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ORIGINAL PAPER

New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments

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Abstract Partial resistances, often controlled by quantitative trait loci (QTL), are considered to be more durable than monogenic resistances. Therefore, a precursor to developing efficient breeding programs for polygenic resistance to pathogens should be a greater understanding of genetic diversity and stability of resistance QTL in plants. In this study, we deciphered the diversity and stability of resistance QTL to *Aphanomyces euteiches* in pea towards pathogen variability, environments and scoring criteria, from two new sources of partial resistance (PI 180693 and 552), effective in French and USA infested fields. Two mapping populations of 178 recombinant inbred lines each, derived from crosses between 552 or PI 180693 (partially resistant) and Baccara (susceptible), were used to identify QTL for Aphanomyces root rot resistance in controlled and in multiple French and USA field conditions using several resistance criteria. We identified a total of 135 additive-effect QTL corresponding to 23 genomic regions and 13 significant epistatic interactions associated with partial resistance to *A. euteiches* in pea. Among the 23 additive-effect genomic regions identified, five were consistently detected, and showed highly stable

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effects towards *A. euteiches* strains, environments, resistance criteria, condition tests and RIL populations studied. These results confirm the complexity of inheritance of partial resistance to *A. euteiches* in pea and provide good bases for the choice of consistent QTL to use in marker-assisted selection schemes to increase current levels of resistance to *A. euteiches* in pea breeding programs.

Introduction

Partial polygenic resistance to plant diseases has recently gained more interest with the pursuit of more durable genetic control of plant pathogens (Kou and Wang 2010; Palloix et al. 2009; Parlevliet 2002), either intrinsically or used in combination to increase the durability of major resistance genes (Brun et al. 2010). In past years, the latter have been overcome, when used alone, by virulent isolates appearing in pathogen populations (Caffier and Laurens 2005; Castagnone-Sereno 2002; Kousik and Ritchie 1998; Sprague et al. 2006a, b). However, classical phenotypic breeding for partial resistance in crop plants has often proven difficult due to the polygenic inheritance of resistance. In the last 20 years, the increasing amount of data about QTL controlling disease resistance in major crops greatly contributed to facilitate breeding for partial polygenic resistance through the development of marker-assisted selection (MAS) strategies. Several examples of gene pyramiding combining resistance QTL and major genes using MAS have thus been published, for example, in legumes (Ender et al. 2008; Faleiro et al. 2004), pepper (Palloix et al. 2009) or barley (Richardson et al. 2006). However, MAS for quantitative traits, especially polygenic disease resistance, was not always consistently successful (Hospital 2009; Xu and Crouch 2008). The instability of QTL even with reasonably large effects, has been one of the main causes of unexpected results of MAS experiments (Hospital 2009). Moreover, many negative epistatic interactions between genes may have reduced the efficiency of MAS or prevented response to MAS (Hospital 2009). In order to optimize the probability of success in MAS, extensive genetic dissection of complex traits, including stability studies of QTL effects and genomic positions towards multiple environments and genetic backgrounds and analyses of genetic effects (additive, epistatic, etc.) of loci controlling complex traits, has become a necessary prerequisite.

Aphanomyces root rot, caused by the oomycete *Aphanomyces euteiches* Drech., is one of the most damaging pea diseases worldwide (Pfender et al. 2001). This soilborne pathogen can attack peas at any stage of plant development, causing a rotting of the roots and epicotyls that result in stunted seedlings, yellow leaves and even dead plants. As no sufficient chemical or cultural methods are available yet to manage the disease, the release of pea cultivars with acceptable levels of resistance, which have been thus far unavailable, is of high priority for the development of a sustainable pea crop in Europe and the United States of America (USA). Genetic resistance to *A. euteiches* has been shown to be partially and polygenically inherited with quite low heritability and large environmental effects (Shehata and Pfleger 1983; Pilet-Nayel et al. 2002). Despite the fact that phenotypic breeding for partial resistance to *A. euteiches* has been difficult in the past 30 years, primarily due to the complex inheritance of resistance, partially resistant germplasm of agronomic types have been released in the USA (Gritton 1990; Kraft 1992; Kraft and Coffman 2000a, b, c; Davis et al. 1995) and in France (Roux-Duparque et al. 2004) to breeders. Genetics of partial resistance to *A. euteiches* in pea have not been extensively studied yet. From a recombinant inbred line (RIL) mapping population derived from the cross Puget (susceptible) × 90-2079 (partially resistant in the USA), Pilet-Nayel et al. (2002, 2005) identified a total of 14 QTL associated with partial resistance evaluated in field experiments over 2 years and two locations in the USA and in greenhouse assays using a USA and a French isolate. Out of the 14 QTL, a major-effect locus, named *Aph1*, was highly and consistently detected over years, locations and isolates on linkage group (LG) IV, and colocalized with a major QTL identified for field tolerance to *A. euteiches* in the pea germplasm MN313 (Weeden et al. 2000). Two other QTL, named *Aph2* and *Aph3*, were also consistently identified from field and greenhouse data, colocalizing with the *R* (round/wrinkled seeds) and *af* (afila/normal leaves) genes on LGV and LGI, respectively. However, the integration of these QTL for MAS in European breeding programs has been questionable, since partial resistance of 90-2079 was not effective in French field conditions (Pilet-Nayel et al. 2002). The reduced effectiveness of resistance contributed by 90-2079 in France is postulated to be related to the predominance of *A. euteiches* isolates from pathotype I in Europe. Pathotype I occurs in the USA together with isolates from the other main pathotype (III) described

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on pea (Wicker and Rouxel 2001; Onfroy, personal communication). A greater understanding of the genetic loci conferring Aphanomyces root rot partial resistance effective in France is required to further progress in breeding using MAS. For the last 10 years, a few partially resistant pea germplasm lines effective both in France and the USA have been identified, as they have expressed stable levels of resistance over the course of multiple years in infested nurseries (Pilet-Nayel et al. 2007). Pea accessions 552 and PI 180693, primarily identified in the USA (Gritton 1995; Lockwood 1960), have shown the most promise, as they exhibit the highest and most stable levels of partial resistance among the different sources of resistance identified and observed (Wicker et al. 2003; Pilet-Nayel et al. 2007).

The objectives of this study were (1) to identify new genetic loci controlling resistance to *A. euteiches* in two new pea sources of resistance effective both in France and the USA and (2) to study the stability of genomic positions and effects of the resistance QTL identified across environments and *A. euteiches* pathotypes. Genetic analysis of resistance to *A. euteiches* was conducted in two new pea RIL mapping populations derived from crosses between the two partially resistant germplasm, 552 and PI 180693 and the susceptible French cultivar Baccara. The RIL populations were (1) genotyped using molecular markers to create a genetic consensus map and (2) evaluated for Aphanomyces root rot resistance in controlled conditions and in multiple French and USA environments using several resistance criteria. Additive- and epistatic-effect QTL were detected for resistance to *A. euteiches*, and the stability of their effects were analyzed across environments, pathotypes and resistance criteria studied. The results identified a selection of consistent QTL and markers potentially useful for MAS and developing pyramiding strategies of resistance alleles for a more highly effective and durable partial resistance in pea.

Materials and methods

Plant material

Two pea RIL populations from the crosses Baccara × PI 180693 (178 F_8-derived RILs) and Baccara × 552 (178 F_9-derived RILs) were produced by single seed descent at INRA-Le Rheu (France). Baccara (Florimond-Desprez, France, registered in 1992) is a dry pea cultivar with afila leaves, round seeds and white flowers and is susceptible to *A. euteiches*. PI 180693 (USDA Plant Introduction Station, USA) and 552 (Gritton 1995) are pea germplasm accessions both partially resistant to *A. euteiches* (Wicker et al. 2003). PI 180693 is a landrace, originating in Germany, with normal leaves, round seeds and pink flowers, which was identified as a source of resistance by Lockwood (1960) as part of a screening program of 805 accessions of the USDA pea collection using one isolate of *A. euteiches* from Michigan. 552 is a garden pea breeding line with normal leaves and white flowers, which was derived from the 8th cycle of a recurrent selection program conducted for Aphanomyces root rot resistance by Dr. E. T. Gritton (University of Wisconsin, WI, USA) (Lewis and Gritton 1988). No genealogical relationship between PI 180693 and 552 has been found (Wicker 2001). Control lines used for controlled conditions and field experiments included the RIL parental lines and the additional pea germplasm MN 313 (Davis et al. 1995), which is resistant to pea pathotype III isolates of *A. euteiches* and susceptible to pathotype I isolates (Wicker and Rouxel 2001; Onfroy, personal communication). An additional check line, the pea cultivar Solara (Cebeco Zaden, Holland, registered in 1986), was used as a susceptible control in the field experiments.

Pathogen material

Two pure culture pea-infecting strains of *A. euteiches* were used for disease experiments in controlled conditions, namely RB84, isolated from an infested pea field at Riec-sur-Belon (Finistère, France) (Moussart et al. 2007) and Ae109, isolated from an infested pea field in Wisconsin (USA) and referred to as the Ae467 strain in Malvick et al. (1998) and Malvick and Percich (1999) (Wicker and Rouxel 2001). The RB84 and Ae109 strains were derived from single spores and belong to pathotypes I and III, respectively, identified by Wicker and Rouxel (2001), based on their differential reactions on a set of six pea genotypes (Wicker et al. 2003).

Controlled condition disease experiments

The RIL populations were evaluated on 14-day-old seedlings for resistance to pure-culture strains of *A. euteiches* in controlled conditions. All the RILs from the two mapping populations and the control lines were assayed in one test. In each test, five seeds of a single line were sown in one pot and pots were arranged in a completely randomized design. Each test was repeated four times for each strain, i.e. each line was assayed with four replicates. Screening tests were conducted in a growth chamber at 25°C for 16 h day and 23°C for 8 h night for 2 weeks, as described in Moussart et al. (2001), using an inoculum concentration of 10^3 zoospores per plant. Seven days after inoculation, the plants were uprooted and disease severity was scored on each plant using the 0–5 disease scoring scale adapted from Moussart et al. (2007) (Pilet-Nayel et al. 2009). A root rot

index (RRI) ranging from 0 to 5 was then calculated as the mean disease score on all plants in a pot.

