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Quantitative trait loci for partial resistance to Aphanomyces root rot in pea

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Abstract Aphanomyces root rot, caused by *Aphanomyces euteiches* Drechs, is the most-important disease of pea (*Pisum sativum* L.) worldwide. No efficient chemicals are available to control the pathogen. To facilitate breeding for Aphanomyces root rot resistance and to better understand the inheritance of partial resistance, our goal was to identify QTLs associated with field partial resistance. A population of 127 RILs from the cross Puget (susceptible) × 90-2079 (partially resistant) was used. The lines were assessed for resistance to *A. euteiches* under field conditions at two locations in the United States (Pullman, Wash. and LeSueur, Minn.) in 1996 and 1998 for three criteria based on symptom intensity and disease effects on the whole plant. The RILs were genotyped using automated AFLPs, RAPDs, SSRs, ISSRs, STSs, isozymes and morphological markers. The resulting genetic map consisted of 324 linked markers distributed over 13 linkage groups covering 1,094 cM (Kosambi). Twenty seven markers were anchored to other published pea genetic maps. A total of seven genomic regions were associated with Aphanomyces root rot resistance. The first one, located on LG IVb and named *Aph1*, was considered as "major" since it was highly consistent over the years, locations and resistance criteria studied, and it explained up to 47% of the variation in the 1998 Minnesota trial. Two other year-specific QTLs, namely *Aph2* and *Aph3*, were revealed from different scoring criteria on LG V and Ia, respectively. *Aph2* and *Aph3* mapped near the *r* (wrinkled/round seeds) and *af* (normal/afila leaves) genes, and accounted for up to 32% and 11% of the variation, respectively. Four other "minor" QTLs, identified on LG Ib, VII and B, were specific to one environment and one resistance criterion. The resistance alleles of *Aph3* and the two "minor" QTLs on LG Ib were derived from the susceptible parent. Flanking markers for the major Aphanomyces resistance QTL, *Aph1*, have been identified for use in marker-assisted selection to improve breeding efficiency.

Keywords *Pisum sativum* · *Aphanomyces euteiches* · Partial resistance · QTL mapping · Automated AFLP

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Introduction

Aphanomyces root rot, caused by *Aphanomyces euteiches* Drechs. f.sp. *pisi* W.F. Pfender & D.J. Hagedorn, is one of the most destructive diseases of peas (*Pisum sativum* L.) worldwide. It was first reported in the 1920s in Wisconsin, United States, and has been widely observed in many pea-growing areas in North America, Australia, New Zealand, Japan and Europe (Kraft and Pfleger 2001). *A. euteiches* can attack peas at any stage of plant development, causing rotting of the roots and epicotyls that result in stunted seedlings, yellow leaves or dead plants. It can be responsible for serious damage and entire fields may be lost under conditions of severe disease development.

No effective fungicides are currently available to control the disease. Several cultural and prophylactic methods of disease management are recommended to prevent losses and include: (1) avoidance of pea crops in moder-

ately to highly infested fields; prediction tests have been developed to identify the pathogen in infested soils and assess the root rot potential of soils (Kraft et al. 1990; Williams-Woodward et al. 1998; Vandemark et al. 2000); (2) prevention of inoculum buildup through the use of long-term rotations and growing of nonhost crops between pea crops, and (3) avoidance of contamination in uninfested fields through the transport of infected farming tools or water streaming.

Genetic control will probably become a key component in an integrated strategy for Aphanomyces root rot control. Since the 1950s, breeding pea varieties with resistance to Aphanomyces root rot has been difficult due to: (1) the polygenic inheritance of resistance and low heritability (Shehata et al. 1983; Lewis and Gritton 1992); (2) the availability of only partial resistance; (3) variability in disease reaction tests (Papavizas and Ayers 1974; Rao et al. 1995) and in the pathogen (Malvick and Percich 1998), and (4) interactions with other root-invading pathogens under field conditions (Papavizas and Ayers 1974). Despite these difficulties, breeding lines with improved partial resistance to Aphanomyces root rot were developed using recurrent selection-based strategies. Lewis and Gritton (1992) suggested that recurrent selection would be an effective strategy to accumulate favorable factors for resistance to *A. euteiches* and to break linkages between Aphanomyces resistance and undesirable traits (Marx et al. 1972). In two cycles of phenotypic recurrent selection, they demonstrated substantial gains of dry seed yield and plant survival in F_2 populations assessed under highly root rot-infested field conditions. Kraft (1988) reported that resistance to *A. euteiches* was incorporated into breeding lines with desirable horticultural traits, which suggested that unfavorable linkages with genes for resistance were overcome. In the last decade, commercial type pea germ plasm with partial resistance or tolerance to *A. euteiches* has been released in the United States. These releases include 90-2079, 90-2131, 96-2052, 97-261, 97-363 (Kraft 1992; Kraft and Coffman 2000a, b, c), Wis 8901 to Wis 8905 (Gritton 1990), MN313 and MN314 (Davis et al. 1995).

The development of molecular markers in the 1990s offers a new approach for improving partial resistance to *A. euteiches* and combining it with favorable agronomic traits. Molecular markers are of great interest for dissecting quantitative resistance by identifying associated genomic regions or QTLs (Quantitative Trait Loci) (Young 1996). At the present time, little is known about genetic factors controlling the quantitative partial resistance to *A. euteiches*. Coyne et al. (1997) identified one major QTL that explained 28% of the resistance variation. However, the QTL linkage group was not anchored to published pea maps. Weeden et al. (2000) reported that a gene in MN313 had a significant influence on the expression of tolerance to Aphanomyces root rot in the field at only one location in the United States. Molecular markers are also useful tools for transferring genetic factors using marker-assisted selection (MAS) (Lee 1995; Mohan et al. 1997; Stuber et al. 1999; Young 1999). In breeding for Aphanomyces root rot resistance, molecular markers could be particularly helpful for increasing the degree of resistance by accumulating different positive alleles and for breaking undesirable linkages with the resistance.

In order to better understand the genetics of quantitative resistance to *A. euteiches* and to assist breeding efforts for resistance, the objectives of this study were to: (1) identify QTLs for Aphanomyces root rot resistance in the field, and (2) assess QTL consistency for several resistance criteria over different environments in the United States. We used a mapping population of recombinant inbred lines (RILs), which allowed for replicated trials at several environments. One of the two parents of the RILs was the line 90-2079 (Kraft 1992), which is partially resistant to *A. euteiches* in the United States (Washington, Minnesota, Wisconsin) and New Zealand (Kraft 1992).

