in Magenta boxes (one plantlet per box) containing Murashige and Skoog medium (4.4 g l ^{1}), gelified by 2.8 g l ^{1} Phytagel (Sigma). Experiments were conducted in a growth chamber (26 ± 1 °C days, 19 ± 1 °C night) with 75 80% relative humidity and 14 h photoperiod with 200 μEm- ^{2} s ^{1} light intensity provided by NAV-T 600W lamps Osram-Vialox, Molsheim, France. Twelve-day-old plantlets were inoculated by 5 μl of a pycniospore suspension (10^6 pycniospores per ml of water containing 0.25% gelatin) added to the medium near the root using a syringe. The development of symptoms was scored on a 1 9 scale for the length of necrotic area seven days after inoculation, where: 1 = 0 0.5 cm, 2 = 0.6 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, more than 4.7 cm necrosis area on the roots.

2.4. Data analysis and QTL mapping

Analysis of variance was carried out with SAS package (SAS Institute Inc. North Carolina, USA) [16]. The most resistant RIL to each isolate (lowest value) was compared with the most resistant parent (lowest value) and the mean of 10% selected RILs (the mean of 10% RILs with low values) was compared with the mean of their parents. Additive and environmental variances as well as narrow-sense heritability were calculated according to Kearsey and Pooni [17] using least-square estimates of genetic parameters.

The linkage map used in this study is the improved map presented recently by Poormohammad Kiani et al. [18]. In summary, using a set of 123 RILs coming from the cross between ‘PAC2’ and ‘RHA266’, a linkage map with 191 SSR

Table 1
Genetic variability for partial resistance to *Phoma macdonaldii* basal stem necrosis isolates (TA6 and TA9) and root necrosis isolates (TA2 and TA4) in sunflower recombinant inbred lines (RILs)

Parameter	Basal stem		Root	
	'TA6'	'TA9'	'TA2'	'TA4'
'PAC2' (P1)	1.92	7.69	6.67	2.66
'RHA266' (P2)	5.76	4.48	3.00	7.00
P1 P2	3.48^{**}	3.21^{**}	3.67^{**}	4.44^{**}
$\bar{X}$P (P1 + P2)/2	3.84	6.08	4.83	4.33
Example of RILs				
C81 (partial resistant to four isolates)	1.37	2.16	4.00	4.00
C137 (partial resistant to four isolates)	1.27	2.05	3.00	4.33
C75 (partial resistant to three isolates)	2.34	7.67	2.00	2.00
C150 (susceptible to four isolates)	7.32	8.83	9.00	5.67
BRIL	1.26	1.80	2.00	2.10
BRIL BP	0.66^{**}	2.68^{**}	1.00^{**}	0.56^{ns}
10% SRILs	1.47	2.13	2.12	2.20
10% SRILs $\bar{X}$P	2.37^{**}	3.95^{**}	2.71^{**}	2.13^{**}
$LSD_{0.05}$	0.51	0.92	0.67	1.09
Coefficient of variation (%)	6.11	10.40	9.96	13.04
h^2	0.48	0.46	0.46	0.44

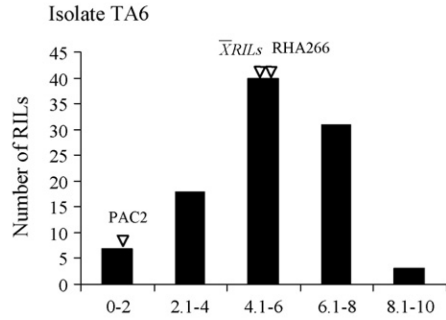
'PAC2' (P1) and 'RHA266' (P2): parental lines. $\bar{X}$P: The mean of two parental lines. BRIL: The best RIL. BP: The best parent. 10% SRILs: The mean of 10% selected RILs. $LSD_{0.05}$: Least significant differences calculated using $t_{0.05}$ and error mean square of each trait. The double asterisks (**) and ns: Significant at 0.01 probability level and non significant. h^2: Narrow sense heritability.

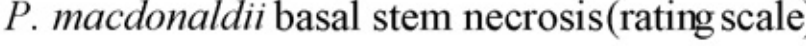
and 304 AFLP markers with the mean density of 3.7 cM per locus was constructed. The linkage groups were numbered according to sunflower reference map [19] and presumably correspond to one of the 17 chromosomes in the haploid sunflower genome ($x = 17$).