Field disease experiments

The two RIL populations and control lines were evaluated for Aphanomyces root rot resistance in an international Aphanomyces field disease network, consisting of infested nurseries established at three locations in France [Riec-sur-Belon, Finistère (RI); Dijon-Epoisses, Côte d'or (DI) and Templeux, Somme (TPX)] and three locations in the United States (Pullman, WA (PLM); Athena, OR (ATH) and LeSueur, MN (LS)). The RI, DI and TPX French nurseries were established on naturally infested fields on experimental farms of Union Nationale Interprofessionelle des Légumes Transformés (UNILET) (Moussart et al. 2009), Institut National de la Recherche Agronomique (INRA) and Groupement des Sélectionneurs de Pois Protéagineux (GSP), respectively, having a history of growing peas and moderate to high levels of soil-borne pathogens, mainly *A. euteiches*. The PLM and LS USA nurseries were described in Pilet-Nayel et al. (2002). The ATH USA nursery was established on a naturally infested grower's field with pea growing history. From the establishment of each nursery (1970 for LS, 1986 for PLM, 2001 for RI, DI, TPX and 2005 for ATH), peas have been grown continuously (until 2005 in the TPX nursery) for the evaluation of Aphanomyces root rot resistance. All the nurseries, but TPX, had irrigation facilities to favor the disease development when necessary. Geographical and climatic characteristics of the French and USA Aphanomyces-infested nurseries used for field evaluation of the RIL populations are summarized in Table 1. Pathogenicity characteristics of isolates sampled from the six nurseries were recently studied (Onfroy, personal communication) using the differential set of six pea lines from Wicker et al. (2003).

The Baccara × 552 RIL population was evaluated for Aphanomyces root rot resistance over a total of six environments distributed over 2 years and four locations (in 2004: RI, DI, TPX, PLM; in 2005: RI, PLM). In Pullman (WA, USA), a subset of 121 RILs chosen randomly out of the 178 RILs was evaluated in 2004 and 2005. The Baccara × PI 180693 RIL population was evaluated for Aphanomyces root rot resistance over a total of eight environments over 3 years and three locations (in 2006: DI, ATH; in 2007: RI, DI, LS; in 2008: RI, DI, LS). The population was also assayed in a healthy nursery at Le Rheu (LR, Brittany, France) in 2008, for scoring comparisons with 2008 data from RI infested nursery (Brittany, France).

Field experiments were carried out using randomized complete block designs with three replicates of RILs and

Table 1 Geographical and climatic characteristics of infested experimental sites used for field evaluation of Aphanomyces root rot resistance on pea

Country	Location	Altitude (m)	Geographical localisation	Type of climate	Type of soil	Year of evaluation	Annual rainfall (mm)	Annual average temperature (°C)	RIL population evaluated	Abbreviation
France	Riec-sur-Belon, Finistère	77	47°50′N, 3°42′W	Oceanic	Well drained sandy silt	2004	1,083	11.9	Baccara × 552	B552_RI04
						2005	931	12.1		B552_RI05
						2007	1,230	12.3	Baccara × PI 180693	BPI_RI07
						2008	1,303	11.6		BPI_RI08
	Dijon-Epoisses, Côte d'Or	211	47°19′N, 5°02′E	Continental	Clay loam	2004	789	11.0	Baccara × 552	B552_DI04
						2006	726	11.5	Baccara × PI 180693	BPI_DI06
						2007	788	11.6		BPI_DI07
						2008	850	11.0		BPI_DI08
	Templeux, Somme	90	49°57′N, 3°08′E	Continental	Clay silt	2004	603	10.5	Baccara × 552	B552_TPX04
USA	Pullman, WA	600	46°43′N, 117°10′W	Continental	Silty loam	2004	329	9	Baccara × 552	B552_PLM04
						2005	168	9		B552_PLM05
	Athena, OR	690	45°48′N, 118°29′W	Continental	Silty loam	2006	352	11	Baccara × PI 180693	BPI_ATH06
	LeSueur, MN	318	44°27′N, 93°54′W	Continental	Clay loam	2007	641	9	Baccara × PI 180693	BPI_LS07
						2008	530	6		BPI_LS08

Annual rainfall and annual average temperature data were obtained from the websites http://www.meteociel.fr/ (French data) and http://weather.org/ (American data)

control lines. Each plot in each replicate consisted of 2-m-long twin rows in France and 2-m-long single rows in the United States with 30 plants per row. The design included systematic check plots of the susceptible cultivar Solara every 2–10 plots of test lines, to control for heterogeneity of *A. euteiches* inoculum within the nursery and to adjust disease scores for local disease variations in the soil (Baker and McKenzie 1967).

Three disease criteria were used to assess resistance:

1. Root rot index (RRI) from the fourth node stage was determined by digging ten successive plants per plot, washing the roots in water and rating diseased roots and epicotyl on each plant using a 0–5 disease scoring scale adapted from Moussart et al. (2001) similar to the one used in controlled conditions. A mean RRI was calculated for each plot from individual scores obtained on the 10 plants.
2. Aerial decline index (ADI) was evaluated on each whole plot once, twice or three times every 10–15 days from the late-flowering stage. The 1–5 disease scoring scale described for the Above Ground Index in Pilet-Nayel et al. (2002) was used for evaluating the two RIL populations in the US nurseries. The 1-9 disease scoring scale described in Duparque and Boitel (2001), adapted from Lewis and Gritton (1992), considering plant yellowing and stunting was used for the Baccara × 552 RIL population in the French nurseries. A 1–8 disease scoring scale slightly modified from Duparque and Boitel (2001), only considering plant yellowing was used for assessing the Baccara × PI 180693 RIL population.
3. Dwarfism (DW) was determined at maturity by calculating the percentage of average height loss in an infested plot compared to a non-infested plot, as follows: $DWij = (HNi - HIij)/HNi$, where $HIij$ is the mean height of five plants of the ith RIL in the jth replicate in infested conditions (3 replicates) and HNi is the mean height of five plants of the ith RIL in non-infested conditions (1 replicate).

For each RIL population, RRI was assessed only in French nurseries and ADI was evaluated both in French and USA nurseries. DW was only assessed for the Baccara × PI 180693 RIL population from 2008 RI and LR data (Brittany, France).

The RRI, ADI and HI scores of the ith RIL test plot within a replicate were adjusted for local disease variation measured by scores on the adjacent cv. Solara susceptible check using the formula: $x''i = xi - [(ix_{01} + (n + 1 - i)x_{02})/(n + 1) - \overline{x0}]$ where x_i'' is the adjusted disease score of the ith RIL plot located between two Solara check plots, x_i is the non-adjusted disease score of the ith RIL plot, x_{01} and x_{02} are the disease scores of the two Solara check plots flanking the ith RIL plot, $\overline{x0}$ is the mean disease score of all the Solara check plots from the replicate, n is the total number of RIL plots between two Solara check plots and i is the value of the ith RIL plot (Dagnelie 2003).

Consensus genetic map

Molecular markers

The two RIL populations were genotyped using simple sequence repeat (SSR) from Loridon et al. (2005), random amplified polymorphic DNA (RAPD), and a resistance gene analog (RGA) marker from Prioul-Gervais et al. (2007). Three morphological trait markers segregating in one or both populations were also scored: "*af*" for leaf morphogenesis, "*P1*" for hilum color on seeds and "*A*" for anthocyanin production.

From each RIL and parental line, young leaves were harvested on 2-week-old plants (10 plants/line) and DNA was extracted from approximately 1 g of leaf tissue according to Doyle and Doyle (1990).

SSR amplifications were carried out using primer pairs and the procedure described in Loridon et al. (2005), with fluorescently (IRD 700 or IRD 800) labelled universal M13 forward primers. Amplified products were electrophoresed on a 6.5% denaturing polyacrylamide gel and visualized on a LICOR IR^2 automated Sequencer (Li-Cor Inc., Lincoln, NE, USA). Polymorphic bands were scored manually. SSR markers were coded using the SSR primer name. Whenever SSR primers amplified more than one locus in a segregating population, a different letter for each locus was added to the primer name.

RAPD amplifications and identifications were carried out from Operon primers (Operon Technologies, Alameda, CA, USA) according to the procedure described by Laucou et al. (1998). RAPD markers were generated following polymorphism tests using 32 Operon RAPD primers from which RAPD markers were mapped in Laucou et al. (1998). RAPD markers were named using the Operon primer name (kit letter and primer number) followed by the length of the fragment in base pairs.

IJB174 RGA amplification and revelation was carried out using primer pairs and the procedure described by Prioul-Gervais et al. (2007).

Map construction

For each locus, adjustment of allelic segregation to the expected 1:1 Mendelian ratio was analyzed using a χ^2 test

($\alpha = 0.01$). Genotyping data from the two segregating populations were assembled in a unique file to elaborate the consensus map. Genetic linkage analyses were performed using the "group" and "order" commands of MAPMAKER/EXP, version 3.0b (Lander et al. 1987; Lincoln et al. 1992), with a minimum LOD score threshold of 3.0 and a recombination frequency ≤ 0.4. Marker order was refined using the "annealing 100 50 0.01 0.99" command of CarthaGene software (De Givry et al. 2005). Kosambi function was used to calculate centiMorgan (cM) distances between markers. MapChart 2.2 was used to draw the map (Voorrips 2002).

Statistical analyses

Phenotypic data analysis

Statistical analyses of phenotypic data obtained from field and controlled condition experiments were carried out for each resistance scoring variable using a generalized linear model [(PROC GLM of Statistical Analysis System (SAS) software (SAS Institute Inc., 2000)], as follows: $Pij = \mu + Li + Rj + eij$, where Pij is the mean disease score of the ith RIL located in the jth replicate, μ is the mean of all the data, Li is the RIL i effect, Rj is the replicate j effect and eij is the residual. Normality of residuals and homogeneity of variances were checked using Skewness, Kurtosis and Shapiro–Wilk ($P \geq 0.05$) statistics and Bartlett's test ($P > 0.05$), respectively. Broad sense heritability (h^2) was estimated from ANOVA using the formula $h^2 = \sigma^2 g / [\sigma^2 g + (\sigma^2 e / n)]$, where $\sigma^2 g$ is the genetic variance, $\sigma^2 e$ is the residual variance and n is the number of replicates per line. RIL least-square means were estimated from ANOVA and used for linkage analysis. Pearson correlation coefficients (r^2) between phenotypic data were calculated from adjusted means, obtained from the different field and controlled condition experiments for the two RIL populations, using the PROC CORR procedure of the SAS software.

For each population and country, additional statistical analyses of phenotypic data were performed to evaluate genotype-by-environment interactions for field experiments or genotype-by-strain interactions for controlled condition tests, using a generalized linear model. Estimated variance components were calculated for genotype, environment, and genotype-by-environment or genotype-by-strain interaction.

