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ORIGINAL ARTICLE

Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines

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Abstract

Key message **Marker-assisted backcrossing was used to generate pea NILs carrying individual or combined resistance alleles at main Aphanomyces resistance QTL. The effects of several QTL were successfully validated depending on genetic backgrounds.**

Abstract Quantitative trait loci (QTL) validation is an important and often overlooked step before subsequent research in QTL cloning or marker-assisted breeding for disease resistance in plants. Validation of QTL controlling partial resistance to Aphanomyces root rot, one of the most damaging diseases of pea worldwide, is of major interest for the future development of resistant varieties. The aim of this study was to validate, in different genetic backgrounds, the effects of various resistance alleles at seven main resistance QTL recently identified. Five backcross-assisted selection programs were developed. In each, resistance alleles at one to three of the seven main Aphanomyces resistance QTL were transferred into three genetic backgrounds, including two agronomically important spring (Eden) and winter (Isard) pea cultivars. The subsequent near-isogenic lines (NILs) were evaluated for resistance to two reference strains of the main *A. euteiches* pathotypes under controlled conditions. The NILs carrying resistance alleles at the major-effect QTL *Ae-Ps4.5* and *Ae-Ps7.6*, either individually or in combination with resistance alleles at other QTL, showed significantly reduced disease severity compared to NILs without resistance alleles. Resistance alleles at some minor-effect QTL, especially *Ae-Ps2.2* and *Ae-Ps5.1*, were also validated for their individual or combined effects on resistance. QTL × genetic background interactions were observed, mainly for QTL *Ae-Ps7.6,* the effect of which increased in the winter cultivar Isard. The pea NILs are a novel and valuable resource for further understanding the mechanisms underlying QTL and their integration in breeding programs.

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Introduction

A large number of Quantitative trait loci (QTL) have been identified in different plant species, but very few have been utilized in plant breeding programs. Difficulties integrating QTL information into breeding strategies have been due to the large number of QTL identified, the time it takes to generate improved populations

(St Clair 2010), but also to unexpected results obtained in QTL introgression programs (Hospital 2005). Thus, a very important step is to validate QTL effects before further research is pursued into QTL cloning, genomics studies, or Marker-Assisted Selection (MAS) to introgress QTL into new genetic backgrounds. Marker-Assisted Backcrossing (MAB) has been frequently used for QTL introgression (Collard and Mackill 2008; Hospital 2009). The goal of MAB is to incorporate a major gene or QTL from an agronomically inferior source (the donor parent) into an elite cultivar or breeding line (the recurrent or recipient parent). The desired outcome is a near-isogenic line (NIL) to the recurrent parent, containing the major allele at the gene or QTL from the donor parent, with the recurrent parent genotype present everywhere else in the genome (Hospital 2003; Kaeppler et al. 1993). NILs have been used advantageously to identify and validate QTL, as well as to produce fine maps, especially in tomato (Brouwer and St Clair 2004), wheat (Ma et al. 2011), maize (Koester et al. 1993), rice (Yu et al. 1991), barley (Schmalenbach et al. 2008), chickpea (Castro et al. 2010) and rapeseed (Delourme et al. 2008). In many studies, NILs allowed the QTL effect associated with introgressed segments to be confirmed. However, in some cases, after MAS the effect of a QTL was reduced or nullified. The loss of QTL effects can occur when the QTL (1) is a false positive, (2) is specific to some environments, (3) includes several genes and recombination between those genes modifies the effect of the introgressed segments or (4) has an epistatic effect with another QTL or with the genetic background (Collard and Mackill 2008; Hospital 2005). For example, Ahmadi et al. (2001) reported that MAS was particularly useful for validating epistatic effects between two QTL for resistance to rice yellow mottle virus, in introgression lines carrying both QTL compared to lines hosting only one of the two QTL which displayed no effects. The rate of unexpected results in MAS was reported to increase when QTL are used rather than known genes, when the number of QTL increases and when manipulating genetic components of more complex traits [complexity comes from the large number of genes controlling the trait, interactions between genes due to linkage and epistasis, low heritability and interaction between genes and environment (Hospital 2005)]. Disease resistances have often been reported to be inherited as simple traits or complex traits with major-effect QTL (St Clair 2010). There are many examples of successful validation of major resistance QTL effects in introgression or pyramiding lines (St Clair 2010). Subsequently, an increasing number of fine mapping studies of major-effect resistance QTL have been recently described (Keller et al. 2015). Several less successful attempts were also reported, such as QTL detected with minor effects in specific environments (Delourme et al. 2008; Li et al. 2003).