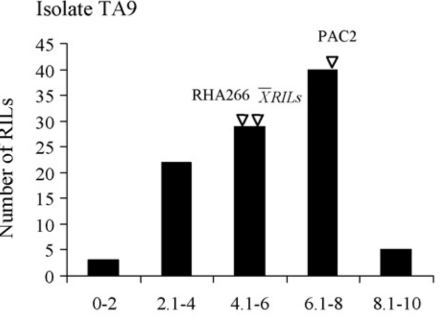
QTL mapping of the studied traits were performed by composite interval mapping (CIM) of QTL Cartographer 1.16 [20] with model 6 of Zmapqtl and a LOD score threshold of 3.0. This model integrated two parameters for CIM: the number of markers which control the genetic background ($n_m = 15$) and a window size ($w = 15$) that will block out a region of the genome on either side of the markers flanking the test site. The inclusion of background markers makes the analysis more sensitive to the presence of a QTL in the target interval. QTL results of different isolates were compared on the basis of overlapping support intervals: a decrease in the LOD score of 1.0 determined the end point of the support interval for each QTL. Additive effects of the detected QTLs, the percentage of phenotypic variation explained by each QTL (R^2), as well as the total phenotypic variation explained in the model of composite interval mapping (TR^2) were estimated using the Zmapqtl programme of QTL Cartographer [20].

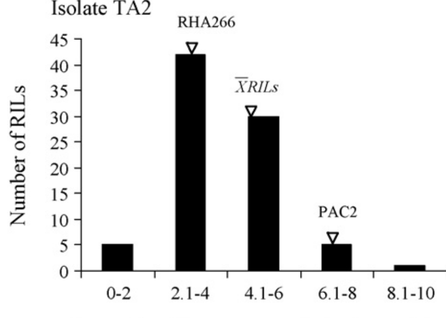
3. Results

Phenotypic values of RILs and their parents 'PAC2' and 'RHA266' for partial resistance to *Phoma macdonaldii* isolates are presented in Table 1. Significant differences were observed between parents for partial resistance to all four isolates. Parental lines showed contrasting levels of partial resistance to









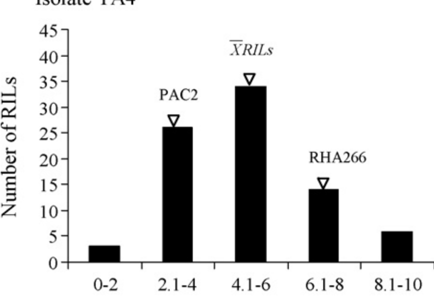


Fig. 1. Frequency distribution of phenotypes for resistance to *Phoma macdonaldii* isolates on basal stem ('TA6' and 'TA9') and root ('TA2' and 'TA4') in recombinant inbred lines (RILs). Phenotypic values of parental lines (PAC2 and RHA266) and mean of the population ($\bar{X}$RILs) are shown by *arrows*.

Table 2
Map positions and effects of QTLs detected for partial resistance to *Phoma macdonaldii* basal stem and root necrosis isolates in sunflower recombinant inbred lines (RILs)