Additive-effect QTL analysis

For QTL analysis, Composite Interval Mapping (Zeng 1994) was performed using Windows QTL Cartographer 2.5 software (Wang et al. 2005). We used the standard model 6 of the program with ten cofactors selected by forward–backward regression ($P < 0.1$) and a window size of 10 cM. Walk speed was set at 2.0 cM to scan the entire genome. This procedure estimated the log-likehood (LOD) score, additive effect and percentage of phenotypic variance (R^2) every 2.0 cM along each linkage group. Using the permutation test with 1,000 permutations, a minimum 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 confidence interval of a QTL was defined by the region within one LOD from the QTL peak.

Additive-effect QTL were named "*Ae-Ps*" (for ***A**phanomyces **e**uteiches **P**isum **s**ativum*), followed by the linkage group number and the QTL number within the linkage group.

Epistatic-effect QTL analysis

The most significant pairwise epistatic interactions were detected among all possible marker couples of the genetic map, using a two-way ANOVA model, as described in Hamon et al. (2010). The significance level threshold used to detect epistatic-effect QTL was $P < 9.10^{-6}$ for marker pairs mapped from the Baccara × PI 180693 RIL population and $P < 1.3.10^{-5}$ for those mapped from the Baccara × 552 RIL population, corresponding to the probability of getting 0.1 false positive interactions out of the total number of marker pairs tested. Epistatic-effect QTL were named "*Ae-PsE*" (for ***A**phanomyces **e**uteiches **P**isum **s**ativum **E**pistasis*), followed by the epistatic interaction number.

Results

Consensus genetic map

The consensus genetic map, constructed from the two segregating populations Baccara × PI 180693 and Baccara × 552 (in total, 356 RILs), was comprised of 224 markers, including 146 SSR, 74 RAPD, one RGA and three morphological markers distributed over seven linkage groups (Fig. 1). The map covered 1652 cM Kosambi, which was slightly greater than the size of the previously published pea consensus genetic map (Loridon et al. 2005).

For seven of the 224 markers (3%), a non-Mendelian allelic segregation was observed ($\alpha = 0.01$), with a higher frequency of Baccara alleles at three markers on LGVII and of PI 180693 or 552 alleles at four other markers on LGV and LGVII (Fig. 1).

Out of the 224 markers, 149 and 126 markers segregated with reliably scorable polymorphism in Baccara × PI 180693 and Baccara × 552 populations, covering 85%

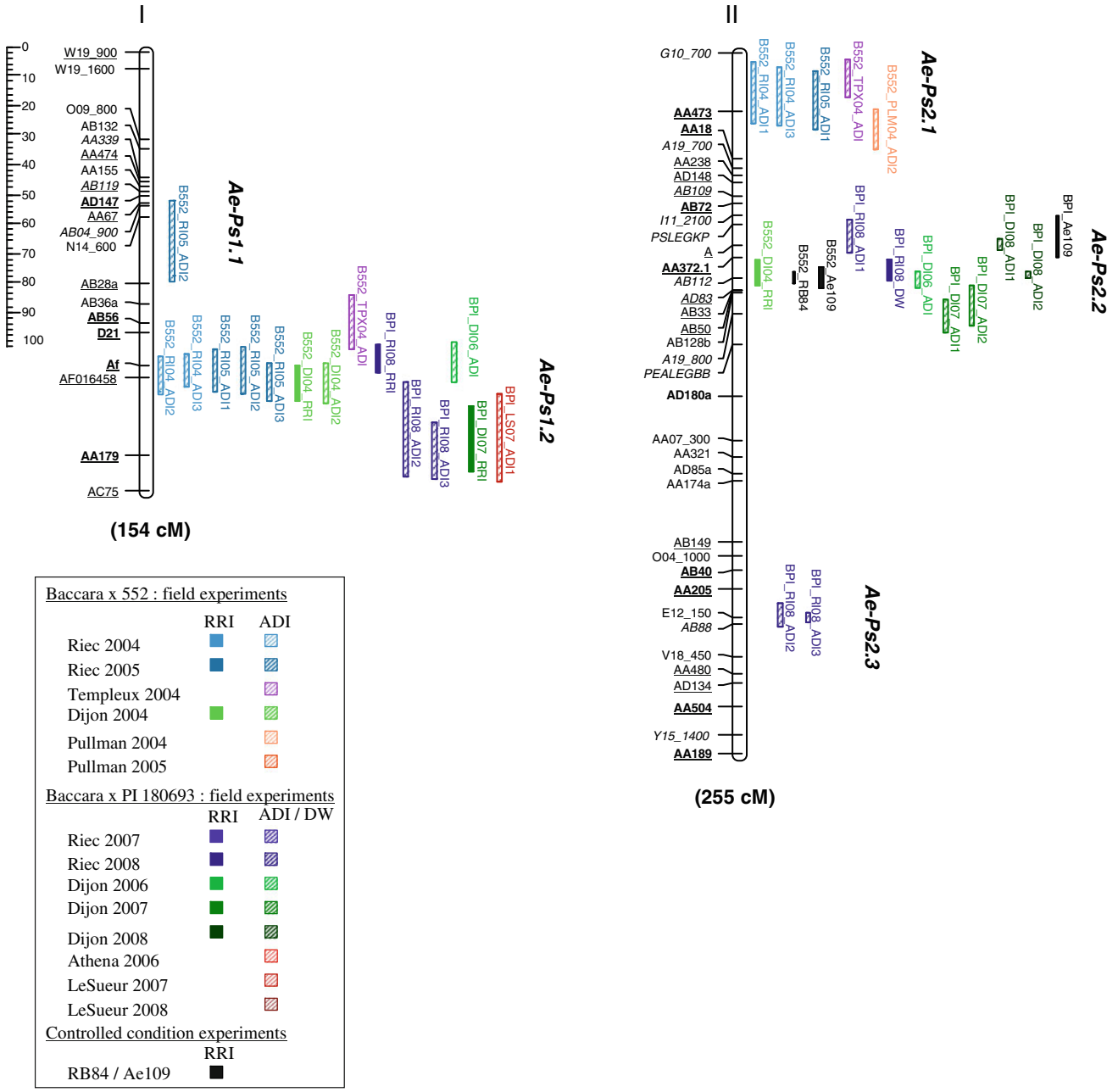

Fig. 1 Consensus genetic consensus map constructed from 356 RILs derived from the crosses Baccara × 552 (178 RILs) and Baccara × PI 180693 (178 RILs) and genomic localization of additive-effect QTL detected for Aphanomyces root rot resistance in different field and controlled conditions, using three resistance criteria (root rot index, aerial decline index and dwarfism). Linkage groups (LG) assigned to the SSR pea reference map (Loridon et al. 2005) are named from I to VII. The size of each LG is indicated in cM Kosambi. Markers with biased allelic segregation are indicated by one *asterisk*. Markers common between the two Baccara × 552 and Baccara × PI 180693 RIL populations are in *bold*. Markers common to the SSR pea reference map (Loridon et al. 2005) are underlined. Specific markers to the Baccara × 552 and the Baccara × PI 180693 RIL populations are indicated in *italic* and in *normal characters*, respectively. Length of additive-effect QTL *boxes* correspond to the LOD-1 support interval (from the peak marker)

(1,505 cM) and 65% (1,361 cM) of the reference genetic map (Loridon et al. 2005), respectively. A total of 51 mapped markers (23%) segregated in both populations. They were well distributed on the consensus genetic map with 5–12 common markers per linkage group, except on LGVI (two common markers) (Fig. 1).

Among the 224 markers, 113 markers (50%), regularly distributed over the seven LG, were common to the previously published pea consensus genetic map (Loridon et al. 2005), including 99 SSR, 11 RAPD and the three morphological markers ("*af*", "*A*" and "*Pl*") (Fig. 1). Their order was well conserved between the present

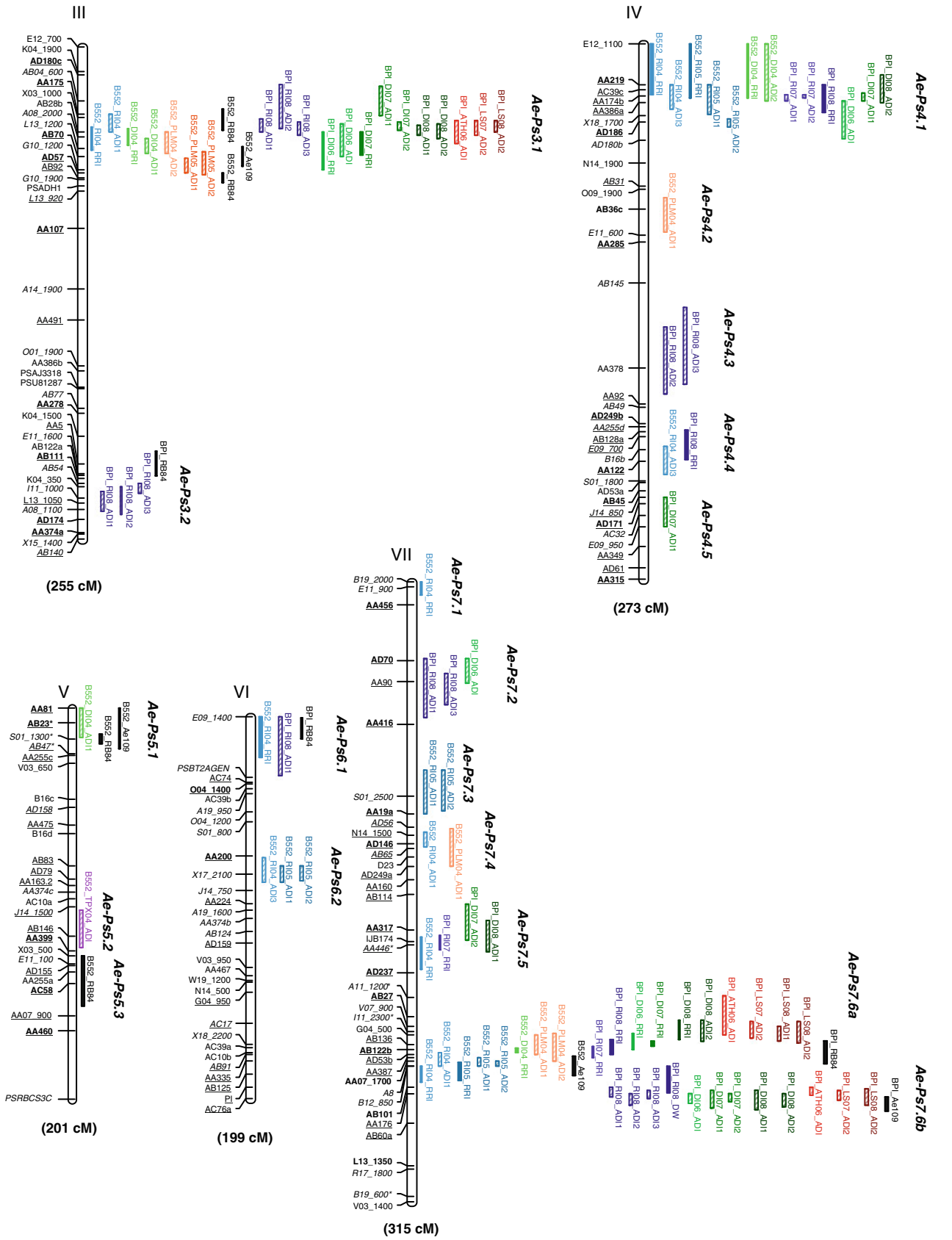

Fig. 1 continued

genetic map and the reference genetic map from Loridon et al. (2005), except in the distal part of LGII, where the order of a block of 10 markers, from markers AB40 to AA189, was inversed. Based on these common markers, only the distal part of LGIII [covering the "*Le*" (internode length) locus region] remains uncovered by the present genetic map.