Aphanomyces root rot of pea, caused by the soil-borne oomycete *Aphanomyces euteiches* (Jones and Drechsler 1925), is one of the most devastating diseases of peas worldwide. In Europe, the disease has spread since the 1990s due to the intensification of pea production (Wicker and Rouxel 2001) and can lead to total devastation of the crop in infested fields (Didelot and Chaillet 1995). Symptoms of the disease are translucent lesions on the rootlets, which evolve into brown rot affecting the entire root system up to the epicotyl (Moussart et al. 2008). Above ground, the disease causes stunted seedlings, yellow leaves and even dead plants. Two main *A. euteiches* pathotypes have been reported in pea (Wicker and Rouxel 2001) based on their differential reactions on a set of six genotypes (Wicker et al. 2003). Pathotype I is predominant in Europe and was observed in the United States. Pathotype III was observed at some locations in the United States [Onfroy et al. (2015) submitted]. The use of partial polygenic resistance to *A. euteiches* has been considered as a key component of disease management for forty years (Papavizas and Ayers 1974). Breeding of pea lines resistant to *A. euteiches* was first developed in the United States. Genetic resources and agronomic lines with partial levels of resistance were identified, including the breeding lines 90-2079 and 90-2131 (Kraft 1992), 552 (Gritton 1990) and the landrace PI180693 (Lockwood and Ballard 1960). The latter three lines were then confirmed among the highest partially resistant lines in France (Pilet-Nayel, unpublished data). Using Recombinant Inbred Line (RIL) populations derived from crosses involving the four sources of resistance, a comprehensive meta-QTL mapping program identified seven QTL out of 27 meta-QTL associated with resistance to *A. euteiches* (Hamon et al. 2011, 2013; Pilet-Nayel et al. 2002, 2005). Each of the seven QTL was detected consistently in two to four RIL populations from at least 12 initial variables out of the total 29 field environment and 12 controlled condition variables studied. In particular, one major-effect QTL, *Ae-Ps7.6* located on Linkage Group VII (LGVII), was consistently identified and explained up to 60 % of the phenotypic variation, depending on environments and isolates. The other QTL contributed up to 15–44 % of the variation depending on the QTL (Hamon et al. 2013). A quite low diversity of non-specific QTL to sources of resistance was observed, suggesting a common genetic origin between the partially resistant parental lines studied. A low specificity of resistance QTL was also shown according to the strains, environments and scoring criteria studied. Negative associations between resistance and undesirable morphological alleles (normal leaves and flower color) for dry pea breeding, especially alleles originating from PI180693, were found. Epistatic interactions contributing to increased

resistance were also identified, especially between the two main QTL *Ae-Ps7.6* and *Ae-Ps2.2*. SSR markers linked to the seven main QTL identified have been made available for use in MAS (Hamon et al. 2013). However, although the main QTL were detected over different RIL populations, their effect remains to be validated when introgressed into new genetic backgrounds as no knowledge is available about Aphanomyces resistance QTL × genetic background interactions.

The aims of this study were to validate individual or multiple Aphanomyces resistance QTL effects in different genetic backgrounds using introgression lines. First, an extensive backcross-assisted selection program was conducted to generate NILs carrying resistance alleles at one to three of the seven main resistance QTL in three genetic backgrounds. Second, the generated NILs were phenotyped for resistance to two reference strains of the main *A. euteiches* pea pathotypes, for validating QTL effects, individually or in combination, in the different genetic backgrounds under controlled conditions. This study provides unprecedented pea resources and knowledge for QTL validation, future QTL genomic studies and demonstrates the potential of MAS for creating resistant agronomic germplasm for release.

Materials and methods

Plant material for MAB

Five parallel MAB schemes were developed, each scheme aiming to simultaneously introgress resistance alleles at one to three QTL from one donor line into three recipient lines. The five donor lines used in the MAB schemes included four RILs (RIL 831.08, RIL 847.50, RIL BAP8.70 and RIL BAP8.195) from previously studied mapping populations (Hamon et al. 2013) and the partially resistant garden pea breeding line 552 also analyzed in Hamon et al. (2011) (Table 1). Each donor RIL was chosen to carry allele(s) from its resistant parent at the one to three target QTL and alleles from its susceptible parent at a maximum number of markers outside the target QTL (Supplementary data file 1). Eleven of the 16 resistance alleles identified at the seven main resistance QTL (Table 3 in Hamon et al. 2013) were targeted for introgression from the five donor lines, based on their level and/or consistency of effects on resistance previously identified. These included three resistance alleles (90-2131, PI180693 and 552) at the major-effect QTL *Ae-Ps7.6*, two resistance alleles (PI180693 and 552) at QTL *Ae-Ps2.2* and *Ae-Ps3.1* and one resistance allele (90-2079, 90-2131 or PI180693) at each of the four other main QTL.

The three recipient parents used in each MAB scheme included (1) a reference line, which was the susceptible parent of the RIL population in which QTL were detected, i.e., Baccara (Hamon et al. 2011), DSP (Hamon et al. 2013) or Puget (Pilet-Nayel et al. 2002) and (2) two dry pea agronomic lines of different sowing types, i.e., the spring cultivar Eden and the winter cultivar Isard. A fourth recipient parent, the winter dry pea cultivar Enduro, was also used in one of the five MAB schemes (donor line RIL 847.50), due to its higher agronomic value (Table 2).

NIL production

The breeding scheme for introgression of the target QTL is shown in Fig. 1. Sixteen bi-parental crosses between donor (as male) and recipient (as female) lines were carried out. Plant progenies from each cross were repeatedly backcrossed with the recipient parent in greenhouse conditions, with two generations per year.