Materials and methods

Plant material

A total of 127 RILs, developed by single-seed descent from the cross Puget × 90-2079 were used for QTL mapping. Puget (also designated as PI 608010 or JI 2434), selected as the susceptible parent, is a freezer-type spring pea cultivar with normal leaves, white flowers and wrinkled seeds. 90-2079 (Kraft 1992) (PI 557500), selected as the partially resistant parent, is a germplasm line with "afila" leaves, white flowers and round seeds. The two parental lines were chosen to flower at similar nodes (15th to 16th) in order to avoid possible bias during scoring for resistance in the derived RILs population due to maturity differences.

For field-disease trials, progenitors of released pea germplasm described as tolerant or resistant to *A. euteiches* were used as controls and included PI 180693 (Lockwood 1960), PH-14-119 (Kraft et al. 1972), 79-2022 (Kraft 1981), 86-2236 (Kraft 1989) and 90-2131 (Kraft 1992). Susceptible lines used as controls included Little Marvel, Dark Skin Perfection, Alaska-81, WSU-28 and M410.

Field experiments

The RILs and controls were tested for Aphanomyces root rot resistance in field disease nurseries in 1996 (F_5-derived RILs) and 1998 (F_{10}-derived RILs), at two locations in the United States (Spillman Farm, Pullman, Wash., and Pinney Farm, LeSueur, Minn.). At Pullman, the disease nursery was established in 1986 by addition of soil taken from an infested field near Potlatch, Idaho. The LeSueur nursery was established in 1970 on a farm with a long history of pea growing and a high level of soil-borne pathogens. Since the establishment of each nursery, peas have been grown continuously for evaluation of soil-borne disease tolerance. At Pullman in 1998, RILs and controls were grown both in a disease nursery and in a non-disease nursery, in order to compare the effects of the disease on biomass production. In both disease and non-disease nurseries, plantings were carried out using a randomized complete block design with three replications, each plot had approximately 20 plants in 1996 and 35 plants in 1998. The parental and control cultivars were replicated six times, with two plots in each of three replicates. Seeds were treated at planting against *Pythium*.

Three disease criteria were used to assess the resistance:

1. Root rot index (RRI) at the flat pod stage determined by digging ten plants per plot, washing the roots in water and rating diseased roots and epicotyl using a 1 to 5 scale as described in Rao et al. (1995). A mean root rot index was calculated for each plot.

2. Above ground index (AGI) at the flat pod stage, evaluated on each whole plot using a 1 to 5 scoring scale (1 = healthy plants,

Table 1 Coding of the AFLP primer combinations employed

Primer	M-CAA[a]	M-CAC	M-CAG	M-CAT	M-CTA	M-CTC	M-CTG	M-CTT
E-AAG[b]	E1M1	E1M2	E1M3	E1M4	E1M5	E1M6	E1M7	E1M8
E-ACC	E2M1	E2M2	E2M3	E2M4	E2M5	E2M6	E2M7	E2M8
E-ACT	E3M1	E3M2	E3M3	E3M4	E3M5	E3M6	E3M7	E3M8
E-AGC	E4M1	E4M2	E4M3	E4M4	E4M5	E4M6	E4M7	E4M8
E-AAC	E5M1	E5M2	E5M3	E5M4	E5M5	E5M6	E5M7	E5M8
E-ACA	E6M1	E6M2	E6M3	E6M4	E6M5	E6M6	E6M7	E6M8
E-ACG	E7M1	E7M2	E7M3	E7M4	E7M5	E7M6	E7M7	E7M8
E-AGG	E8M1	E8M2	E8M3	E8M4	E8M5	E8M6	E8M7	E8M8

[a] M indicates *Mse*I primer 5′-GAT GAG TCC TGA GTA A+CNN-3′
[b] E indicates *Eco*RI primer 5′-GAC TGC GTA CCA ATT C+ANN-3′

2 = slight yellowing of lower leaves, 3 = necrosis of the lower leaves up to the 3rd or 4th node, some stunting, a few dead plants, 4 = necrosis of at least half or more of the plants with stunting, more than half of the row dead, 5 = all plants dead or nearly so).

3. Percentage of dried weight losses (DWL) per plant at the flat pod stage, by drying and weighing ten plants per plot grown in both disease and non-disease nurseries.

Both AGI, RRI and DWL were assessed at Pullman in 1998 whereas only RRI was evaluated at Pullman in 1996 and AGI at LeSueur in 1996 and 1998.

Genotyping with molecular markers

The F_{10}-derived RILs and parental lines were genotyped using AFLP (Amplified Fragment Length Polymorphism) markers, RAPD (Random Amplified Polymorphic DNA), SSRs (Simple Sequence Repeats), ISSRs (Inter Simple Sequence Repeats), STSs (Sequence Tagged Sites), isozymes and genes for morphological traits (afila/normal leaves, round/wrinkled seeds).

DNA was extracted from 1 g of leaf tissue following the modified CTAB method of Murray and Thompson (1980).

AFLP reactions were conducted using a modified protocol of Vos et al. (1995), as described in Qiu et al. (1999), with *Mse*I and *Eco*RI restriction enzymes and adapters. In the final amplification, *Eco*RI plus three selective nucleotide primers were labeled with infrared dye IRD 700 or IRD 800 (Li-Cor, Lincoln, Neb., USA). Polymorphism was detected with a two-dye Li-Cor IR^2 4200 automated DNA sequencer. Electrophoresis on 25-cm plates and analysis of AFLP fragments using RFLPscan software (version 3.0, Scanalytics) were carried out as reported in Remington et al. (1999). Out of 64 *Eco*RI-ANN/*Mse*I-CNN primer pairs tested for parental polymorphism, 29 were selected for genotyping the RILs, based on the quality of fingerprint patterns and the number of scoreable polymorphic markers produced (Coyne et al. 2000a). Each AFLP marker generated was named using a code for each *Eco*RI-ANN and *Mse*I-CNN primer (Table 1), followed by the length of the fragment (in base pairs) estimated at single-base resolution by RFLPscan software.

RAPD genotyping was carried out using two sets of markers. The first one included markers generated by 49 primers from University of British Columbia (UBC, Vancouver, Canada) and 4 primers from Sigma (St-Louis, MO, US). The amplification procedure was similar to that described in Williams et al. (1990), using the following PCR profile: 40 cycles of 1 min at 94 °C, 20 sec at 36 °C, 1 min at 72 °C, final extension cycle of 8 min at 72 °C. Amplification products were electrophoresised in 2% agarose gels, then visualized with ethidium bromide stain and transmitted UV light. Nomenclature for these markers was composed of the letter U or CS (for UBC or Sigma primers, respectively), followed by the primer number then the size of the amplified fragment. The second set of RAPD markers comprised fragments amplified from 15 Operon primers (Operon Technologies, Alameda, CA, USA) as described in Laucou et al. (1998). These markers were named using the Operon primer name (kit letter + primer number) followed by the length of the fragment (in base pairs).