Organ	QTL	Linkage group	Marker	Position (cM)[a]	LOD	Additive effect	R^{2b}	TR^{2c}
Basal stem	'TA6'							
	psnTA6.1.1	LG1	E32M61 10	42.90	3.11	0.587	0.07	0.62
	psnTA6.5.1	LG5	**E32M49 23**	100.21	7.63	0.889	0.15	0.88
	psnTA6.6.1	LG6	**E33M48 26**	71.66	3.13	0.797	0.18	0.78
	psnTA6.7.1	LG7	ORS331 2	0.01	6.69	0.633	0.09	0.84
	psnTA6.9.1	LG9	ORS805	0.01	8.17	0.733	0.13	0.82
	psnTA6.10.1	LG10	SSL133	6.92	6.75	0.492	0.16	0.85
	psnTA6.10.2	LG10	E37M49 3	126.96	7.67	0.971	0.17	0.85
	psnTA6.15.1	LG15	HA3102	26.23	4.62	0.930	0.09	0.76
	'TA9'							
	psnTA9.1.1	LG1	E33M62 11	47.76	4.13	0.674	0.10	0.73
	psnTA9.2.1	LG2	E35M60 23	28.86	4.20	0.920	0.15	0.65
	psnTA9.6.1	LG6	**E33M48 26**	77.66	7.91	1.090	0.29	0.91
	psnTA9.8.1	LG8	**HA3278**	109.11	3.73	0.620	0.08	0.69
	psnTA9.12.1	LG12	**HA3555**	59.11	6.85	0.780	0.25	0.67
	psnTA9.13.1	LG13	**E33M48 20**	24.46	5.74	1.070	0.27	0.89
Root	'TA2'							
	prnTA2.5.1	LG5	**E32M49 23**	100.21	8.27	0.789	0.19	0.88
	prnTA2.6.1	LG6	**E33M48 26**	79.66	4.24	0.435	0.20	0.92
	prnTA2.12.1	LG12	**HA3555**	59.11	6.44	0.359	0.12	0.77
	prnTA2.13.1	LG13	**E33M48 20**	28.86	7.62	0.533	0.24	0.88
	prnTA2.15.1	LG15	**E38M48 4**	75.16	5.61	0.328	0.10	0.77
	prnTA2.16.1	LG16	SSL22 1	99.00	6.65	0.340	0.12	0.78
	prnTA2.17.1	LG17	E33M50 4	91.81	5.81	0.367	0.12	0.78
	'TA4'							
	prnTA4.4.1	LG4	E41M62 6	0.01	11.16	0.594	0.22	0.79
	prnTA4.5.1	LG5	ORS1024 2	64.41	3.71	0.825	0.15	0.60
	prnTA4.8.1	LG8	**HA3278**	113.11	4.80	0.463	0.11	0.77
	prnTA4.10.1	LG10	E35M62 3	141.71	4.91	0.595	0.09	0.79
	prnTA4.12.1	LG12	**HA3555**	59.11	3.95	0.328	0.07	0.78
	prnTA4.15.1	LG15	**E38M48 4**	75.16	6.49	0.376	0.11	0.77

The markers linked to QTL common between different isolates are presented as *bold face*.
[a] From the north of linkage group.
[b] Percentage of phenotypic variance explained by each QTL (QTL Cartographer, Version 1.16, Basten et al. [20]).
[c] Percentage of phenotypic variance explained by the QTLs given all the covariants (QTL cartographer, Version 1.16, Basten et al. [20]).

different isolates. 'PAC2' presented a significantly higher level of partial resistance to basal stem isolate 'TA6' (1.92) and root isolate 'TA4' (2.66). By contrast, 'RHA266' showed a significantly higher partial resistance to basal stem isolate 'TA9' (4.48) and root isolate 'TA2' (3.00). Some RILs presented high partial resistance to all isolates, and some others were resistant to three or two out of four isolates. In contrast, genotypes susceptible to all isolates were also observed. For example, C81 and C137 RILs are partially resistant to all four isolates and C75 is resistant to three out of four isolates. By contrast, the C150 genotype was susceptible to all four isolates (Table 1). Comparison between the best parent (lowest value) and the best RIL (lowest value) as well as between the mean of 10% selected RILs with lowest values (10% SRILs) and the mean of parents ($\bar{X}$P) showed transgressive segregation for partial resistance to all basal stem and root necrosis isolates.

Fig. 1 shows the frequency distributions of resistance to different basal stem and root necrosis isolates. The 'TA9' basal stem necrosis isolate is probably the most aggressive isolate considering the phenotypic value of 'PAC2' (7.69) and 'RHA266' (4.48) and its high RILs population mean ($\bar{X}$RILs = 5.46). In addition, 40% of RILs presented a note from 6.1 to 8 for this isolate. Transgressive RILs, which are more susceptible and/or more resistant than their parents, were observed for partial resistance to all four isolates.

Quantitative trait loci (QTL) associated with resistance to different Phoma isolates are presented in Table 2. QTLs are designated as phoma basal stem necrosis and phoma root necrosis ('*psn*' and '*prn*', respectively) followed by the isolate name, linkage group and QTL number, respectively. For an easier overview of overlapping QTL between isolates, images of linkage groups having QTL regions are presented in Fig. 2. A decrease in the LOD score of 1.0 determined the end point of support interval for each QTL.