At each backcross generation, 45–91 15-day-old plants were genotyped using microsatellite markers, to select plants carrying one, two or three QTL introgressions, respectively. During genotyping, growth of the young plants was slowed down in a growth chamber at 5 °C and 8-h photoperiod for about 1 month. Plants carrying the donor introgressions at all the target QTL (heterozygous at

Table 1 Donor lines used for MAB

Donor line	Donor	RIL cross of origin	Target QTL for MAB (Hamon et al. 2013)	Resistance enhancing alleles at target QTL	References
RIL 831.08	USDA, USA	Puget * 90-2079	*Ae-Ps4.5*	90-2079	Pilet-Nayel et al. (2002, 2005)
RIL 847.50	USDA, USA	DSP * 90-2131	*Ae-Ps5.1, Ae-Ps7.6*	90-2131	Hamon et al. (2013); McGee et al. (2012)
RIL BAP8.70	INRA, France	Baccara * PI180693	*Ae-Ps4.1, Ae-Ps7.6*	PI180693	Hamon et al. (2011)
RIL BAP8.195	INRA, France	Baccara * PI180693	*Ae-Ps1.2, Ae-Ps2.2, Ae-Ps3.1*	PI180693	Hamon et al. (2011)
552	University of Wisconsin, USA		*Ae-Ps2.2, Ae-Ps3.1, Ae-Ps7.6*	552	Gritton (1990)

Table 2 Recipient lines used for MAB

Recipient parent	Parents type for MAB	Cultivar type	Registration year	Breeder
Puget	Reference	Spring garden pea	1967	Brotherton Seed Company, UK
Dark Skin Perfection (DSP)	Reference	Spring garden pea	1960	Unilever limited, UK
Baccara	Reference	Spring dry pea	1992	Florimond-Desprez, France
Eden	Agronomic	Spring dry pea	2004	Nickerson, France
Enduro	Agronomic	Winter dry pea	2007	Florimond-Desprez, France
Isard	Agronomic	Winter dry pea	2005	Agri-Obtentions, France

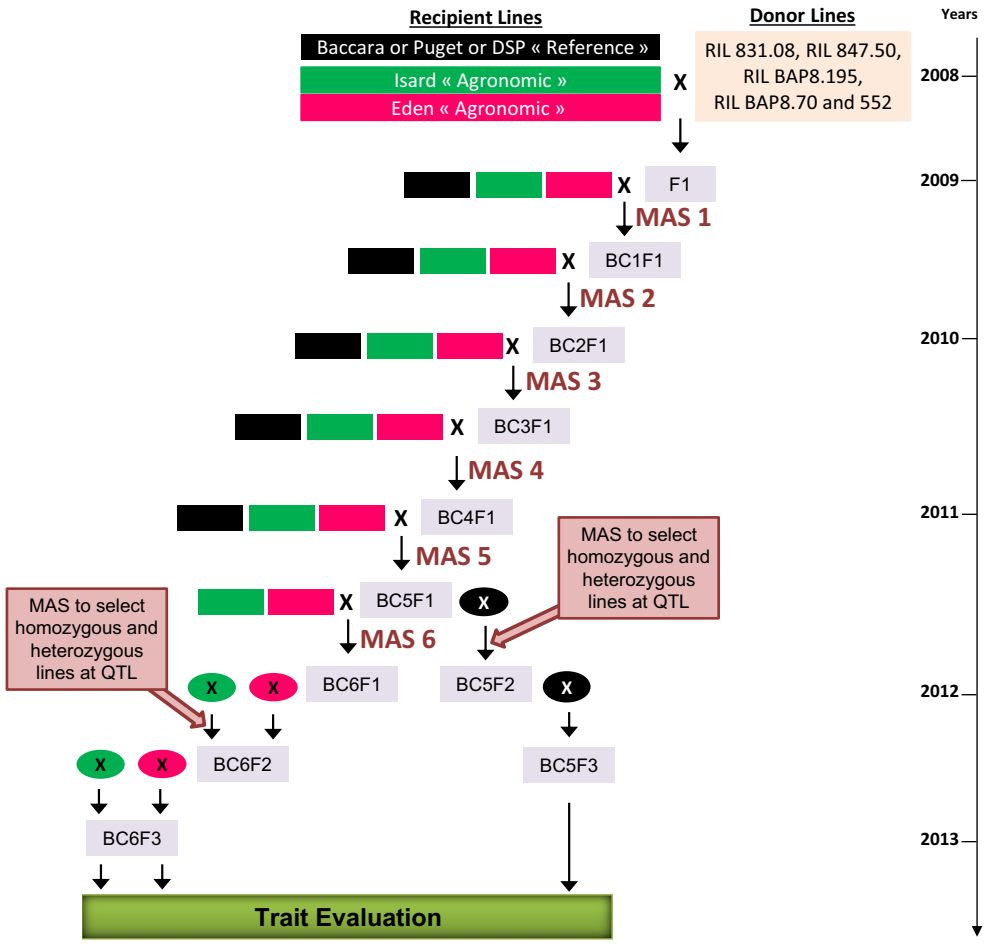


Fig. 1 The marker-assisted backcrossing scheme used to construct NILs at the main Aphanomyces resistance QTL. A fourth recipient parent (not presented), the winter cultivar Enduro, was also used in one of the five MAB schemes (donor line RIL 847.50)

markers linked to QTL) and the highest level of recipient genome outside the target QTL were selected and transplanted in greenhouse conditions. One to five selected plants were used for producing the next backcross progeny, by manual outcrossing with the recipient parent.

Five backcrossed generations were produced from four crosses (Puget × RIL 831.08, DSP × RIL 847.50, Baccara × RIL BAP8.70 and Baccara × RIL BAP8.195), in which the donor lines initially carried portions of the reference recipient line genomes. Six backcrossing generations were performed from the other crosses to improve recovery of genomes of the recipient lines, except for the Isard × RIL BAP8.70 and Enduro × RIL 847.50 crosses for which only five generations of backcrosses could be carried out (Table 3).