ISSR markers were also generated from UBC primers, as described for UBC RAPD primers, with the following PCR profile: 40 cycles of 30 sec at 94 °C, 30 sec at 55 °C, 30 sec at 72 °C, final extension cycle of 8 min at 72 °C. They were designated as U followed by the primer number.

Pea SSR primers, developed by Agrogène Company (Moissy Cramayel, France), were used to amplify markers following the protocol presented in Burstin et al. (2001). Each marker was named PSMPSAA followed by the primer number.

STS markers were generated using primers developed from sequence-characterized pea cDNA by Gilpin et al. (1997) and from cloned genes by Weeden et al. (2001a). STS assays were carried out as reported by the respective authors.

Isozyme analyses were conducted using starch gel electrophoresis according to Wenzel and Weeden (1989).

Genetic map construction

For each segregating marker, a chi-square analysis (α =1%) was used to test for deviations from the expected Mendelian ratios (1:1). Markers that did not fit the expected ratios at the threshold of 0.1% were excluded from the first linkage analysis. The genetic map was constructed using MAPMAKER/EXP v3.0 software (Lincoln et al. 1992). The RIL (ri self) genetic model, minimum LOD (Logarithm of likelihood ratio) score of 4.0, Kosambi genetic distances estimation and maximum recombination of 30% were used for map elaboration. Linkage groups and marker orders were established using the 'group', 'order', 'compare' and 'try' commands, then verified using the 'ripple' and 'pair' commands. After mapping, the 'error detection' option of MAPMAKER was used. Possible errors (single loci flanked by double crossovers) were examined again by rereading the molecular marker patterns and replacing some original scorings with missing data or removing problematic markers from the analysis.

Field data analysis

Analysis of field data for each resistance criterion within each year, location and nursery, as well as analysis of genotype × environment interactions for AGI and RRI resistance criteria, were performed using the generalized linear model (PROC GLM) of SAS package (SAS Institute 1988). For each resistance criterion assessed in one experiment, differences among RILs and replicates were tested using the following analysis of variance (ANOVA) model: $P_{ij} = \mu + G_i + R_j + e_{ij}$, where P_{ij} is the score of the i^{th} RIL located in the j^{th} replicate, μ the mean of all the data, G_i the i^{th} RIL effect, R_j the j^{th} replicate effect and e_{ij} the residual. The assumption of homogeneity of variances by genotype and replicate was tested with Bartlett's test (Snedecor and Cochran 1980), carried out with HOVTEST option in MEANS statement of PROC GLM. Normality of residual distribution was checked by the PROC UNIVARIATE procedure (Skewness, Kurtosis, Shapiro-Wilk statistics) (Shapiro and Wilk 1965). RILs adjusted means were estimated from

ANOVA (/SOLUTION option of GLM procedure) and used for QTL analysis.

Percentages of dried weight losses (weights from disease/non-disease nurseries) and Pearson correlation coefficients between resistance criteria, years and locations (PROC CORR procedure) were calculated from adjusted means.

Mean-based heritability (h^2) was calculated from ANOVA (1) for each resistance criterion in one environment and (2) for RRI and AGI across several environments (year or location), using the formulas: (1) $h^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_e^2/r)]$ and (2) $h^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_{GE}^2/E) + (\sigma_e^2/rE)]$, respectively, with σ_G^2 the genetic variance, σ_{GE}^2 the genotype × environment interaction variance, σ_e^2 the residual variance, E the number of environments and r the number of replicates per line. The 90% confidence interval of h^2 was calculated by the method of Knapp et al. (1985).

QTL mapping

For each resistance criterion × year × location data combination, QTL mapping was conducted by Composite Interval Mapping (CIM) (Zeng 1994), using the program QTL-CARTOGRAPHER v1.21 for MS-Windows (Basten et al. 1994; Wang et al. 2001). CIM was run with model 6 of the program. A forward–backward stepwise regression was run to select background markers having a significance level of $P < 0.10$. The ten markers with highest F-values were considered as co-factors in the CIM model. The window size, representing the region around the test site not considered as background, was set to 10 cM, and the walking speed to 2 cM. Using the permutation test with 1,000 permutations, a mean LOD threshold of 2.8 was chosen for all the traits to declare a putative QTL significant, corresponding to a genome-wide α error risk of 5%. The QTLs detected with QTL-CARTOGRAPHER were checked using one-way ANOVA (SAS Institute 1988) and the mixed linear model approach used in QTL-Mapper v1.0 software (Wang et al. 1999) with main effect markers selected as co-factors by stepwise regression ($P < 0.10$). The confidence interval of a QTL was defined by the region within one-LOD from the QTL peak. For each single QTL, estimates of phenotypic variance (R^2) and additive effects at the LOD peak were obtained from QTL-CARTOGRAPHER. QTL × environment interaction effects of the QTLs revealed from multiple environments for AGI and RRI criteria were estimated using the Jackknife re-sampling procedure of QTL-Mapper v1.0 ($P < 0.01$) (Wang et al. 1999).

Results

Genetic map

The genetic map constructed comprised 331 markers, including 203 AFLPs, 100 RAPDs, 11 SSRs, 7 STSs, 6 isozymes, 2 ISSRs and 2 genes for morphological traits. Among them, 324 were distributed over 13 linkage groups with a mean spacing of 3.5 ± 5.0 cM (Kosambi) between flanking markers. Seven markers remained unlinked. The map covered a total of 1,094 cM (Fig. 1), which is quite consistent with previous-published pea genetic maps (Gilpin et al. 1997; Laucou et al. 1998) but slightly higher than the length expected (nearly 800 cM) according to the pea consensus genetic map (Weeden et al. 1998) and cytogenetic studies (Hall et al. 1997). Automated AFLPs were particularly useful for quickly establishing the genetic-map framework. About half of the AFLP markers mapped in clusters (with an average distance between two markers of less than 1.0 cM) distributed over six linkage groups.