Aphanomyces root rot resistance data

Controlled condition experiments

The pea controls ranked as expected for adjusted RRI scores obtained with the RB84 and Ae109 strains, confirming pathotypes of the two strains as described in Wicker (2001) (Fig. 2). Specifically, MN313 was resistant to Ae109 (adjusted RRI = 1.65) and susceptible to RB84 (adjusted RRI = 3.50).

Variance analysis of RRI scores obtained in each of the two RIL populations with the two strains indicated significant genotype-by-strain interaction effects ($P < 0.02$, $MS_{interaction} = 0.2$, $MS_{genotype} = 0.5$). For each strain in each population, ANOVA of RRI scores showed highly significant genotypic and replicate effects ($P < 0.0001$). Residuals after ANOVA were normally distributed according to the Shapiro–Wilk ($P \geq 0.05$) and/or Skewness and Kurtosis statistics (data not shown).

In the RB84 strain test, mean-based heritabilities of RRI in the Baccara × PI 180693 and Baccara × 552 RIL populations were high ($h^2 = 0.83$) and moderate ($h^2 = 0.66$), respectively, whereas they were moderate and similar in the Ae109 strain test ($h^2 = 0.59$ and 0.60, respectively).

RRI adjusted mean scores for each population and strain were distributed according to a normal curve, with the exception of RRI scores obtained in the Baccara × PI 180693 population with the RB84 strain, which tended

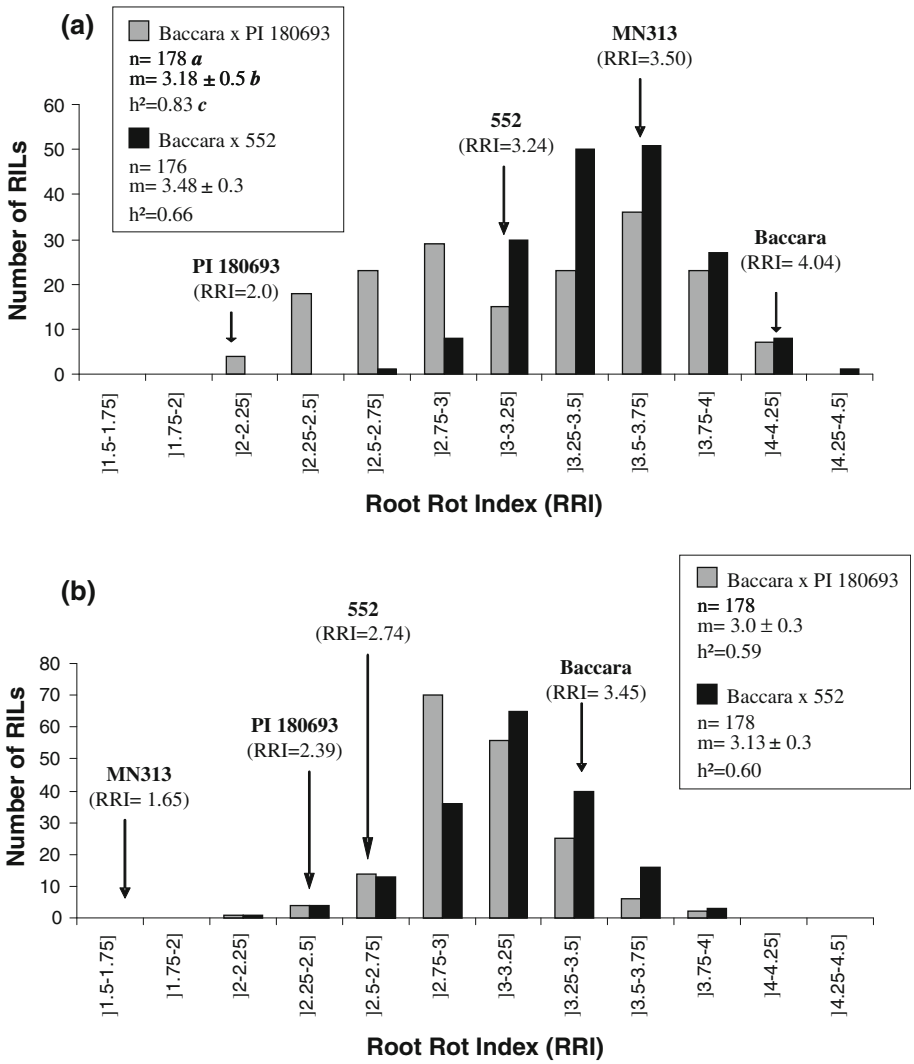

Fig. 2 Frequency distribution of adjusted mean root rot index scores for resistance to two strains of *A. euteiches*, in the Baccara × PI 180693 and Baccara × 552 RIL populations. Values of the parental and control lines are shown by *arrows*. *n* total number of RILs assessed, *m* mean ± standard deviation of the RIL population, h^2 heritability estimate. **a** RB84 strain of *A. euteiches*. **b** Ae109 strain of *A. euteiches*

to fit a bimodal curve (Fig. 2a). The phenotypic range observed in the Baccara × PI 180693 population inoculated with RB84 was slightly higher than those obtained in this population with Ae109 or in the Baccara × 552 population with either strain. Compared with the parental values, transgressive RIL segregants with increased resistance or susceptibility to the Ae109 strain were observed in the two populations. Transgressive RIL segregants with increased resistance or susceptibility to the RB84 strain were observed in the Baccara × 552 population but not in the Baccara × PI180693 population.

Field experiments

For each population, variance analysis of RRI and ADI scores obtained in each country (France or USA) showed highly significant genotype-by-environment interaction effects ($P < 0.0001$, $0.2 < MS_{interaction} < 1.7$ and $0.5 < MS_{genotype} < 4.9$).

Analysis of variance of each field disease variable revealed highly significant genotypic effects ($P < 0.0001$) for all the resistance criteria assessed in the two RIL populations and significant replicate effects ($P < 0.001$) for half of the resistance variables evaluated, mostly ADI. Residuals after each ANOVA were normally distributed according to the Shapiro–Wilk ($P \geq 0.05$) and/or Skewness and Kurtosis statistics (data not shown).

Mean-based heritabilities of the resistance traits scored in the experimental designs established ranged from 0.48 (ADI1, Dijon, 2004) to 0.74 (ADI2, Riec-sur-Belon, 2005) in the Baccara × 552 RIL population and from 0.28 (RRI, Riec-sur-Belon, 2008) to 0.80 (ADI3, Riec-sur-Belon, 2008) in the Baccara × PI 180693 RIL population (supplementary Figs. S1, S2). Heritability values were superior to 0.60 for 73% of all the variables scored in the two populations. Lower heritabilities were mostly observed for RRI and ADI1 variables. A high heritability was obtained for the DW variable ($h^2 = 0.90$), which was calculated from the highly heritable plant height trait.

Frequency distributions of the adjusted mean scores for each variable in the Baccara × 552 or Baccara × PI 180693 RIL population fitted a normal distribution, confirming the quantitative inheritance of partial resistance to *A. euteiches*, except the ADI2 score distribution of the Baccara × PI180693 RILs which more tended to fit a bimodal curve (supplementary Figs. S1, S2).

In each of the two RIL populations, differences were observed between RRI or ADI means and ranges scored in different years and locations. For example, in the Baccara × PI 180693 RIL population, ranges for RRI-scoring were larger at Riec-sur-Belon than at Dijon. In the USA infested fields, disease severity was much higher at LeSueur than at Athena. For most variables in most environments, transgressive segregation was observed, with lines more resistant and/or susceptible than the parental lines. Susceptibility scores were obtained for the control line MN313 in all the environments tested (data not shown).

Correlations

In the Baccara × 552 RIL population, most of the phenotypic data from different field criteria and environments were significantly ($P < 0.001$) and positively correlated, with the exception of the ADI scores assessed in Pullman (USA) in 2004 (supplementary Table S3). Correlations between different field data were markedly higher than between field and controlled condition data. The RB84 strain controlled condition data were generally positively but moderately correlated with some root rot or aerial decline scores assessed in French and USA field conditions. The Ae109 strain data were significantly correlated with the RB84 strain data ($r^2 = 0.37$; $P < 0.001$) but moderately (with RRI scores) or poorly (with ADI scores) correlated with traits scored in French and/or USA field conditions.

In the Baccara × PI 180693 RIL population, correlation coefficients were most significant ($P < 0.001$) between RRI data scored at different French locations, as well as between ADI data scored at different French and USA locations in different years, excluding Riec-sur-Belon 2007 data (supplementary Table S4). Correlations between field ADI and RRI data were more often lower than those between different ADI data. The RB84 and Ae109 strain data were poorly correlated with each other ($r^2 = 0.18$, $P < 0.05$) and were poorly to moderately positively correlated with the root rot scores obtained in French field conditions. The RB84 and Ae109 strain data were negatively correlated with most of the aerial decline scores obtained in French and USA field conditions. The 2008 DW data obtained at Riec-sur-Belon were not significantly correlated with all the other variable data except the 2008 RRI data from the same location. The DW data have to be considered with caution since they correspond to ratios less accurately estimated (one healthy replicate, infested and healthy nurseries at different locations) than the other semi-quantitative variable data.

Additive-effect QTL mapping

From the Baccara × 552 and the Baccara × PI 180693 RIL populations, a total of 60 and 75 additive-effect QTL, respectively, distributed over the seven linkage groups of the consensus genetic map established, was detected for Aphanomyces root rot resistance. The QTL were identified (1) from 16 and 21 field disease variables obtained over six and eight environments in France and the USA for each of

the two populations, respectively, and (2) from two strain data (RB84 and Ae109) in controlled conditions. All the QTL detected clustered in 23 genomic regions, comprising individual QTL with overlapping LOD-1 confidence intervals, covering genomic segments of 8–65 cM. Characteristics and genomic localization of the QTL detected are detailed in Table 2 and Fig. 1. The 23 genomic regions were classified into three groups according to the stability of their effects on resistance to *A. euteiches* in the different conditions and environments studied.