BC5/6F1 progenies were then self-pollinated to select, in subsequent QTL pseudo-F2 populations, homozygous plants carrying resistance alleles at zero, one, two or three

Table 3 Near-isogenic lines (NILs) developed at Aphanomyces resistance QTL

Donor Line	Recipient Line	NIL set number	Target QTL	Fixity generation[a]	Genetic divergence generation[b]	No. of markers for MAS[c]		RGC %[d] (min–max)	No. of NILs selected[e]			
						QTL	Outside QTL (min–max)		0 QTL	1 QTL	2 QTL	3 QTL
RIL 831.08	Puget	1	*Ae-Ps4.5*	BC_5F_2	BC_5F_2	3	43–46	98–100	2	2		
	Isard	2	*Ae-Ps4.5*	BC_6F_2	BC_6F_2	4	68–72	99–100	2	2		
	Eden	3	*Ae-Ps4.5*	BC_6F_2	BC_6F_2	5	65–70	100	2	2		
RIL 847.50	DSP	4	*Ae-Ps5.1* + *Ae-Ps7.6*	BC_5F_2	BC_5F_2	5-3	57–65	94–100	2	4	2	
	Isard	5	*Ae-Ps5.1* + *Ae-Ps7.6*	BC_6F_2	BC_4F_1	4-5	60–69	98–100	2	4	2	
	Eden	6	*Ae-Ps5.1* + *Ae-Ps7.6*	BC_6F_2	BC_6F_1	4-4	65–73	99–100	2	4	2	
	Enduro	16	*Ae-Ps5.1* + *Ae-Ps7.6*	BC_5F_2	BC_5F_1	3-4	47–55	100	2	4	2	
RIL BAP8.70	Baccara	7	*Ae-Ps4.1* + *Ae-Ps7.6*	BC_5F_2	BC_5F_1	2-3	57–62	97–100	2	4	2	
	Isard	8	*Ae-Ps4.1* + *Ae-Ps7.6*	BC_5F_2	BC_4F_1	2-5	43–51	98–100	2	4	2	
	Eden	9	*Ae-Ps4.1* + *Ae-Ps7.6*	BC_6F_2	BC_6F_1	2-4	29–35	100	2	4	2	
RIL BAP8.195	Baccara	10	*Ae-Ps1.2* + *Ae-Ps2.2* + *Ae-Ps3.1*	$BC_5F_{2/3}$	BC_2F_1	3-3-3	68–74	99–100	4●●●	8●●●	2●	0●
	Isard	11	*Ae-Ps1.2* + *Ae-Ps2.2* + *Ae-Ps3.1*	$BC_6F_{2/3}$	BC_6F_1	3-3-3	63–72	97–100	2	6	6	3●●●
	Eden	12	*Ae-Ps1.2* + *Ae-Ps2.2* + *Ae-Ps3.1*	$BC_6F_{2/3}$	BC_6F_1	3-3-3	45–54	96–100	2	6	4●●	2
552	Baccara	13	*Ae-Ps2.2* + *Ae-Ps3.1* + *Ae-Ps7.6*	$BC_6F_{2/3}$	BC_6F_1	4-2-4	57–68	100	2	6	4●	2
	Isard	14	*Ae-Ps2.2* + *Ae-Ps3.1* + *Ae-Ps7.6*	$BC_6F_{2/3}$	BC_4F_1	4-1-4	52–61	98–100	2	6	5●●	1●●
	Eden	15	*Ae-Ps2.2* + *Ae-Ps3.1* + *Ae-Ps7.6*	$BC_6F_{2/3}$	BC_5F_1	4-1-4	49–58	98–100	2	6	6	2
Total no. of NILs selected									34	72	41	10

[a] Generation from which homozygous NILs at QTL was selected

[b] Generation from which the selected homozygous NILs carrying same allele combinations (two sister lines per NIL type) diverged

[c] Total number of markers used in each cross for controlling (1) each introgression at the target QTL at each MAB generation and (2) return to the recipient line genome in regions outside the QTL over all the MAB generations, in each homozygous NIL set at the fixity generation (minimum and maximum per NIL set)

[d] Minimal and maximal percentages of Recipient Genome Content in each homozygous NIL set at the fixity generation, calculated by the ratio between the number of markers outside the target QTL for which NILs are homozygous for the recipient alleles and the total number of polymorphic markers tested outside the target QTL in the NIL set. RGC values are relative to the number of markers tested outside the target QTL in each NIL set

[e] Number of NILs selected, homozygous at 0, 1, 2 or 3 resistance QTL. Two sister NILs with same introgressions were usually selected in each cross. QTL allelic combinations for which at least three, one or no NIL could be selected in each cross are indicated by ●●●, ●● and ●, respectively. In Baccara x RIL BAP8.195, the number of each NIL type selected was higher since the MAB scheme was divided into two parts from BC2 for bi-QTL introgressions (*Ae-Ps1.2* + *Ae-Ps2.2* and *Ae-Ps2.2* + *Ae-Ps3.1*), due to no identification of plants carrying the three target introgressions

QTL. A total of 91–273 BC5/6F2 plants were screened, depending on the number of segregating QTL. In some crosses, a second generation of self-pollination was necessary to select BC5/6F3 homozygous lines carrying resistance alleles at three QTL. In each cross, two of the selected homozygous plants per allelic combination at target QTL, named "sister" lines, were self-pollinated to increase seed and the BC5/6F3/4 seeds were used for pathological tests.