Twenty seven markers, including 13 RAPDs, 6 STSs, 6 isozymes and 2 genes for morphological traits, were common to other pea genetic maps previously reported (Gilpin et al. 1997; Laucou et al. 1998; Weeden et al. 1998) (Table 2). The RAPD markers proved to be trans-

Table 2 Markers anchored to other published linkage maps, used to assign (Puget × 90-2079) linkage groups to those defined by the international nomenclature. Identity of the loci identified over different crosses were checked by the size of the bands, and for RAPD markers, by comparing amplification patterns segregating between the parents from the different crosses

(Puget × 90-2079) linkage group	Marker	Literature citation	Reference linkage group
Ia	*af*	(Weeden et al. 1998)	I
Ib	A04.430	(Laucou et al. 1998)	I
II	Pgmp	(Weeden et al. 1998)	II
II	Q363.HinfI	(Gilpin et al. 1997; Weeden et al. 1998)	II
III	I11.1020	(Laucou et al. 1998)	III
III	L109.Sau3A	(Gilpin et al. 1997; Weeden et al. 1998)	III
Iva	U01.1200	(Laucou et al. 1998)	IV
Ivb	J14.850	(Laucou et al. 1998)	IV
Ivb	C12.650	(Laucou et al. 1998)	IV
Ivb	A08.1250	(Laucou et al. 1998)	IV
Ivb	A19.630	(Laucou et al. 1998)	IV
Ivb	P628.HinfI	(Gilpin et al. 1997; Weeden et al. 1998)	IV
V	*r*	(Weeden et al. 1998)	V
V	P108.AseI	(Gilpin et al. 1997; Weeden et al. 1998)	V
V	O09.800	(Laucou et al. 1998)	V
V	Px1	(Weeden et al. 1998)	V
V	Pgdc	(Weeden et al. 1998)	V
VI	A19.870	(Laucou et al. 1998)	VI
VI	B07.1750	(Laucou et al. 1998)	VI
VI	Gsp.HaeIII	(Weeden et al. 1998; 2001a)	VI
VI	E11.600	(Laucou et al. 1998)	VI
VI	O01.700	(Laucou et al. 1998)	VI
VII	I7.AluI	(Gilpin et al. 1997; Weeden et al. 1998)	VII
VII	Aldo	(Weeden et al. 1998)	VII
VII	Aatm	(Weeden et al. 1998)	VII
VII	C12.500	(Laucou et al. 1998)	VII
VII	Pgdp	(Weeden et al. 1998)	VII

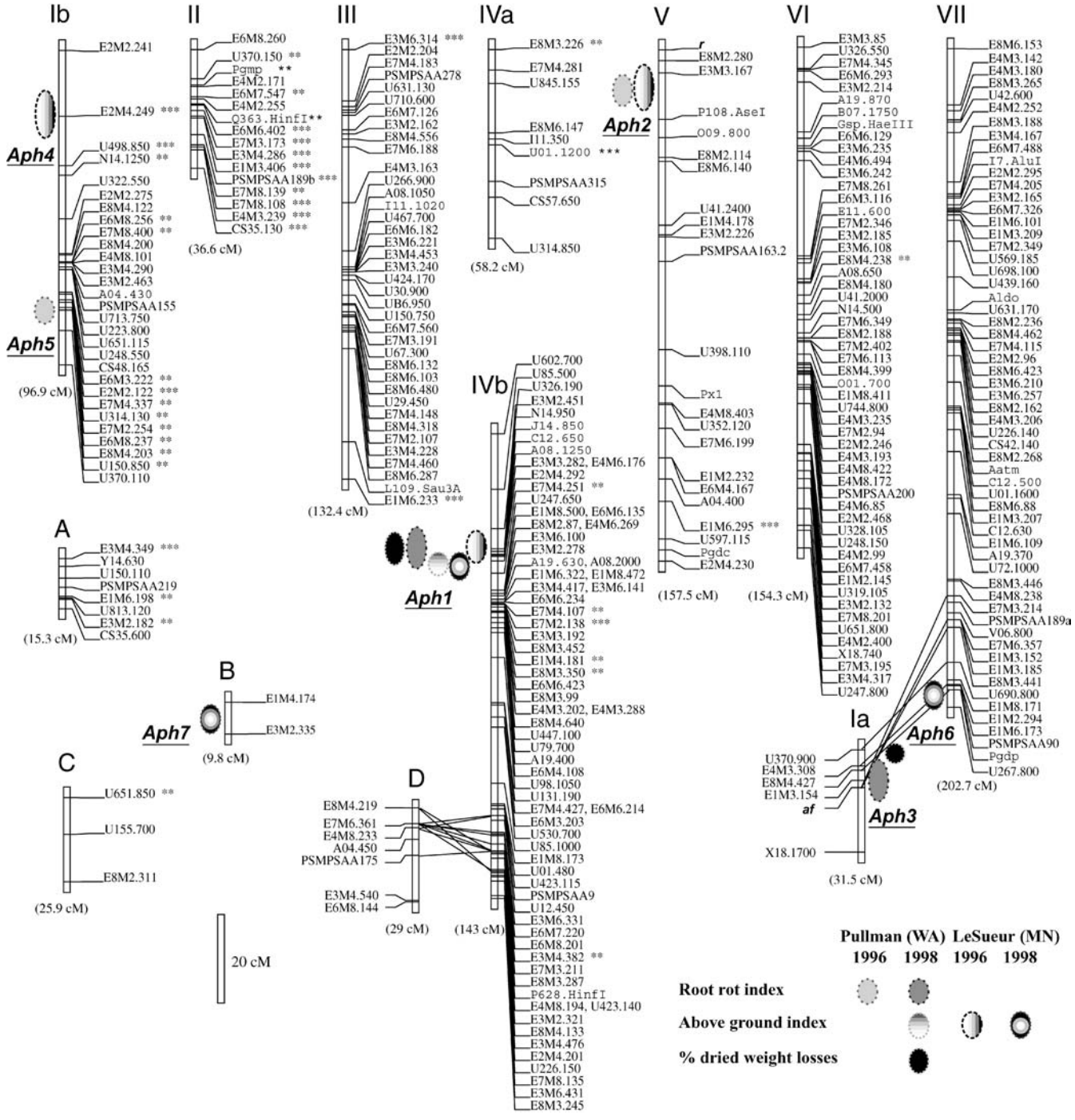

Fig. 1 Genetic map constructed from 127 RILs (Puget × 90-2079) and genomic localization of QTLs detected for field Aphanomyces root rot resistance in 2 years (1996 and 1998) at two locations (LeSueur, Minn., and Pullman, Wash.), using three resistance scoring criteria (above ground index, root rot index and percentage of dried weight losses). Linkage groups assigned to published pea genetic maps are named from I to VII and unassigned linkage groups from A to D. The size of each linkage group is indicated *in parentheses* below each group, in cM Kosambi. Markers displayed in *bold* are anchored to other published genetic maps (Laucou et al. 1998; Weeden et al. 1998) and were used to assign the I to VII linkage groups. Markers with biased segregation at $P \leq 0.01$ and $P \leq 0.001$ are indicated by ** and ***, respectively. Linkages observed with a LOD ≥ 3 between markers of linkage groups Ia and VII, and D and IVb, are represented by *lines* connecting the different linkage groups. QTL length corresponds to its confidence interval defined within ten-fold (1 LOD) of its peak LOD

Table 3 Heritability estimates (h^2) and their confidence interval *in parentheses* for three resistance scoring criteria evaluated over 2 years and two locations in the (Puget × 90-2079) RILs mapping population