A total of 27 QTL were detected for partial resistance to four isolates (Table 2). The number of QTL detected was from six to eight for each isolate (Table 2 and Fig. 2). QTLs involved in resistance to basal stem necrosis isolate 'TA6' are located on linkage groups (LGs) 1, 5, 6, 7, 9, 10, 15; and those for resistance to 'TA9' are located on linkage groups 1, 2, 6, 8, 12

and 13. As far as Phoma root necrosis is concerned, QTLs involved in resistance to 'TA2' are located on linkage groups 5, 6, 12, 13, 15, 16, 17 and those of resistance to 'TA4' are located on linkage groups 4, 5, 8, 10, 12 and 15. The percentages of phenotypic variation explained by each QTL (R^2) ranged from 7 to 29%, whereas a high percentage (60 92%) of total phenotypic variance (TR^2) is explained for partial resistance to different isolates when considering all the covariants in composite interval mapping. The sign of additive gene effects showed that the favorable alleles come from both parental lines. For QTLs detected for partial resistance to basal stem necrosis isolates ('TA6' and 'TA9'), favorable alleles come from 'RHA266' and 'PAC2' equally (seven QTL for each parents); whereas for QTLs controlling partial resistance to root necrosis isolates ('TA2' and 'TA4') 'RHA266' provided more favorable alleles than 'PAC2' (Table 2).

4. Discussion

The RILs studied in our experiment presented high genetic variation for partial resistance to *Phoma macdonaldii* isolates on basal stem and root (Table 1). Genetic variability for partial resistance to Phoma black stem has been previously reported in both field and controlled conditions [10,11]. Each parent has partial resistance to two out of four isolates whereas some RILs are resistant to all four isolates or three out of four isolates, indicating the importance of recombination as a source of resistance to the diseases (Table 1). Significant differences between the best RIL (BRIL) and the best parent (BP) as well as between 10% selected RILs and the mean of parents, are evidence for transgressive segregation of resistance to *Phoma macdonaldii* (Table 1 and Fig. 1). The positive and negative signs of additive gene effect at the different loci (Table 2) indicate the contribution of both parental lines and confirm the transgressive segregation observed at the phenotypic level. Trangressive segregation has previously been reported by Rachid Al-Chaarani et al. [10] for partial resistance to black stem and downy mildew in sunflower. The method of evaluation of partial resistance to basal stem necrosis isolates developed in our department has been tested under field conditions by the French Technical Centre for Oil Seed Crops (CETIOM). Preliminary experiments showed identical reactions in adult plants in the field and test plantlets in some sunflower genotypes. Root contamination of sunflower varieties by the pathogen is also observed by 'CETIOM' in field conditions but artificial contamination as we have presented in plantlets is not applied.

Narrow-sense heritability for partial resistance to different isolates on basal stem and root varied from 0.44 to 0.48, which is close to 0.58 presented by Rachid Al-Chaarani et al. [10].

Regarding the position of QTL involved in partial resistance to basal stem and root necrosis isolates, in several cases overlapping QTLs were found for resistance to different isolates of each tissue and also across tissues (Table 2 and Fig. 2). This demonstrates that partial resistance to *Phoma macdonaldii* is governed by both shared loci (isolate-non-specific) and 'isolate-specific' loci. One QTL detected for basal stem necrosis isolate 'TA6' (*psnTA6.6.1*) is overlapped with a