Marker-assisted selection

Over all the backcross and selfing generations during NIL production, 152 microsatellite markers were used for MAS, including 99 SSR markers (Simple Sequence Repeats) from Loridon et al. (2005) and 53 EST-SSR markers (Expressed Sequence Tags-Simple Sequence Repeats, named "eSSRs"; Mohamadi, unpublished data).

A total of 37 markers (32 SSRs and 5 eSSRs) previously associated with the seven main resistance QTL to *A. euteiches* (Hamon et al. 2013) were used to control introgressions at each MAS generation (Fig. 2). One to five markers, depending on their level of polymorphism between the donor and recipient lines, were used for tracing each QTL, the size of which was estimated at 9–37.5 cM when projected onto the consensus genetic map of Duarte et al. (2014). Most of the QTL were controlled with at least three markers (Supplementary data file 1).

A total of 115 markers (67 SSRs and 48 eSSRs), regularly distributed on the genome (Supplementary data file 1; Fig. 2), was used over all the MAS generations to control that the genome had returned to the recipient type. One to three markers per QTL carrier- or non-carrier linkage group were used at each generation from BC1F1 to BC4F1. All the remaining polymorphic markers were used to check the recipient genome in BC5F2 and BC6F2/3. A Recipient Genome Content (RGC) value was calculated for each homozygous NIL as the percentage of markers among all those tested outside the target QTL, which revealed alleles from the recipient parent.

SSR genotyping

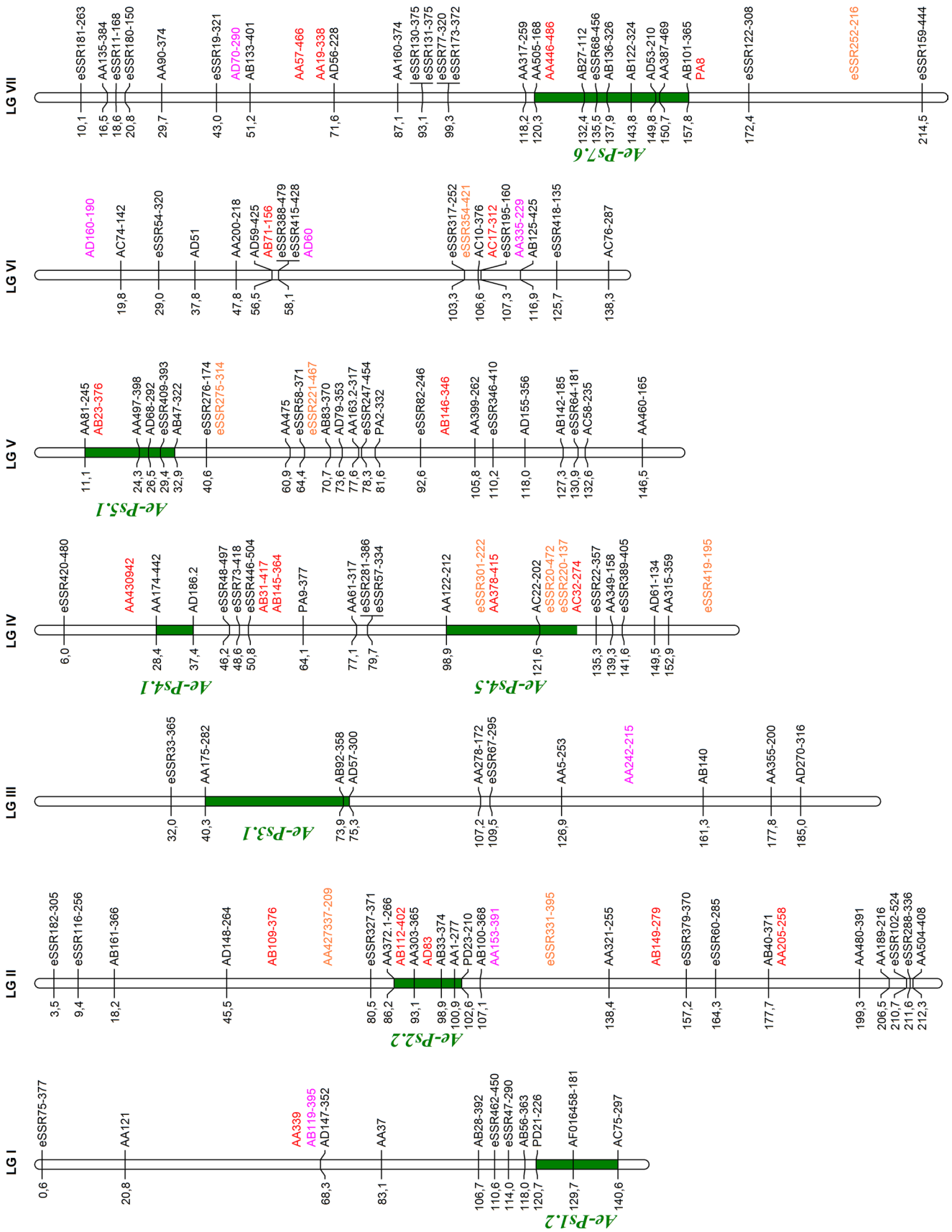
At each MAS generation, foliar tissues were harvested from each plant at the four-leaf stage and freeze-dried. Total DNA was extracted from freeze-dried leaf material using a NucleoSpin 96 Plant II Core Kit (Macherey–Nagel GmbH & Co. KG, Germany), following the manufacturer's instructions. PCRs were carried out in a total volume of 7 µL, containing 20 ng genomic DNA template, 1X PCR Polymerization Buffer, 0.2 mM of dNTPs, 2 mM of $MgCl_2$, 0.13 pM of the forward primer coupled to a 19-base M13 tail in 5′, 0.26 pM of the reverse primer and 0.5 Units of *Taq* DNA polymerase (reagents from Promega, USA). PCR products were labeled by adding a universal M13 oligonucleotide labeled with the fluorescent dye, 6-FAM, to the PCR reaction. Thermocycling was carried out as follows: denaturation at 94 °C for 4 min; followed by 10 cycles at 94 °C for 30 s, annealing at the required temperature for 45 s and extension at 72 °C for 1 min; then, 30 cycles at 94 °C for 30 s, annealing at 51 °C for 45 s and extension at 72 °C for 1 min; followed by a final extension step at 72 °C for 10 min. The annealing temperature (T_a) was optimized for each locus (Supplementary data file 1). PCR products were sent to the GENTYANE genotyping platform (INRA Clermont-Ferrand, France) to be loaded on an ABI3730 XL DNA Analyzer (Applied Biosystems®, USA). PCR amplification results were analyzed using GeneMapper® Software v.4.0 (Applied Biosystems®, USA).