Resistance criteron	h^2 (confidence interval)			
	Pullman (Wash., USA)		LeSueur (Minn., USA)	
	1996	1998	1996	1998
Root rot index (RRI)	0.45 (0.29–0.57)	0.40 (0.22–0.54)		
Above ground index (AGI)		0.41 (0.24–0.55)	0.71 (0.63–0.78)	0.53 (0.40–0.63)
Dried weights (DWL)				
Infected nursery		0.53 (0.39–0.64)		
Healthy nursery		0.43 (0.26–0.56)		

Table 4 Pearson phenotypic correlation coefficients between the different scoring criteria assessed in 2 years at two locations for Aphanomyces root rot resistance in the (Puget × 90-2079) RILs population

Trait[a]	AGIMN96	RRIWA96	AGIMN98	AGIWA98	RRIWA98	DWLWA98
AGIMN96		0.30***	0.43***	0.12	0.03	0.10
RRIWA96			0.02	–0.16	0.01	–0.01
AGIMN98				0.19*	0.16	0.29**
AGIWA98					0.48***	0.50***
RRIWA98						0.66***
DWLWA98						

*, **, ***: significant correlation at the 0.05, 0.01 and 0.001 probability level, respectively

[a] Traits are coded as follows: scoring resistance criterion (AGI: above ground index; RRI: root rot index; DWL: % dried weight losses), location of the scoring (MN: LeSueur, Minnesota; WA: Pullman, Washington) then year of the scoring (96; 98)

ferable to the present cross. These common markers allowed nine linkage groups (named with Roman numerals) to be assigned to the pea consensus genetic map (Weeden et al. 1998) (Fig. 1). Four linkage groups (LGs), named from "A" to "D", remained unassigned. Each assigned group included at least two markers common to other published pea genetic maps, except LGs Ia, Ib and IVa which had a more putative assignment with only one common marker. In each linkage group, the order of the common markers was well conserved between our genetic map and the reference maps we used.

Several loci on LG Ia and LG D tended to be associated (LOD ≥ 3.0) with loci within LG VII and LG IVb, respectively, as described in Fig. 1. These marker associations between different linkage groups were also described by Ellis et al. (1992), who suggested the occurrence of translocation events in the regions of these associations. Translocated segments have been reported as a source of the difficulties encountered in constructing a pea consensus genetic map (Temnykh and Weeden 1993).

Out of the 331 markers mapped, 42 (12.7%) did not segregate according to expected Mendelian ratios, among which 57% favored the resistant parent alleles and 43% the susceptible parent ones. Markers showing segregation distortion were mainly clustered on LG Ib and LG II.

Field data

Analysis of experimental data showed significant genotype × environment interaction effects for RRI at Pullman over the 2 years ($P = 0.011$) and for AGI over three environments (LeSueur 1996, 1998; Pullman 1998) ($P = 0.0001$).

Analysis of variance of each field trial revealed highly significant genotypic effects for AGI, RRI and DWL resistance criteria ($P \leq 0.0005$). Distributions of residuals after analysis of variance were normal according to the Shapiro-Wilk ($P \geq 0.05$) and/or Skewness and Kurtosis statistics. Variances of genotypes and replicates were homogeneous according to Bartlett's test ($P > 0.05$). Within-year or location heritability estimates ranged from 0.40 (RRI, Pullman 1998) to 0.71 (AGI, LeSueur 1996) depending on the resistance criterion used, the year and the location (Table 3). Heritability values across environments were 0.30 for RRI (Pullman 1996, 1998) and 0.51 for AGI (LeSueur 1996, 1998; Pullman 1998).

Distributions of the estimated adjusted means after analysis of variance are represented in Fig. 2 for each resistance criterion × year × location data combination. They did not differ from normal distributions according to the S statistics given by QTL-CARTOGRAPHER ($\alpha = 5\%$), confirming the quantitative inheritance of the partial resistance. Ranges for AGI-scoring in the RILs population were larger at Pullman than at LeSueur. At LeSueur, disease severity was much greater in 1996 than in 1998. Compared with the parental values, transgressive segregants with increased resistance and susceptibility were observed for all resistance traits over years and locations.

Pearson correlation coefficients (Table 4) were highly significant between resistance criteria evaluated within the same year (AGI, RRI and DWL at Pullman in 1998) and for AGI between the 2 years at LeSueur. However, a