First, five genomic regions, namely *Ae-Ps1.2*, *Ae-Ps2.2*, *Ae-Ps3.1*, *Ae-Ps4.1* and *Ae-Ps7.6* (*a* and *b*), were classified as highly stable, since they were identified using more than five variables per RIL population, in at least one of the two populations. These five regions were detected within the two populations using two to three resistance criteria (RRI, ADI and/or DW). Three of them (*Ae-Ps1.2*, *Ae-Ps3.1*, and *Ae-Ps7.6*) were identified from French and USA field condition data, while the two others (*Ae-Ps2.2*, *Ae-Ps4.1*) were detected from French field condition data only. The three genomic regions *Ae-Ps2.2*, *Ae-Ps3.1*, and *Ae-Ps7.6* were identified for resistance both to RB84 and Ae109 strains in controlled conditions, whereas the regions *Ae-Ps1.2* and *Ae-Ps4.1* were not detected from controlled condition data. *Ae-Ps7.6* was the most consistently detected region, having been detected from a total of 24 and 9 variables in the Baccara × PI 180693 and Baccara × 552 RIL populations, respectively. In the Baccara × PI 180693 RIL population, the *Ae-Ps7.6* genomic region was divided into two sub-genomic regions, *Ae-Ps7.6a* and *Ae-Ps7.6b*, which differ from each other (1) in their resistance allele content (PI 180693 alleles at *Ae-Ps7.6a*, explaining up to 14.4% of the phenotypic variation, and Baccara alleles at *Ae-Ps7.6b*, explaining up to 42.2% of the phenotypic variation for the BPI_DI07_ADI2 trait in accordance with the bimodal distribution trend) and (2) by their specificity (*Ae-Ps7.6b*) or non specificity (*Ae-Ps7.6a*) to the ADI and DW resistance criteria. *Ae-Ps3.1*, which accounted for up to 29.9% of the phenotypic variation, was also consistently detected from 13 and 9 variables in the Baccara × PI 180693 and Baccara × 552 RIL populations, respectively. In addition, *Ae-Ps3.1* would colocalize with a locus responsible for sensitivity to the photoperiod for floral initiation, *Hr*, previously mapped at 2.6 cM of the SSR marker AA175 by Lejeune-Henaut et al. (2008). *Ae-Ps1.2*, which accounted for up to 14.3% of the phenotypic variation and was detected from a total of 14 variables within the two populations, cosegregated with the *af* locus that controls leaf morphogenesis, with resistance alleles in coupling phase with the normal leaf allele, *Af*. *Ae-Ps2.2* and *Ae-Ps4.1*, which accounted for up to 26.9 and 24.5% of the phenotypic variation, respectively, were identified from 11 and 13 French field and/or controlled condition variables within the two RIL populations, respectively. *Ae-Ps2.2* cosegregated with the *A* locus that controls anthocyanin production, as the resistance alleles couple with the flower color alleles. In these five genomic regions, alleles of the resistant parental lines (552 or PI 180693) contributed to the resistance, except in the *Ae-Ps7.6b* region, in which the Baccara alleles accounted for the resistance. In the *Ae-Ps1.2* and *Ae-Ps4.1* regions, Baccara alleles also contributed to the resistance for two and three variables, respectively.

Second, 11 genomic regions (*Ae-Ps2.1, Ae-Ps2.3, Ae-Ps3.2, Ae-Ps4.3, Ae-Ps5.1, Ae-Ps6.1, Ae-Ps6.2, Ae-Ps7.2, Ae-Ps7.3, Ae-Ps7.4* and *Ae-Ps7.5*) were classified as moderately stable, since they were identified using two to five variables per population from at least one of the two RIL populations. Out of the 11 regions, only *Ae-Ps6.1* and *Ae-Ps7.5* were identified from both RIL mapping populations and were also detected using both RRI and ADI field and/or controlled condition data. Among the nine other regions detected from one RIL population, *Ae-Ps3.2* and *Ae-Ps5.1* were identified from RRI controlled condition and ADI field data whereas the seven other regions were specifically detected from ADI field data. All 11 regions were specifically detected in French field environments, excluding two regions (*Ae-Ps2.1* and *Ae-Ps7.4*) that were revealed from both French and USA environments. Most of the 11 regions were not associated with resistance to the two strains tested in controlled conditions, except *Ae-Ps5.1*, detected for resistance to RB84 and Ae109, and *Ae-Ps3.2* and *Ae-Ps6.1*, detected for resistance to RB84. Each of the 11 genomic regions accounted for up to 15.7% of the phenotypic variation, excluding *Ae-Ps6.1*, which accounted for up to 49.4% of the variation for resistance to the RB84 strain, in accordance with the bimodal trend of the trait distribution in the Baccara × PI 180693 population. Parental allelic contribution to the resistance was supplied by 552 or PI 180693 in three regions (*Ae-Ps4.3, Ae-Ps6.2* and *Ae-Ps7.5*), by Baccara in three other regions (*Ae-Ps2.3, Ae-Ps7.2* and *Ae-Ps7.3*) and by both resistant and susceptible parents, depending on the variable, at the five last regions.

Third, seven genomic regions (*Ae-Ps1.1, Ae-Ps4.2, Ae-Ps4.4, Ae-Ps4.5, Ae-Ps5.2, Ae-Ps5.3* and *Ae-Ps7.1*), accounting for 5.2–17% of the variation, were classified as poorly stable, since they were specifically identified using no more than one variable per population, in at least one of the two RIL populations. Resistance alleles were contributed by the resistant parents in all the seven genomic regions except *Ae-Ps4.2* and *Ae-Ps7.1*.

Epistatic-effect QTL mapping

A total of 13 significant pairwise epistatic interactions were identified, including 12 interactions from the "Baccara ×

Table 2 Additive-effect QTL identified from the Baccara × 552 and Baccara × PI 180693 RIL populations for resistance to *A. euteiches* in different field environments, using three resistance scoring criteria, and towards different strains in controlled conditions (values obtained by Windows QTL Cartographer 2.5, LOD threshold ≥2.8)

LG	QTL name (most probable and maximal sizes)[a]	Scoring criterion[b]	Position (cM)[c]	Left marker[d]	LOD[e]	LOD-1 support interval (cM)[f]	R^2 (%)[g]	Additive effect[h]
I	*Ae-Ps1.1* (0.1–26 cM)	B552_RI05_ADI2	63.2	N14_600	2.9	52.9 78.5	6.1	0.20
	Ae-Ps1.2 (46–65 cM)	B552_DI04_ADI2	118.1	AF0164458	3.3	110.4 124.8	13.7	0.33
		B552_DI04_RRI	118.1	AF0164458	3.4	111.1 124.3	14.3	0.14
		B552_RI04_ADI2	113.5	Af	6.2	108.4 120.7	13.6	0.28
		B552_RI04_ADI3	111.5	Af	7.3	108.4 120.7	13.2	0.46
		B552_RI05_ADI1	109.6	D21	5.2	105.3 121.4	7.7	0.22
		B552_RI05_ADI2	116.1	AF0164458	6.3	104.6 122.1	13.8	0.30
		B552_RI05_ADI3	116.1	AF0164458	3.1	110.3 123.9	10.4	0.28
		B552_TPX04_ADI	96.2	AB56	3.2	86.3 106.0	5.2	−0.19
		BPI_DI06_ADI	115.5	Af	3.5	103.0 128.5	3.8	−0.21
		BPI_DI07_RRI	136.1	AF0164458	5.7	126.1 147.2	12.0	0.15
		BPI_LS07_ADI1	138.1	AA179	3.3	121.8 151.7	8.0	0.25
		BPI_RI08_ADI2	136.1	AF0164458	5.2	119.1 148.3	9.9	0.24
		BPI_RI08_ADI3	141.8	AA179	3.1	130.4 149.9	3.7	0.19
		BPI_RI08_RRI	109.6	D21	3.4	104.2 114.3	5.8	0.16
II	*Ae-Ps2.1* (14–30 cM)	B552_PLM04_ADI2	24.0	AA473	3.3	20.0 33.0	10.7	−0.15
		B552_RI04_ADI1	12.0	G10_700	3.3	3.4 24.6	8.7	0.11
		B552_RI04_ADI3	12.0	G10_700	4.2	4.7 25.7	9.6	0.39
		B552_RI05_ADI1	14.0	G10_700	5.0	7.3 27.5	9.5	0.24
		B552_TPX04_ADI	10.0	G10_700	4.9	3.2 15.2	15.4	0.35
	Ae-Ps2. (30–45 cM)	B552_Ae109	80.4	AA372.1	4.6	76.0 83.3	8.4	0.09
		B552_DI04_RRI	80.4	AA372.1	4.2	74.9 84.3	5.6	0.08
		B552_RB84	82.3	AB112	5.3	80.0 84.3	6.4	0.08
		BPI_Ae109	66.0	PSLEGKP	3.7	58.4 74.0	8.7	0.08
		BPI_DI06_ADI	82.3	AB112	16.7	79.8 86.1	23.2	0.37
		BPI_DI07_ADI1	95.7	A19_800	9.3	90.2 102.9	26.9	0.47
		BPI_DI07_ADI2	86.7	AB128b	6.8	84.6 98.7	7.9	0.32
		BPI_DI08_ADI1	70.0	A	12.9	67.3 71.9	20.9	0.45
		BPI_DI08_ADI2	80.4	AA372.1	9.2	78.4 80.7	17.5	0.28
		BPI_RI08_ADI1	66.0	PSLEGKP	3.6	59.7 72.5	6.8	0.17
		BPI_RI08_DW	80.4	AA372.1	5.2	76.4 81.0	13.1	0.05
	Ae-Ps2.3 (0.1–8 cM)	BPI_RI08_ADI2	204.1	E12_150	3.0	200 207.8	4.5	−0.16
		BPI_RI08_ADI3	204.1	E12_150	6.6	203.8 206.6	8.4	−0.39
III	*Ae-Ps3.1* (45–54 cM)	B552_Ae109	62.1	AB70	5.2	62.1 64.4	10.4	0.11
		B552_DI04_ADI1	54.0	A08_2000	5.4	51.9 56.9	11.1	0.17
		B552_DI04_RRI	48.0	A08_2000	6.0	43.5 52.2	7.4	0.09
		B552_PLM04_ADI2	52.0	A08_2000	3.2	45.3 56.8	9.5	0.16
		B552_PLM05_ADI1	65.5	G10_1200	3.5	64.0 68.5	9.1	0.22
		B552_PLM05_ADI2	62.1	AB70	5.3	55.8 68.5	14.1	0.24
		B552_RB84	37.4	X03_1000	6.5	32.8 44.1	9.4	0.10
		B552_RB84	69.9	AD57	4.1	68.7 73.1	5.6	0.09
		B552_RI04_ADI1	41.4	X03_1000	5.6	35.2 45.4	13.0	0.13
		B552_RI04_RRI	47.0	A08_2000	4.7	41.1 55.1	7.3	0.09
		BPI_ATH06_ADI	41.4	X03_1000	7.5	38.6 45.0	15.5	0.23