Fig. 2 Genetic map positions of the seven main Aphanomyces resistance QTL and markers used in the Marker-assisted backcrossing scheme for NIL production. Positions (in cM Haldane) of markers indicated in black on each Linkage Group (LG) were determined from the consensus genetic map reported in Duarte et al. (2014). Positions of markers indicated in *red*, *purple*, and *orange* were estimated from Hamon et al. (2013), Loridon et al. (2005) and Mohamadi (unpublished data), respectively. Positions of target fragments at the seven main QTL, named "*Ae-Ps*-.-", are indicated by green bars and were estimated from Hamon et al. (2013)

Evaluation of NILs for resistance

Two single spore strains of *A. euteiches* were used to assess NIL disease resistance in tests under controlled conditions. The RB84 strain was isolated from a French infested soil (Riec-sur-Belon, Finistère) (Moussart et al. 2007) and is the reference for pathotype I. The Ae109 strain was isolated from American soil (Wisconsin) (Malvick et al. 1998) and is the reference for pathotype III. Fourteen pea control lines were used in the disease test, including the five donor and six recipient parents of the MAB scheme and three partially resistant parental lines of RIL populations (PI180693, 90-2131 and 90-2079).

Disease resistance tests were performed in a growth chamber, using a modified version of the standardized test developed for evaluating pea resistance to *A. euteiches* (Moussart et al. 2001). For each strain, all the lines were evaluated within two tests, in a randomized complete block design with two blocks as replicates per test. In each block, five seeds of a single genotype were sowed in one pot containing unsterilized vermiculite. Plants were grown under constant conditions at 25 °C for 16 h of day and 23 °C for 8 h of night. Seven days after sowing, seedlings were inoculated with 5 ml per plant of a suspension of zoospores concentrated at 200 spores per ml, from a pure culture of the French or American strain. Zoospores were produced as previously described by Moussart et al. (2001). The