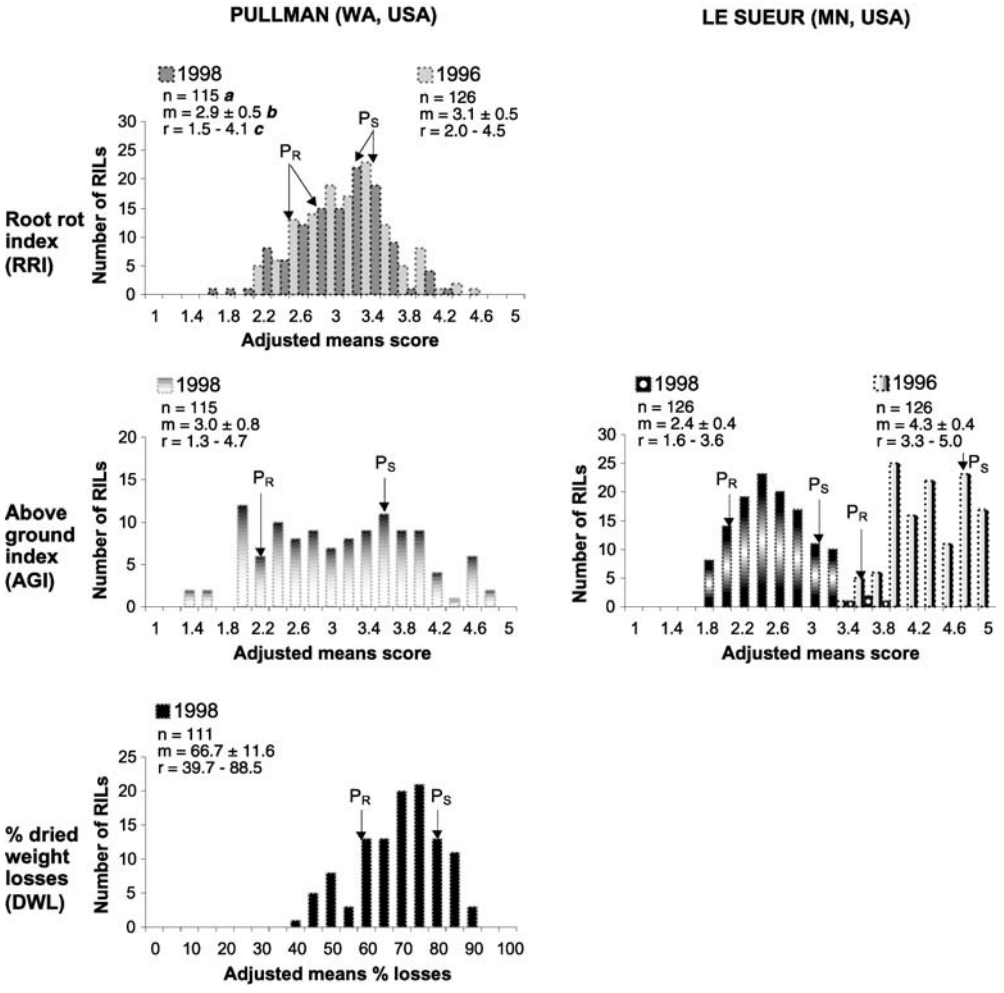

Fig. 2 Adjusted means frequency distributions of the (Puget × 90-2079) RILs population for three field Aphanomyces root rot resistance criteria (root rot and above ground indexes, percentage of dried weight losses) assessed over 2 years and two locations in the United States. Adjusted means values of the resistant (90-2079) and susceptible (Puget) parents, named P_R and P_S, respectively, are shown by *arrows*. [a]n = total number of RILs assessed [b]m = mean ± standard deviation of the RILs population [c]r = range of scoring variation in the RILs population

poor inter-year correlation was observed at Pullman between the different traits evaluated, but only RRI was scored in 1996 at Pullman. Correlations between the two locations were highly and moderately significant in 1996 and 1998, respectively.

QTL mapping

A total of seven genomic regions, distributed over six linkage groups, were associated with field Aphanomyces root rot resistance using three resistance criteria, over years and locations. The characteristics and localization of these QTLs are indicated in Table 5 and Fig. 1.

The first QTL identified was located on LG IVb, in the region of a cluster of AFLP markers that covered about 15–20 cM. This particular QTL was designated *Aph1* and was considered as "major" for two main reasons. (1) It was significant ($2.7 < \text{LOD} < 20.4$) for all the resistance criteria over the years and locations tested but only for RRI at Pullman in 1996 (Fig. 3). From this RRI WA 96 data set, *Aph1* was revealed by QTL-CARTOGRAPHER with a LOD score of 2.4, but it was not detected by one-way ANOVA or by QTL-Mapper (data not shown); (2) *Aph1* was identified with major additive effects and explained up to 47% of the variation for AGI at LeSueur in 1998. At *Aph1*, the resistance allele was derived from the partially resistant parent, 90-2079. *Aph1* displayed significant additive × environment interaction effects for AGI over years and locations [interaction effect due to LeSueur (1996), $P = 0.001$] and for RRI over the 2 years ($P = 0.0054$).

Two other QTLs, *Aph2* and *Aph3*, were identified using two trait measures on LG V and LG Ia, respectively (Fig. 3).

(1) *Aph2* was revealed in 1996, from AGI scores collected at LeSueur and RRI data at Pullman. It accounted for 32% of the variation at Pullman and 8% at LeSueur. *Aph2* co-segregated with the *r* locus, controlling the wrinkled/round seed trait. *Aph2* resistance and *R* (round seeds) alleles were both derived from the partially resistant parent, 90-2079.

(2) *Aph3* was identified using RRI and DWL data from Pullman in 1998. *Aph3* accounted for 14 and 11% of the variation for RRI and DWL, respectively. The resistance-enhancing allele at *Aph3* originated from the