Table 2 continued

LG	QTL name (most probable and maximal sizes)[a]	Scoring criterion[b]	Position (cM)[c]	Left marker[d]	LOD[e]	LOD-1 support interval (cM)[f]	R^2 (%)[g]	Additive effect[h]
		BPI_DI06_ADI	43.4	X03_1000	12.4	40.9 43.5	16.6	0.31
		BPI_DI06_RRI	62.1	AB70	3.8	45.0 65.0	7.9	0.09
		BPI_DI07_ADI1	27.0	AD180c	4.9	20.9 35.6	12.9	0.31
		BPI_DI07_ADI2	41.4	X03_1000	20.5	39.4 43.5	29.9	0.61
		BPI_DI07_RRI	52.0	A08_2000	4.6	44.8 58.0	8.6	0.13
		BPI_DI08_ADI1	43.4	X03_1000	9.9	41.2 46.4	18.0	0.42
		BPI_DI08_ADI2	43.4	X03_1000	15.5	40.4 48.1	26.2	0.34
		BPI_LS07_ADI2	41.4	X03_1000	8.2	38.6 45.0	16.0	0.37
		BPI_LS08_ADI2	41.4	X03_1000	14.3	38.9 47.0	26.7	0.39
		BPI_RI08_ADI1	41.4	X03_1000	7.6	37.5 43.4	13.0	0.23
		BPI_RI08_ADI2	25.0	AD180c	7.6	19.2 32.7	21.6	0.35
		BPI_RI08_ADI3	41.4	X03_1000	7.4	38.7 47.1	10.9	0.35
	Ae-Ps3.2 (21–38 cM)	BPI_RB84	213.6	AA5	2.9	203.2 218.0	5.3	0.14
		BPI_RI08_ADI1	234.1	L13_1050	3.1	228.7 239.4	4.4	−0.14
		BPI_RI08_ADI2	232.1	K04_350	3.0	226.1 241.3	5.3	−0.18
		BPI_RI08_ADI3	224.2	AB54	5.9	223.8 229.0	8.6	−0.41
IV	*Ae-Ps4.1* (31–49 cM)	B552_DI04_ADI2	14.0	E12_1100	3.1	0.0 29.5	9.2	0.25
		B552_DI04_RRI	12.0	E12_1100	4.6	0.0 27.7	11.4	0.11
		B552_RI04_ADI3	26.9	AC39c	4.9	20.1 34.1	11.0	0.41
		B552_RI04_RRI	10.0	E12_1100	5.2	0.0 18.0	20.9	0.14
		B552_RI05_ADI1	28.9	AA174b	4.2	19.6 36.0	8.4	0.23
		B552_RI05_ADI2	40.7	AD186	5.5	39.3 43.8	7.7	0.23
		B552_RI05_RRI	19.7	AA219	6.8	0.0 27.6	12.2	0.12
		BPI_DI06_ADI	36.1	AA386a	6.0	28.8 48.7	7.5	0.21
		BPI_DI07_ADI1	26.7	AA174b	6.3	25.1 27.3	10.5	−0.30
		BPI_DI08_ADI2	24.7	AC39c	4.0	15.3 30.2	6.0	0.16
		BPI_RI07_ADI1	26.9	AA174b	7.5	25.9 28.6	12.9	−0.35
		BPI_RI07_ADI2	26.9	AA174b	13.2	25.9 27.9	24.5	−0.58
		BPI_RI08_RRI	24.7	AC39c	3.1	20.0 36.1	5.0	0.13
	Ae-Ps4.2 (0.1–18 cM)	B552_PLM04_ADI1	90.4	AB36c	5.3	80.5 98.1	17.0	−0.30
	Ae-Ps4.3 (7–39 cM)	BPI_RI08_ADI2	164.5	AA378	2.9	140.5 173.7	6.3	0.19
		BPI_RI08_ADI3	157.7	AB145	3.7	134.3 168.6	5.6	0.23
	Ae-Ps4.4 (13–24 cM)	B552_RI04_ADI3	211.1	AA122	3.2	200.8 216.5	5.3	0.29
		BPI_RI08_RRI	197.9	PSAJ3318	3.6	192.2 207.2	6.9	0.17
	Ae-Ps4.5 (0.1–18 cM)	BPI_DI07_ADI1	236.4	AD171	3.9	226.9 244.9	8.8	0.26
V	*Ae-Ps5.1* (9–30 cM)	B552_Ae109	14.3	AB23	3.0	0.0 22.8	6.1	−0.08
		B552_DI04_ADI1	8.0	AA81	3.2	0.0 15.6	6.6	0.13
		B552_RB84	16.8	S01_1300	11.7	14.4 30.2	15.7	−0.13
	Ae-Ps5.2 (0.1–21 cM)	B552_TPX04_ADI	117.2	J14_1500	3.9	107.7 128.4	11.7	0.30
	Ae-Ps5.3 (0.1–27 cM)	B552_RB84	141.8	AA255a	4.1	131.2 157.9	5.9	0.07
VI	*Ae-Ps6.1* (10–32 cM)	B552_RI04_RRI	8.0	E09_1400	3.9	0.0 19.3	11.2	−0.10
		BPI_RB84	0.0	E09_1400	5.2	0.0 10.0	49.4	0.39
		BPI_RI08_ADI1	10.0	E09_1400	3.0	0.0 31.8	18.1	−0.27
	Ae-Ps6.2 (4–13 cM)	B552_RI04_ADI3	75.3	AA200	4.7	69.8 82.8	8.3	0.36
		B552_RI05_ADI1	79.2	AA200	5.1	74.5 83.1	7.2	0.22
		B552_RI05_ADI2	79.2	X17_2100	6.2	74.3 82.1	8.7	0.25

Table 2 continued

LG	QTL name (most probable and maximal sizes)[a]	Scoring criterion[b]	Position (cM)[c]	Left marker[d]	LOD[e]	LOD-1 support interval (cM)[f]	R^2 (%)[g]	Additive effect[h]
VII	*Ae-Ps7.1* (0.1–9 cM)	B552_RI04_RRI	2.7	E11_900	3.4	0.0 8.7	5.2	−0.07
	Ae-Ps7.2 (11–29 cM)	BPI_DI06_ADI	42.1	AD70	3.9	37.8 50.4	4.9	−0.17
		BPI_RI08_ADI1	44.1	AD70	3.0	37.8 66.3	5.7	−0.15
		BPI_RI08_ADI3	53.3	AA90	4.0	45.0 60.5	6.4	−0.25
	Ae-Ps7.3 (5–22 cM)	B552_RI05_ADI1	104.4	S01_2500	4.3	89.5 111.3	6.5	−0.20
		B552_RI05_ADI2	99.9	AA416	3.1	89.8 110.3	5.1	−0.18
	Ae-Ps7.4 (0.1–9 cM)	B552_PLM04_ADI1	126.9	N14_1500	2.9	125.2 130.5	8.1	0.20
		B552_RI04_ADI1	126.9	N14_1500	6.9	121.3 129.9	12.2	−0.14
	Ae-Ps7.5 (22–35 cM)	B552_RI04_RRI	187.5	AA446	2.9	177.5 195.1	6.3	0.08
		BPI_DI07_ADI2	165.4	AB114	4.6	160.0 178.5	7.4	0.31
		BPI_DI08_ADI1	175.5	AA317	5.0	168.3 184.6	7.5	0.29
		BPI_RI07_RRI	179.5	AA317	4.9	175.8 184.3	9.9	0.18
	Ae-Ps7.6 (14–17 cM)	B552_Ae109	245.2	AA07_1700	3.6	240.1 251.3	7.0	0.08
		B552_DI04_RRI	238.2	AB122b	11.1	236.2 238.4	19.9	0.15
		B552_PLM04_ADI1	235.9	AB136	4.5	228.8 239.8	11.8	0.26
		B552_PLM04_ADI2	238.2	AD53b	3.7	236.2 243.5	11.6	0.16
		B552_RI04_ADI1	241.3	AA387	3.1	238.7 248.3	5.9	0.10
		B552_RI04_RRI	250.3	B12_850	6.3	246.4 252.9	12.8	0.11
		B552_RI05_ADI1	245.2	AA07_1700	5.6	241.2 246.5	8.0	0.22
		B552_RI05_ADI2	245.2	AA07_1700	4.0	243.1 246.4	5.9	0.20
		B552_RI05_RRI	248.3	B12_850	3.2	243.9 253.5	6.6	0.09
	Ae-Ps7.6a (23–38 cM)	BPI_ATH06_ADI	222.3	AB27	4.4	208.0 228.5	9.6	0.20
		BPI_DI06_RRI	238.2	AB122b	3.0	236.2 239.0	6.0	0.08
		BPI_DI07_RRI	233.9	AB136	8.9	232.4 235.3	13.7	0.19
		BPI_DI08_ADI2	226.0	V07_900	4.9	220.7 231.5	8.3	0.22
		BPI_DI08_RRI	224.0	V07_900	5.5	220.3 231.6	13.6	0.11
		BPI_LS07_ADI2	226.0	V07_900	5.2	221.1 230.6	10.8	0.34
		BPI_LS08_ADI1	228.5	G04_500	4.0	223.8 232.1	7.9	0.22
		BPI_LS08_ADI2	226.0	G04_500	3.7	221.3 232.8	7.2	0.23
		BPI_RB84	245.2	AA07_1700	6.2	234.0 245.5	14.4	0.21
		BPI_RI07_RRI	238.2	AB122b	5.4	234.6 241.3	11.0	0.20
		BPI_RI08_RRI	235.9	AB136	6.8	231.6 239.6	10.9	0.21
	Ae-Ps7.6b (24–28 cM)	BPI_Ae109	266.8	AA176	6.0	259.4 270.5	13.8	0.10
		BPI_ATH06_ADI	259.4	AB101	7.6	257.4 261.7	15.4	−0.27
		BPI_DI06_ADI	262.8	AA176	14.8	260.8 265.8	20.9	−0.46
		BPI_DI07_ADI1	262.8	AA176	12.2	258.9 267.9	24.4	−0.44
		BPI_DI07_ADI2	262.8	AA176	25.8	260.8 265.4	42.2	−0.76
		BPI_DI08_ADI1	260.8	AA176	2.9	259.2 269.6	4.9	−0.22
		BPI_DI08_ADI2	262.8	AA176	10.3	260.8 267.6	19.8	−0.34
		BPI_LS07_ADI2	260.8	AA176	9.1	259.2 264.3	16.3	−0.41
		BPI_LS08_ADI2	262.8	AA176	12.3	259.2 267.0	26.0	−0.42
		BPI_RI08_ADI1	259.4	AB101	10.7	257.4 262.3	17.7	−0.31
		BPI_RI08_ADI2	260.8	AA176	5.5	259.2 267.9	9.5	−0.24
		BPI_RI08_ADI3	260.8	AA176	13.1	259.5 263.8	18.3	−0.44