LG I
0,6 eSSR75-377
20,8 AA121
68,3 AA339 AB119-395 AD147-352
83,1 AA37
106,7 AB28-392
110,6 eSSR462-450
114,0 eSSR47-290
118,0 AB56-363
120,7 PD21-226
129,7 AF016458-181
140,6 AC75-297
Ae-PsI.2
LG II
3,5 eSSR182-305
9,4 eSSR116-256
18,2 AB161-366
45,5 AD148-264
AB109-376
AA427337-209
80,5 eSSR327-371
86,2 AA372.1-266 AB112-402
93,1 AA303-365 AD83
98,9 AB33-374
100,9 AA1-277
102,6 PD23-210
107,1 AB100-368 AA153-391
eSSR331-395
138,4 AA321-255
AB149-279
157,2 eSSR379-370
164,3 eSSR60-285
177,7 AB40-371 AA205-258
199,3 AA480-391
206,5 AA189-216
210,7 eSSR102-524
211,6 eSSR288-336
212,3 AA504-408
Ae-PS2.2
LG III
32,0 eSSR33-365
40,3 AA175-282
73,9 AB92-358
75,3 AD57-300
Ae-PS3.1
107,2 AA278-172
109,5 eSSR67-295
126,9 AA5-253
AA242-215
161,3 AB140
177,8 AA355-200
185,0 AD270-316
LG IV
6,0 eSSR420-480
28,4 AA430942 AA174-442
37,4 AD186.2
46,2 eSSR48-497
48,6 eSSR73-418
50,8 eSSR446-504 AB31-417 AB145-364
64,1 PA9-377
77,1 AA61-317
79,7 eSSR281-386 eSSR57-334
Ae-PS4.1
98,9 AA122-212
eSSR301-222 AA378-415
121,6 AC22-202
eSSR20-472 eSSR220-137 AC32-274
135,3 eSSR22-357
139,3 AA349-158
141,6 eSSR389-405
149,5 AD61-134
152,9 AA315-359
eSSR419-195
Ae-PS4.5
LG V
11,1 AA81-245 AB23-376
24,3 AA497-398
26,5 AD68-292
29,4 eSSR409-393
32,9 AB47-322
40,6 eSSR276-174 eSSR275-314
60,9 AA475
64,4 eSSR58-371 eSSR221-467
70,7 AB83-370
73,6 AD79-353
77,6 AA163.2-317
78,3 eSSR247-454
81,6 PA2-332
92,6 eSSR82-246
AB146-346
105,8 AA399-262
110,2 eSSR346-410
118,0 AD155-356
127,3 AB142-185
130,9 eSSR64-181
132,6 AC58-235
146,5 AA460-165
Ae-PS5.1
LG VI
19,8 AD160-190 AC74-142
29,0 eSSR54-320
37,8 AD51
47,8 AA200-218
56,5 AD59-425 AB71-156
58,1 eSSR388-479 eSSR415-428 AD60
103,3 eSSR317-252 eSSR354-421
106,6 AC10-376 AC17-312
107,3 eSSR195-160 AA335-229
116,9 AB125-425
125,7 eSSR418-135
138,3 AC76-287
LG VII
10,1 eSSR181-263
16,5 AA135-384
18,6 eSSR11-168
20,8 eSSR180-150
29,7 AA90-374
43,0 eSSR19-321
51,2 AD70-290 AB133-401
71,6 AA57-466 AA19-338 AD56-228
87,1 AA160-374
93,1 eSSR130-375 eSSR131-375
99,3 eSSR77-320 eSSR173-372
118,2 AA317-259
120,3 AA505-168 AA446-486
132,4 AB27-112
135,5 eSSR68-456
137,9 AB136-326
143,8 AB122-324
149,8 AD53-210
150,7 AA387-469
157,8 AB101-365 PA8
172,4 eSSR122-308
eSSR252-216
214,5 eSSR159-444
Ae-PS7.6

vermiculite was saturated by capillary watering after inoculation to favor disease development and avoid zoospores leaching. Seven days after inoculation, disease severity (DS) on each uprooted and washed plant was assessed according to a 0 to 5 scoring scale (Moussart et al. 2008) (0 = no symptoms; 1 = traces of discoloration on the roots (<25 %); 2 = discoloration of 25–50 % of the roots; 3 = discoloration of 50–75 % of the roots; 4 = discoloration of >75 % of the roots; 5 = plant dead).

Data analysis

Statistical analyses of phenotypic data were performed using R software (R Development Core Team 2014). For each strain, the absence of genotype × test interactions was checked with a linear model, using the 'Anova' function of the 'car' package (John and Sanford 2011). Then, the DS scoring data obtained for all the NILs, or each NIL set per cross or donor line, were analyzed using a linear mixed model [LMM; 'lmer' function, 'lme4′ package (Bates et al. 2014)], considering the DS scoring data as explanatory variables, the genotype as fixed factor and the replicate as random factor. A Wald F test was applied for evaluating genotypic effect ($\alpha = 5$ %). Broad sense heritability (h^2) was estimated from an analysis of deviance table using the formula $h^2 = \sigma^2 g/[\sigma^2 g + (\sigma^2 e/n)]$ ['pbkrtest' package (Halekoh and Højsgaard 2013)], where $\sigma^2 g$ is the genetic variance, $\sigma^2 e$ is the residual variance and n is the number of replicates per line. Least square means (LSMeans) were estimated for each line using the 'lsmeans' function of the 'lsmeans' package (Lenth and Herve 2015). LSMeans values were compared between each other with the pairwise contrast or multiple comparison Tukey HSD or Tukey test ($\alpha = 5$ %), using the 'pairs' or 'cld' functions of the 'MultcompView' package (Graves et al. 2012). Pearson correlation coefficients were calculated between the two strain LSMeans data ($\alpha = 5$ %).

Results

Construction of NILs at the main Aphanomyces resistance QTL

A total of 157 NILs were selected from the 16 MAB crossing programs conducted over 6 years (2008–2013) (Fig. 1). These were homozygous at zero to three of the seven main target resistance QTL (Table 3). Two homozygous NILs per allelic combination at the target QTL, known as sister NILs, could usually be selected in each cross. However, only one or no NIL was found for six allelic combinations at two to three QTL in four different crosses. Five combinations in two crosses were each represented by more than two NILs (Table 3). All the sister NILs diverged from each other in later generations (BC4F1 to BC6F1), except those from Baccara × RIL BAP8.195 which was divided into two bi-QTL introgression schemes from BC2 because no plants were selected carrying introgressions at the three QTL initially targeted.

The 157 homozygous NILs at QTL were coded as follows: NIL"NIL set number"-"0 for zero QTL or QTL number(s)" "sister NIL letter (a or b)" (Supplementary data file 1). They were characterized for two morphological traits, i.e., type of leaves and flower color (Supplementary data file 1). NILs carrying resistance alleles from RIL BAP8.195 at QTL *Ae-Ps1.2* and *Ae-Ps2.2* had normal leaves and purple flowers, respectively.