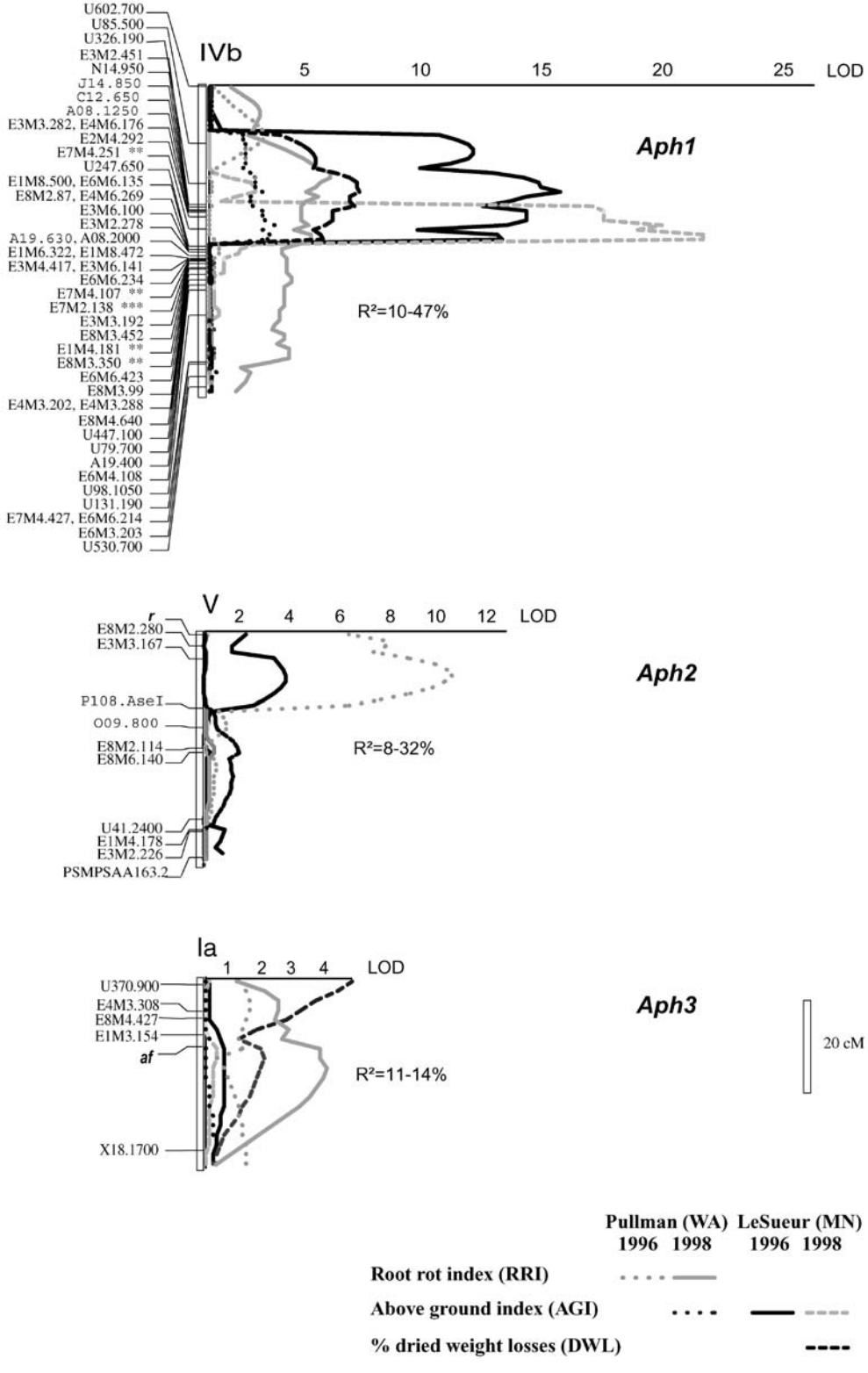

Fig. 3 LOD profiles of *Aph1*, *Aph2* and *Aph3* QTLs identified in (Puget × 90-2079) RILs population for several field Aphano-myces root rot resistance measures. Field resistance was assessed in 2 years (1996, 1998) at two locations in the United States (LeSueur, Minn., Pullman, Wash.) using three resistance criteria (above ground and root rot indexes, percentage of dried weight losses). R^2 is the range of variation explained by each QTL over different resistance scorings. Markers anchored to published genetic maps are indicated in *bold*

susceptible parent, Puget, and co-segregates with the *Af* allele that confers normal leaves.

Aph2 and *Aph3* showed highly significant additive × year interaction effects for RRI (P = 0.0023 and P = 0.0000, respectively).

Finally, four other QTLs, *Aph4*, *Aph5*, *Aph6* and *Aph7*, were identified on LG Ib, LG VII and LG B (Fig. 1). Each of them was detected for one resistance criterion assessed in one environment. These QTLs accounted for 6 to 13% of the variation, depending on the

Table 5 Markers and parameters associated with QTLs detected for field Aphanomyces resistance in 2 years at two locations, for three resistance scoring criteria (values obtained by QTL-Cartographer v1.21/Win)

Year	Location[a]	Scoring criterion[b]	QTL name	Linkage group	Position (cM)[c]	Maximum LOD	Additive effect[d]	R^{2e}
1996	MN	AGI	*Aph4*	Ib	E2M4.249 + 2	3.2	–0.13	0.06
			Aph1	IVb	N14.950 + 0	14.5	0.23	0.26
			Aph2	V	E3M3.167 + 6	3.5	0.11	0.08
1996	WA	RRI	*Aph5*	Ib	E3M3.167 + 6	6.5	–0.20	0.13
			Aph2	V	E7M2.254 + 0	10.1	0.29	0.32
1998	MN	AGI	*Aph1*	IVb	E7M4.251 + 2	20.4	0.30	0.47
			Aph6	VII	Pgdp + 4	3.7	0.11	0.06
			Aph7	B	E1M4.174 + 2	4.2	0.12	0.07
1998	WA	AGI	*Aph1*	IVb	E2M4.292 + 2	2.7	0.28	0.10
1998	WA	RRI	*Aph3*	Ia	*af* + 4	4.0	–0.19	0.14
			Aph1	IVb	U326.190 + 2	5.1	0.20	0.16
1998	WA	DWL	*Aph3*	Ia	U370.900 + 0	4.0	–4.28	0.11
			Aph1	IVb	N14.950 + 0	6.2	4.97	0.16

[a] MN: LeSueur, Minnesota; WA: Pullman, Washington
[b] AGI: above ground index; RRI: root rot index; DWL: percentage of dried weight losses
[c] QTL position at the LOD peak from the first marker of the interval (in centiMorgans)
[d] Effect of substituting '90-2079' alleles for 'Puget' alleles at the LOD peak of the QTL. A positive sign reflects that QTL alleles increasing the resistance were contributed by the resistant parent whereas a negative sign means that resistance alleles were brought by the susceptible parent
[e] Part of the phenotypic variance explained by an individual QTL

QTL. Two of them were located in two distinct regions on LG Ib, with both resistance alleles originating from the susceptible parent. The QTL on LG VII was located in a region where markers tend to co-segregate with *Aph3* on LG Ia, suggesting that this QTL may correspond to a translocated segment that includes *Aph3*. However, *Aph3* and the QTLs on LG VII showed opposite additive effects, meaning that the resistance alleles of these two QTLs did not originate from the same parent and consequently would probably correspond to two different loci.

Discussion

Genetic basis of partial resistance to Aphanomyces root rot

The present study is the first extensive published QTL mapping report for partial resistance to *A. euteiches* in pea. The results obtained over 2 years and two locations in the US showed that field partial resistance to Aphanomyces root rot in pea germplasm line 90-2079 was associated with (1) a "major" QTL, *Aph1*, located on LG IVb that explained up to 47% of the variation, and (2) "minor" QTLs, among which two QTLs revealed from two trait measures, *Aph2* and *Aph3*, and located on LGs V and Ia, respectively. For *Aph3* and the two minor QTLs on LG Ib, the resistance alleles were apparently derived from the susceptible parent. The exploitation of Puget resistance alleles could make it possible to select transgressive segregants with improved resistance. These results could not be biased by the plant maturity trait since this trait did not segregate in the RILs population (days to 50% bloom were June 1st and 4th for 98% of the RILs in 2001).

At least 50% of the phenotypic variation was not explained by the detected QTLs, depending on the trait, year and location studied. Further additive-effect QTLs may have been undetected because of weak effects, particularly when heritabilities were lower, and/or incomplete map coverage as suggested by the QTLs revealed on LG B. The involvement of epistatic QTL in the unexplained portion of the variation could also be possible. A preliminary analysis of digenic epistatic interactions between markers was carried out using a two-way ANOVA model with an interaction component (SAS/IML, SAS 1989) (data not shown). Results showed particularly significant ($P \leq 5.10^{-5}$) interaction effects between *Aph1* and three markers (U30.900 on LG III, U42.600 on LG VII and E8M2.311 on LG C) for the AGI resistance criterion at LeSueur in 1996 and 1998, that explained up to 15% of the variation. These results will have to be verified using methods such as multiple interval mapping recently reported (Kao et al. 1999).

Our results support the observations of Shehata et al. (1983) who reported low heritabilities of partial resistance (0.28 to 0.46) in the breeding line Mn108, suggesting its quantitative mode of inheritance. Marx (1972) showed that tolerance to *A. euteiches* was genetically dominant and associated with several wild-type alleles: *Le* (tall plants) on LG III, *A* (colored flowers) on LG I and *Pl* (black hilum of the seeds) on LG VI, located in different genomic regions than *Aph1*, *Aph2* and *Aph3* identified in this study. In the present study, we demonstrated associations between resistance and morphologi-

cal traits by the co-segregations of *Aph2* and *Aph3* resistance alleles with the *Af* (normal leaves) and *R* (round seeds) alleles. A correlation between partial resistance and the round seed trait has already been reported (Marx et al. 1972) but considered statistically not significant.