Table 2 continued

LG	QTL name (most probable and maximal sizes)[a]	Scoring criterion[b]	Position (cM)[c]	Left marker[d]	LOD[e]	LOD-1 support interval (cM)[f]	R^2 (%)[g]	Additive effect[h]
		BPI_RI08_DW	243.2	AA07_1700	6.1	243.0 256.3	11.6	0.05

[a] The most probable size of the QTL is defined by the interval (in cM Kosambi) covered by LOD peaks of all the individual QTL clustered in the region (set 0.1 cM by default when the region included only one QTL). The maximal size of the QTL is defined by the interval (in cM Kosambi) covered by the overlapping LOD-1 confidence intervals of all the individual QTL clustered in the region

[b] RIL populations: *B552* Baccara × 552, *BPI* Baccara × PI 180693, locations: *RI* Riec-sur-Belon (FR), *DI* Dijon (FR), *TPX* Templeux (FR), *ATH* Athena (US), *PLM* Pullman (US), *LS* LeSueur (US), years: *04* 2004, *05* 2005, *06* 2006, *07* 2007, *08* 2008, resistance criteria: *RRI* root rot index, *ADI* aerial decline index, *DW* dwarfism

[c] QTL position from the first marker of the linkage group (in cM Kosambi)

[d] Nearest marker from the LOD score peak of the QTL

[e] Log of likelihood ratio (LOD) peak value at the QTL position for each variable

[f] Position of the lower and upper ends of the QTL confidence, from the first marker of the linkage group (in cM Kosambi)

[g] Percentage of phenotypic variance explained by an individual QTL

[h] Effect of substituting Baccara alleles for 552 or PI 180693 alleles at the QTL. A positive sign indicates that QTL alleles increasing the resistance for RRI and ADI criteria are contributed by the resistant parent 552 or PI 180693 whereas a negative sign means that resistant alleles are brought by the susceptible parent Baccara, and inversely for DW criterion

PI 180693" RIL population and one interaction from the Baccara × 552 RIL population (Fig. 3; supplementary Table S5). The 13 epistatic QTL individually explained between 10.2 and 14.7% of the phenotypic variation, and 10 of them were detected exclusively using ADI or DW scores. Two of the 13 epistatic QTL, *Ae-PsE3* and *Ae-PsE7*, were identified from two and three different French and/or USA field environments, respectively. The 11 other epistatic QTL were specific to one variable type from a single environment and three of them (*Ae-PsE1*, *Ae-PsE8* and *Ae-PsE13*) involved several linked markers.

Among the 13 epistatic QTL, seven QTL-by-QTL interactions (*Ae-PsE5, Ae-PsE7, Ae-PsE8, Ae-PsE10, Ae-PsE11, Ae-PsE12* and *Ae-PsE13)* were identified, since they were detected between markers linked to nine of the 23 additive-effect genomic regions identified in this study. The most notable QTL-by-QTL interaction, *Ae-PsE5*, involved markers linked to the highly stable *Ae-Ps7.6* and *Ae-Ps2.2* additive-effect genomic regions. Four QTL-by-genetic-background epistatic interactions (*Ae-PsE2, Ae-PsE3, Ae-PsE4* and *Ae-PsE6*) were detected between markers linked to additive-effect genomic regions (*Ae-Ps1.1*, *Ae-Ps2.3* and *Ae-Ps3.1*) and markers not associated with additive-effect QTL. Seven intra-chromosomic interactions, which involve marker pairs from the same linkage groups, were identified out of the 13 epistatic QTL, including four QTL-by-QTL interactions and one intra-QTL interaction (*Ae-Ps7.6*).

For all but two significant epistatic interactions (*Ae-PsE1* and *Ae-PsE12*), PI 180693 or 552 alleles at one or both loci involved in the interaction contributed to an increased resistance to *A. euteiches*.

Discussion

Identification of new consistent genetic loci associated with partial resistance to *A. euteiches* in pea

In this study, we identified a total of 135 additive-effect QTL corresponding to 23 genomic regions and 13 significant epistatic interactions associated with partial resistance to *A. euteiches* within two new pea RIL populations derived from crosses involving two sources of resistance effective in France and in the USA. These results confirm the complexity of inheritance of partial resistance to *A. euteiches*. However, 5 and 11 additive-effect genomic regions were detected with high or moderate consistency, respectively, and five epistatic-effect QTL were also consistently revealed from different variables or marker positions.

Based on a few common markers, genomic positions of the most consistent QTL identified for partial resistance to *A. euteiches* in this study were compared to those reported in previous studies (Pilet-Nayel et al. 2002, 2005). Among the five highly consistent genomic regions identified in this study (*Ae-Ps1.2, Ae-Ps2.2, Ae-Ps3.1, Ae-Ps4.1* and *Ae-Ps7.6*), four were not consistently reported previously. Out of these five regions, only *Ae-Ps1.2* on LGI colocalizes with the *Aph3* QTL previously consistently identified from the Puget × 90-2079 RIL population (Pilet-Nayel et al. 2002) using the common *af* morphological marker. *Ae-Ps4.1* may colocalize with the minor QTL *Aph14* due to the AA219 SSR marker, and *Ae-Ps3.1* do not colocalize with any previously identified QTL due to the AA278 SSR

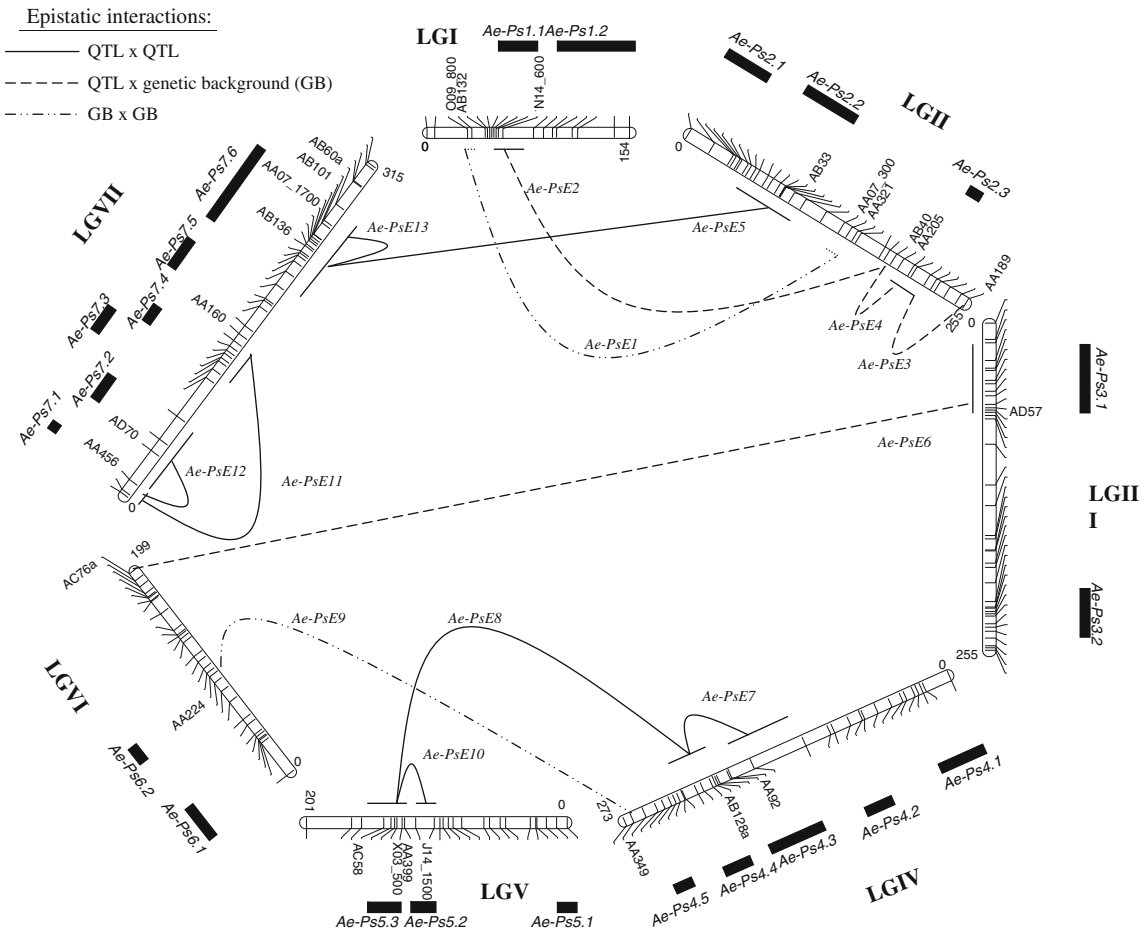

Fig. 3 Genomic localization, on the seven linkage groups (LG) of the pea consensus genetic map, of the 23 additive-effect genomic regions and 13 epistatic-effect QTL detected for Aphanomyces root rot resistance in field and controlled conditions, from the two Baccara × 552 and Baccara × PI 180693 RIL populations. Additive and epistatic-effect QTL are referenced to the Table 2 and supplementary data 5, respectively. Length of each additive-effect genomic region *black box* corresponds to the interval covering overlapping LOD-1 support interval of clustered individual QTL in the region. Names of markers involved in the epistatic interactions are mentioned. The size of each LG is given in cM Kosambi

marker (Pilet-Nayel et al. 2002, 2005). Genomic positions of *Ae-Ps2.2* and *Ae-Ps7.6* could not be precisely estimated using the previously published Puget × 90-2079 genetic map due to a lack of common markers, but no consistent QTL for resistance to *A. euteiches* were previously identified on LGII and LGVII in the cross Puget × 90-2079. However, two other consistent QTL, *Aph1* and *Aph2*, were identified on LGIV and LGV from the Puget × 90-2079 RIL population. Due to the J14_850 RAPD marker, *Aph1* colocalizes with the *Ae-Ps4.5* genomic region, specifically identified for only one variable and therefore considered as poorly stable in this study. In the *Aph2* genomic region, there were no markers in common with the present genetic map, but only moderately to poorly stable QTL were identified on LGV in this study. A fifth less consistent but major-effect genomic region, *Ae-Ps6.1* ($R^2 = 49.4\%$), considered as moderately stable, was also newly detected within French data in this study. This study of new bi-parental RIL populations, generated from new independent resistance sources has brought useful information to get an overview of choice stable QTL. The mapping of additional common markers between the present and the previous genetic maps carrying Aphanomyces resistance QTL will be necessary for further comparisons of resistance QTL between studies. More markers would also be useful on the individual genetic maps used for generating the consensus map described in this study, as they would allow for a higher coverage of the pea genome, especially in the region of the "*Le*" locus on LGIII, reportedly associated with variation for resistance to other stresses such as ascochyta blight (Prioul et al. 2004) or frost (Lejeune-Henaut et al. 2008), in order to ascertain the presence of other QTL responsible for resistance to *A. euteiches*.