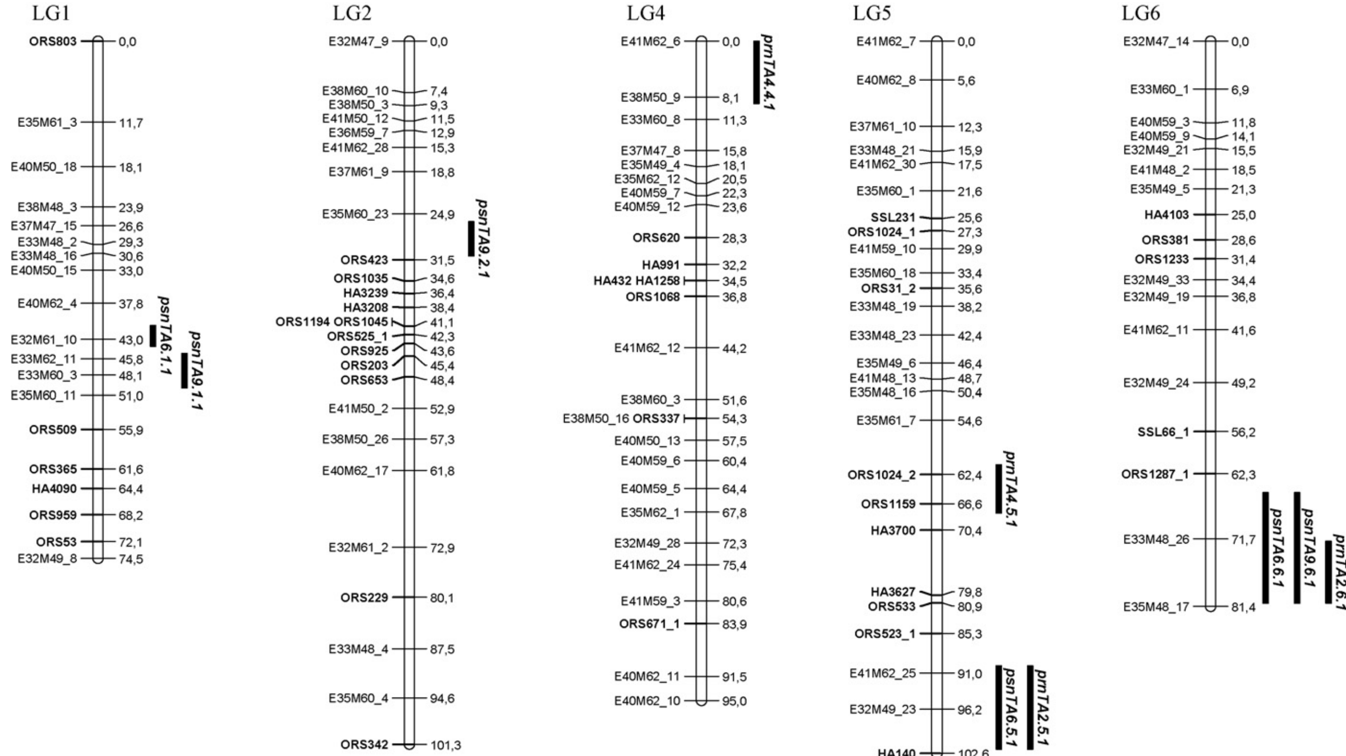


Fig. 2. Genetic linkage map of sunflower based on 123 RILs derived from cross 'PAC2' × 'RHA266' for 495 marker loci defined by Poormohammad Kiani et al. [18]. The linkage groups (LG) including QTLs are presented. Markers in *bold* characters are SSRs and the rest are AFLPs. The position of QTLs involved in resistance to basal stem necrosis isolates (TA6 and TA9) and root necrosis isolates (TA2 and TA4) are presented by vertical bars. Distances (right) are given in Kosambi cM.

Fig. 2. (*Continued*)

QTL of another basal stem necrosis isolate 'TA9' (*psnTA9.6.1*) and with a QTL of root necrosis isolate 'TA2' (*prnTA2.6.1*) on linkage group 6 (Fig. 2). This overlapping QTL is very important as it explain 18, 29 and 20% of phenotypic variance of partial resistance to basal stem necrosis isolates 'TA6' and 'TA9' and root necrosis isolate 'TA2', respectively (Table 2). Favorable alleles for this QTL come from 'RHA266'. Besides this QTL, other overlapping QTLs which are involved in partial resistance to three isolates 'TA9', 'TA2' and 'TA4', located on linkage group 12 (*psnTA9.12.1*, *prnTA2.12.1* and *prnTA4.12.1*), explain 25, 12 and 7% of phenotypic variance of partial resistance to these three isolates, respectively (Table 2 and Fig. 2). Favorable alleles for '*psnTA9.12.1*' come from 'PAC2' but for two others (*prnTA2.12.1* and *prnTA4.12.1*) they come from 'RHA266'. Additional QTLs controlling partial resistance to different isolates are overlapped on: linkage group 5 for resistance to 'TA6' and 'TA2' (R^2 = 15 and 19%), linkage group 8 for resistance to 'TA9' and 'TA4' (R^2 = 8 and 11%), linkage group 13 for resistance to 'TA9' and 'TA2' (R^2 = 27 and 24%), and finally linkage group 15 for resistance to 'TA2' and 'TA4' (R^2 = 10 and 11%) (Table 2 and Fig. 2). These overlapped QTLs are 'isolate-non-specific', which indicates the involvement of the same genomic regions in partial resistance to different isolates on basal stem and root. The