Aph1, a major resistance QTL to Aphanomyces root rot

Aph1 was assigned to LG IVb with the use of five markers anchored to previously published genetic maps. Weeden et al. (2000, 2001b) reported that tolerance to Aphanomyces root rot at LeSueur, Minn., U.S., in the pea line Mn313 was associated with a major gene linked to P393 and $PgmF_{390}$ markers, also located on LG IV. At the $PgmF_{390}$ locus, this gene accounted for 26% of the variation. However, there was no correlation between LG IV markers and root rot tolerance at Pullman, Wash., U.S. Pea lines 90-2079 and Mn313 were selected from a common partially resistant progenitor, 79-2022 (Kraft 1992; Davis et al. 1995), suggesting that *Aph1* and the gene associated with Aphanomyces root rot tolerance in Mn313 may be the same locus. In this study, we could not compare the genomic localizations of *Aph1* and the major gene in Mn313 since the P393 and $PgmF_{390}$ markers did not show any polymorphism between Puget and 90-2079.

QTL consistency over years, locations and resistance criteria

Aphanomyces root rot resistance is strongly influenced by environment (Marx et al. 1972; Shehata et al. 1983). Consistency of the detected QTLs towards environments was different depending on the QTL:

Aph1 was detected for all the variables studied, even if it showed significant QTL × environment interactions due to environments where it was detected with a less significant LOD score. *Aph1* was also the main QTL revealed from AGI data collected at the flowering stage at LeSueur in both years (LOD = 19.8, R^2 = 46% in 1996, or LOD = 8.8, R^2 = 19% in 1998; data not shown).

Aph2 and *Aph3* were both year-specific, because they were only detected from 1996 and 1998 data, respectively, and displayed significant additive × year interaction effects. *Aph3*, identified only from Pullman data, was also specific to location. QTLs detected at *Aph2* and *Aph3* supported the observed pattern of phenotypic correlations (good correlations between AGI and RRI 1996 data at LeSueur and Pullman, and between data collected in 1998 at Pullman).

The phenotypic and mapping results obtained in the present study indicate that the "year" environmental component had a greater effect on the resistance than the "location" component. Weeden et al. (2000, 2001b) reported the "location" component as the one mainly affecting the partial resistance. They observed little correlation between resistance scores at LeSueur and Pullman in Mn313 and a Mn313-derived population of RILs. They suggested that the *A. euteiches* pathogen populations may differ at the two locations. Malvick and Percich (1998) studied the genetic diversity among 114 pea-infecting strains of *A. euteiches* isolated from Minnesota, Wisconsin and Oregon, U.S. Based on pathogenicity assays, they found that the percentage of strains pathogenic on five different pea hosts varied among locations but, based on RAPD analyses, there were no distinct genotypic differences among populations from the different locations.

Our results also showed no QTL specificity towards the resistance criteria used. *Aph1*, *Aph2* and *Aph3* were detected for AGI, RRI and DWL. Good correlations between resistance criteria were observed in the same year. These results suggest that resistance criteria based on foliage symptoms and disease effects on yield, which have been widely used by breeders to screen for field tolerance to *A. euteiches* (Kraft 1992; Lewis and Gritton 1992; Davis et al. 1995), are useful for assessing partial resistance to this soil-borne disease.

Associations between partial resistance to *A. euteiches* and other root pathogens

Other root-invading pathogens can influence the expression and assessment of symptoms due to *A. euteiches*. Kraft and Pfleger (2001) reported that when *Aphanomyces* spp. were present at low or moderate inoculum levels, infection of roots by other pathogens such as *Fusarium* or *Pythium* spp., or nematodes, can increase the severity of Aphanomyces root rot. In the present study, seeds were treated at planting against *Pythium*. At Pullman, the occurrence of *Fusarium oxysporum* was checked using the susceptible line M410 which displayed no Fusarium wilt symptoms. Quantification of *Fusarium solani* in soil samples was also conducted in 1998 at Pullman (Boge, personal communication) and revealed the presence of the pathogen in both disease and healthy nurseries used to evaluate the Puget × 90-2079 RILs (mean values: 886 and 306 colony forming units/g soil, respectively). However, only typical symptoms of *A. euteiches* were observed and scored on the roots. At Le Sueur, the disease nursery was known to be infested with *F. oxysporum* races 1, 2 and *F. solani* in addition to *A. euteiches*, based on the reaction of a partial set of the standard differentials described in Kraft and Pfleger (2001). However, above-ground scorings were carried out from the flowering stage, to primarily score for symptoms due to *A. euteiches* occurring early in the season.

The QTLs identified in this study were located in different genomic regions than the *Fw* and *Fwf* genes conferring resistance to *F. oxysporum* races 1 and 5, which were tagged on LGs III and II, respectively (Dirlewanger et al. 1994; Coyne et al. 2000b). They were also mapped in different regions from the DRR49 gene, known to be involved in the disease resistance response to *F. solani* f. sp. *phaseoli*, located on LG VI (Weeden et al. 2001b).

Conclusion, prospects

These results identify DNA markers linked to a "major" QTL, *Aph1*, which could be used in marker-assisted selection (MAS) for Aphanomyces root rot resistance in the U.S. Out of 25 AFLP and RAPD markers located in the one LOD confidence interval of *Aph1*, ten are in coupling phase with the 90-2079 resistance allele at *Aph1*. Three or four of these markers, as well as markers that would be checked to show interaction effects with *Aph1*, could be converted to codominant specific markers such as SCARs (Sequence Characterized Amplified Regions). Such markers would be useful to introgress *Aph1* and genomic regions showing interaction effects with *Aph1* into enhanced genotypes.

Further mapping studies are needed to investigate and to better understand the genetics of partial resistance to Aphanomyces root rot. The consistency of the resistance QTLs identified in the Puget × 90-2079 cross will have to be analyzed over years, countries, pathogen isolates, genetic backgrounds, screening conditions (pure culture, field) and evaluation criteria in order to validate the usefulness of these QTLs for MAS. Particularly, the usefulness of the resistance alleles identified in 90-2079 will have to be verified in other environments where the disease is particularly severe and damaging. In France, 90-2079 does not show resistance in the field (Roux-Duparque, personal communication), mainly due to greater aggressiveness of French *A. euteiches* isolates compared with U.S. isolates, as demonstrated by Wicker and Rouxel (2001). This observation suggests that other alleles are probably required for the expression of partial resistance under French conditions. The identification of new resistance loci from other sources of resistance will also be an objective in order to accumulate multiple resistance alleles in a genotype and increase the level of partial resistance to this destructive pathogen.

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