In this study, we also identified several previously unreported epistatic interactions, most involving markers linked to additive-effect QTL. Forty percent of these

interactions involved marker pairs from the same linkage groups that have an additive effect on resistance, which may suggests the occurrence of duplication events in regions carrying additive-effect QTL (Ellis and Poyser 2002).

Stability of Aphanomyces resistance QTL effects

In this study, a high stability of QTL effects was observed towards *A. euteiches* strains, environments, resistance criteria, test conditions and RIL populations studied. Such consistency has been infrequently reported for polygenetic disease resistance in prior published studies (e.g. Buerstmayr et al. 2009).

QTL stability over *A. euteiches* strains

Seven genomic regions containing resistance to *A. euteiches* were detected in controlled conditions, among which four were common to the RB84 and Ae109 strains, including the highly consistent regions *Ae-Ps2.2, Ae-Ps3.1* and *Ae-Ps7*.6. Three others were specific to the RB84 strain, including *Ae-Ps6.1*, which expressed a major effect on resistance to RB84 ($R^2 = 49.4\%$) in the Baccara × PI 180693 RIL population. Although RB84 and Ae109 strains belong to two different pathotype groups, all the additive-effect QTL identified for resistance to the Ae109 strain therefore colocalized with additive-effect QTL detected for resistance to the RB84 strain of *A. euteiches*. These results suggest that some common or linked genes in pea would control resistance to the Ae109 and RB84 strains. Such colocalizations of QTL for resistance to *A. euteiches* RB84 and Ae109 strains were also recently observed and discussed in *M. truncatula*, where a major-effect locus, *AER1*, identified for resistance to RB84 also expressed a partial effect on resistance to Ae109 (Hamon et al. 2010). However, preliminary mapping results of bridge gene-based markers in the *AER1* region between pea and *M. truncatula* show that the *AER1* locus would not colocalize with QTL controlling resistance to *A. euteiches* Ae109 and RB84 strains in pea (data not shown). Moreover, in *M. truncatula*, QTL controlling resistance to Ae109 and not to RB84 have been identified. In our study in pea, QTL specific to the RB84 strain and to the original infested field of RB84 (Riec-sur-Belon) (*Ae-Ps3.2* and *Ae-Ps6.1*) were identified, suggesting genetic specificities within RB84 pathogenicity compared to Ae109. Further molecular characterization of the different pathotypes of *A. euteiches* would be required to better understand the genes underlying their pathogenic specificities. The recent development of genomic tools in *A. euteiches* (Gaulin et al. 2008; Madoui et al. 2007) and the ongoing sequencing of the *A. euteiches* genome (Gaulin et al. 2010) will most likely help in discovering *A. euteiches* pathogenicity genes.

QTL stability over environments

Only few QTL identified in this study were specific to locations or years. Among the 16 regions detected from more than one variable in at least one RIL population, most of them were non-specific to the environments tested, in terms of countries, locations or years. Particularly, the five regions detected in USA environments, including the highly consistent regions *Ae-Ps1.2, Ae-Ps3.1* and *Ae-Ps7.6,* were also all found in French environments. These observations are in accordance with the significant correlations discovered in each population between most of the phenotypic data obtained from the different environments studied. They are likewise in accordance with the significant but low genotype-by-environment interaction effects compared to the genotype effects in each of the two populations studied. Stable QTL toward environments may thus correspond to generalist QTL, effective in different soils, climates and crop conditions and potentially towards different strains of the pathogen. In the highly infested nurseries with irrigation possibilities used in this study, climate and soil structure variations characterizing the different test sites and years did not create significant QTL instability factors, contrary to the findings in most literature, where genotype × environment effects (Marx et al. 1972; Shehata and Pfleger 1983) and soil type effects (Weeden et al. 2000, 2001) on expression of *A. euteiches* resistance have been reported. Pathogen populations of *A. euteiches* present in the infested nurseries did not allow the identification of many country or location-specific QTL, especially since few QTL specificities were observed towards pathotypes of *A. euteiches* (all the QTL identified for the Ae109 strain were also detected for the RB84 strain). A recent analysis of *A. euteiches* pathogenic diversity in the different nurseries used in this study showed that only pathotype I isolates were present in most of the French and USA nurseries (Onfroy, personal communication). However, possible genetic differences between pathotype I strains or heterogeneous occurrence of other pathogens of the root rot complex (*Fusarium* spp., *Phoma medicaginis*) in the different nurseries may explain the country- or location-specificity of some resistance QTL, i.e. *Ae-Ps2.2* and *Ae-Ps4.1*, which were consistently specific to French environments, and *Ae-Ps4.4, Ae-Ps6.1* and *Ae-Ps6.2*, which were specifically detected from Riec-sur-Belon data over several years. Further characterization of *A. euteiches* diversity and occurrence of the root rot complex pathogens in the infested nurseries should contribute to a better understanding of the stability of QTL expression towards environments and strains.

QTL stability over resistance criteria and test conditions

All seven genomic regions, with the exception of *Ae-Ps5.3*, detected for resistance in controlled conditions were also associated with resistance in at least one field condition, despite the poor to moderate correlations observed between resistance scores from the two condition types. However, 16 additional genomic regions were specifically identified from field condition data. Out of the total 23 regions identified for Aphanomyces root rot resistance, all but one (*Ae-Ps7.1*) of the nine genomic regions associated with field RRI resistance were also detected for field AGI and/or DW resistance. However, 14 additional ADI and/or DW specific regions, including the consistent QTL *Ae-Ps7.6b*, were also identified in field conditions. These results suggest that (1) most of the genomic regions controlling the reduction of root symptoms of *A. euteiches* at early plant development stages are consequently involved in the reduction of aerial symptoms at later plant development stages and (2) additional genomic regions also control tolerance to *A. euteiches* and, probably, other pathogens of the root rot complex at later plant development stages.

QTL stability over RIL populations

Eight genomic regions, including the five highly stable QTL, were identified from the two RIL populations studied. 552 and PI 180693 provided resistance alleles for most of the variables associated to the resistance in these regions. Although not reported to date, it is likely that the two germplasm lines, PI 180693 and 552, identified or selected for resistance to *A. euteiches* in the USA during the last 30 years would share some common genetic basis. QTL common to the two RIL populations could alternatively correspond to same or different alleles from common or closely linked loci. In this study, first results were obtained for comparing 552 and PI 180693 resistance allelic effects and potentially reducing confidence intervals at some common genomic regions, by conducting a joint QTL analysis of resistance to RB84 and Ae109 strains of the two populations using a multiparental connected model (Blanc et al. 2006) implemented in the MC-QTL software (Jourjon et al. 2005) (data not shown). Results showed that the effects of the PI 180693 and 552 alleles were higher than the other parental allele effects at the *Ae-Ps7.6* region (on resistance to both strains) and the *Ae-Ps2.2* and *Ae-Ps3.1* regions (on resistance to Ae109 strain), respectively.

Choice of QTL for MAS of resistance to *A. euteiches* in pea

This study provides bases for the choice of consistent QTL to use in MAS schemes to increase current levels of resistance to *A. euteiches* in pea. The five highly stable QTL (*Ae-Ps1.2, Ae-Ps2.2, Ae-Ps3.1, Ae-Ps4.1* and *Ae-7.6*) are choice candidates for use in MAS, especially the most consistent QTL, *Ae-Ps7.6,* as they comprise resistance alleles covering a broad spectrum of environments and/or pathotypes and resistance criteria. The epistatic effect of the two QTL *Ae-Ps2.2* and *Ae-7.6* could also be mined by tracing resistance alleles of both QTL in MAS. Codominant polymorphic SSR markers linked to these five genomic regions are available for tracing out resistance alleles in different MAS schemes.

However, further studies would be required for better optimizing the use of these most consistent QTL in MAS programs. First, the reduction of their confidence intervals will be of great interest to breeders, especially in regions where resistance alleles were contributed by resistant or susceptible parents depending on the variables that suggests possible contribution of linked genes to the resistance in the target region. Knowledge of the diversity of resistance alleles and the possible linkages between resistance and undesirable alleles in target regions will also be of high interest. A more extensive view of the diversity of Aphanomyces resistance loci and alleles from different pea sources of resistance and their comparison with loci controlling agronomic traits in pea (Bourion et al. 2010; Lejeune-Henaut et al. 2008; Burstin et al. 2007; Prioul et al. 2004) could thus be obtained, especially by using a QTL meta-analysis approach that could additionally help in reducing QTL confidence intervals (Hanocq et al. 2007). More codominant polymorphic markers would also be necessary to fine map QTL from QTL-NILs (Near Isogenic Lines) segregating progenies and possibly break unfavourable linkages, such as the ones identified in this study between resistance and undesirable foliar type or anthocyanin production alleles. The fast evolution of sequencing technologies should help in developing new markers, such as SNP markers, in target regions in pea (Macas et al. 2007; Deulvot et al. 2010). Second, it would be important to validate target QTL effects in different genetic backgrounds, since QTL effects have often been reported not to be effective in different mapping populations (Liao et al. 2001; Steele et al. 2006) or to be lost when transferred into new genetic backgrounds (Hospital 2009), due to epistatic interactions. The creation of NILs for the target resistance QTL, using a marker-assisted back-cross scheme, will be useful to validate the stability of the main resistance QTL effects in different agronomic backgrounds. Finally, knowledge of the effects of different QTL combinations on levels of resistance and the evolution of pathogen populations will be necessary for identifying main QTL combinations that could durably increase levels of partial resistance to *A. euteiches* in pea.

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