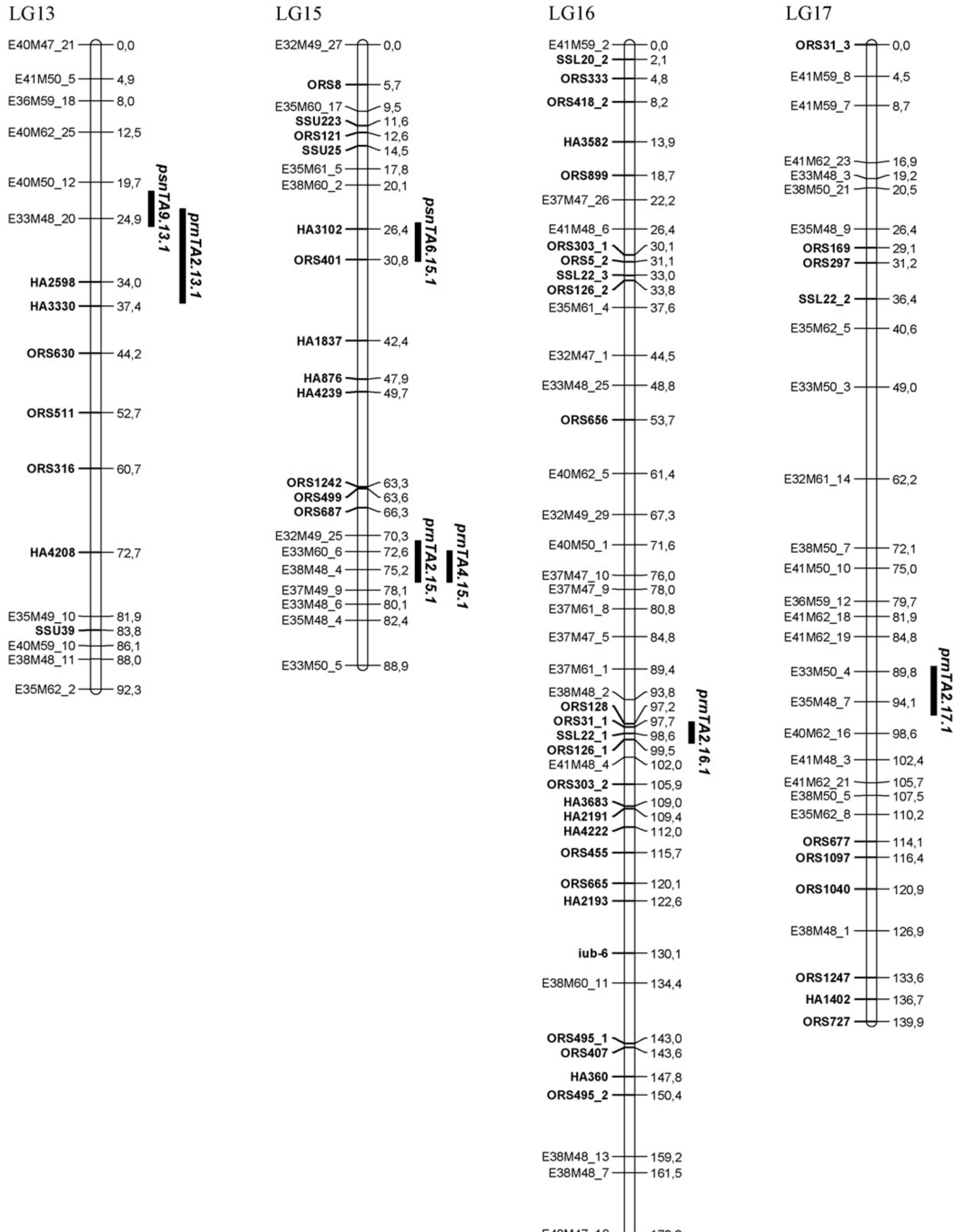


Fig. 2. (*Continued*).

'isolate-non-specific' QTL could be important in general defence mechanisms against different isolates. Above-mentioned overlapped QTL located on linkage groups 6, 12 and 13, are the most important DNA regions, as they confer resistance at two tissues and explain a high level of phenotypic variance (Table 2 and Fig. 2). It is of interest that most QTL common to the different isolates are those with the highest effect in term of phenotypic variance (R^2). Arru et al. [21] reported both common and specific QTL for partial resistance to leaf stripe in barley. They found that common QTL to the different isolates are those with highest effects on resistance. Caranta et al. [22] reported a single major QTL acting against the three potyvirus strains tested.

A total of 13 'isolate-specific' QTL were also detected for partial resistance to four isolates. There were six for partial resistance to 'TA6' on linkage groups 1, 7, 9, 10 and 15; two for partial resistance to 'TA9' on linkage groups 1 and 2; two for partial resistance to 'TA2' on linkage groups 16 and 17; and finally three for partial resistance to 'TA4' on linkage groups 4, 5 and 10. As far as we know, 'isolate-specificity' or 'isolate-non-specificity' of QTL has not been reported for partial resistance to *Phoma macdonaldii*. So far, two studies have been

undertaken to map QTL controlling partial resistance to Phoma black stem in sunflower [10,12]. In both studies only one French isolate (MP6) has been used and disease severity has been evaluated on cotyledon petioles of seedlings. In the first study, using an AFLP map (331 markers) with the RILs from the same cross as reported here, Rachid Al-Chaarani et al. [10] detected seven QTL for partial resistance to Phoma black stem with moderate phenotypic variance (R^2) ranging from 6 to 17% on linkage groups 3, 4, 8, 9, 11, 15 and 17, which correspond to linkage groups 1, 7, 16, 10, 14, 17 and 8 in our new improved map presented here. Our QTL controlling partial resistance to basal stem and root necrosis isolates are on the same linkage groups except LG14 as QTL reported by Rachid Al-Chaarani et al. [10] for black stem isolates. However, the QTLs are detected in different regions, which suggest isolate-specificity for QTL of Phoma. In another study, using an RFLP/AFLP map of F3 families, Bert et al. [12] reported four QTLs for resistance of sunflower to black stem on linkage groups 1, 3, 15 and 17. However, the lack of SSR markers and common linkage group nomenclature in their map makes it difficult to compare the location of QTL detected in our study and those detected by Bert et al. [12]. The incorporation of 191 public SSR markers and the standard linkage group nomenclature in our new map will permit the cross-reference of markers and QTL mapped in other research works. In sunflower, candidate genes for downy mildew [23,24] and QTL for resistance to *S. sclerotiorum* [12 14,25 27] have been also identified. Cross-referencing QTL detected here with QTL controlling resistance to *S. sclerotiorum* reported by Micic et al. [27], showed that the 'ORS31' SSR flanking marker of '*prnTA2.17.1*' controlling partial resistance to root necrosis isolate 'TA2' in our study is linked to two QTL for resistance to *Sclerotinia*.

The use of different isolates allowed us to analyze the specificity of the quantitative resistance to *Phoma macdonaldii* basal stem and root necrosis isolates. Both isolate-specific and isolate-non-specific QTLs were detected for partial resistance to the isolates. Isolate-specific QTLs have been described in other plant-pathogen models, and have been implicated in both partial resistance [21,28] and overall resistance [29], leading to the hypothesis of specific interaction between regions of the genome and specific determinants of the pathogen isolates. According to Rocherieux et al. [30], several hypotheses have been addressed for the meaning of effect and specificity variation of QTLs detected with different isolates: (1) a QTL could be a cluster of resistance genes either belonging or not to the same multigene family, specific to different plant pathogens and/or to different races of the same pathogen [31]; (2) a single gene at the QTL could carry alleles that differ for their quantitative effect; (3) a single gene could also operate in different signaling pathways leading to the resistance. The resistance response implicates a great number of genes that could be interconnected and be required in different signaling pathways [32]. Thus, QTLs detected with different races of a pathogen or with different pathogens could overlap [13,33]. It is difficult to conclude whether putative QTLs in the same genomic region involved just one genetic factor or were the result of linkage between several of them.

Accumulation of QTL for partial resistance in breeding programmes is important to improve crops in modern agriculture, especially in an organic one. The markers such as HA3555 on LG12 and E33M48 26 on LG6 as well as E33M48 20 on LG13 which are each linked to multiple QTL for partial resistance to different basal stem and root necrosis isolates could be used in marker-assisted selection to introduce tolerance to four *Phoma macdonaldii* isolates into elite sunflower breeding lines. Furthermore, SSR markers for individual QTL such as ORS1024 2 (LG5), SSL22 1 (LG16), SSL133 (LG10), and ORS805 (LG9) are also useful for accumulation of QTL for resistance to individual isolates. Breeding sunflower resistant to Phoma black stem is complicated by the fact that the pathogen can attack several parts of the plant and the level of resistance may be different for each part [34]. Pyramiding 'isolate-non-specific' QTL with 'isolate-specific' ones could increase the level of resistance to a wide range of isolates and the likelihood of resistance durability.

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