The size of the genome fragment containing the QTL which was introgressed in NILs could not be estimated precisely due to the low density of available SSR markers. In some cases, the introgression was larger than the initial target fragment since the recipient genome in the QTL flanking regions was not recovered (Supplementary data file 1). In contrast, some portions of target fragments, not controlled by markers, may have been lost in several NILs, e.g., the QTL *Ae-Ps3.1* in three NIL sets (13, 14 and 15). Variations in introgression size resulted from differences in the number of polymorphic markers, between the donor and recipient parents, available for controlling introgressions at the target QTL and return to recipient genomes in QTL flanking region.

In each final selected NIL, a total of 29–74 SSR markers tested outside the target QTL, depending on their level of polymorphism between donor and recipient lines, were used to examine the return to the recipient genome (Table 3, Supplementary data file 1). Each QTL homozygous NIL selected carried the recipient type allele at 94–100 % of the markers tested outside the target QTL. Out of all the QTL homozygous NILs, only six NILs (NIL1-4.5b, NIL7-4.1/7.6b, NIL12-2.2a-b, NIL12-1.2/2.2a and NIL12-1.2/2.2/3.1a) have a fragment of the donor line outside the target QTL according to the SSR markers tested.

Based on all the SSR markers tested in or outside the QTL regions, the genomic composition of sister NILs was identical, except in two NIL sets (11 and 15), which comprised five pairs of sister NILs carrying different introgression sizes at QTL *Ae-Ps2.2* or *Ae-Ps7.6*.

Evaluation of NILs for Aphanomyces resistance under controlled conditions

For both strains, genotype × test interactions were not significant ($P = 0.30$ and 0.99, for RB84 and Ae109 strain, respectively). LMM analysis of DS scoring data for all the NILs showed a highly significant genotypic effect ($P < 0.001$) for each strain test. Heritability estimates of the

trait observed in the tests were high, i.e., 0.93 for the RB84 strain and 0.91 for Ae109. LSMeans of DS scores and standard errors obtained for each NIL with the two strains are detailed in Supplementary data file 2, and averaged 3.24 ± 0.14 for RB84 and 2.93 ± 0.13 for Ae109 for all the NILs tested. Frequency distributions of DS LSMeans values for all the NILs tended to fit normal curves with both strains (Fig. 3). However, a group of 12 highly resistant NILs to the Ae109 strain was separated from the other lines. This group contains (1) all the NILs carrying QTL *Ae-Ps4.5* from RIL 831.08 and (2) six NILs carrying QTL *Ae-Ps7.6*, individually and/or in combination with other QTL, from three donor parents (RIL 847.50, RIL BAP8.70 and 552) in the recipient line Isard. The control lines tested ranked as expected for DS LSMeans with the two strains. In particular, the donor parents RIL 847.50, RIL BAP8.70 and RIL BAP8.195 were the most partially resistant controls to the RB84 strain with scores between 1.7 and 2.7 and RIL 831.08 was the most resistant to the Ae109 strain with a score of 0.6 (Fig. 3). Levels of susceptibility of the recipient parents were similar for the RB84 strain (scores of 3.56–3.87), while they were more variable for the Ae109 strain (score of 2.85–3.4). The Pearson correlation coefficient between RB84 and Ae109 LSMeans data for all NILs was 0.28 ($P < 0.001$).

Histograms of DS LSMeans scores obtained for each of the 16 NIL sets with the two strains are shown in Fig. 4 and Supplementary data file 3, in comparison to parental line values. LSMeans of NILs without introgressions were not significantly different from those of the recipient parents, except for the NIL14-0b.

Three NIL sets showed significant LSMeans differences between NILs and their parental lines in the reference genetic backgrounds (Fig. 4). In two of the three NILs sets, the effects of 90-2131 and PI180693 resistance alleles at QTL *Ae-Ps7.6,* individually and/or in combination with the 90-2131 allele at QTL *Ae-Ps5.1* or PI180693 allele at QTL *Ae-Ps4.1*, were validated for resistance to the RB84 strain in the reference recipient lines. The DSP × RIL 847.50 NILs carrying the single QTL *Ae-Ps5.1* or *Ae-Ps7.6* were significantly more resistant to RB84 than the recipient parent (Fig. 4A). The combination of the two QTL had a significantly increased effect compared to the two single QTL effects in the NILs tested (Figs. 4, 5). The Baccara × RIL BAP8.70 NILs carrying the single QTL *Ae-Ps7.6* were also significantly more resistant to RB84 than the recipient parent, whereas NILs carrying QTL *Ae-Ps4.1* were not significantly different from the recipient parent. The combination of QTL *Ae-Ps7.6* and *Ae-Ps4.1* had a greater effect than the single *Ae-Ps7.6* QTL effect (Fig. 4B). In the third NIL set, the effect of the 90-2079 allele from RIL 831.08 at QTL *Ae-Ps4.5* was also validated for resistance to Ae109, but not to the RB84 strain, in the reference recipient line (Fig. 4C, Supplementary data file 3).

In two other NIL sets, significant but smaller LSMeans differences were observed between NILs carrying either the QTL *Ae-Ps2.2* from RIL BAP8.195 or the QTL *Ae-Ps7.6* (combined or not with *Ae-Ps2.2* and/or *Ae-Ps3.1*) from 552, and the reference recipient line Baccara with the RB84 strain.

Analysis of QTL × genetic background interactions

The main QTL effects observed in the reference recipient lines with each strain were confirmed in the other recipient

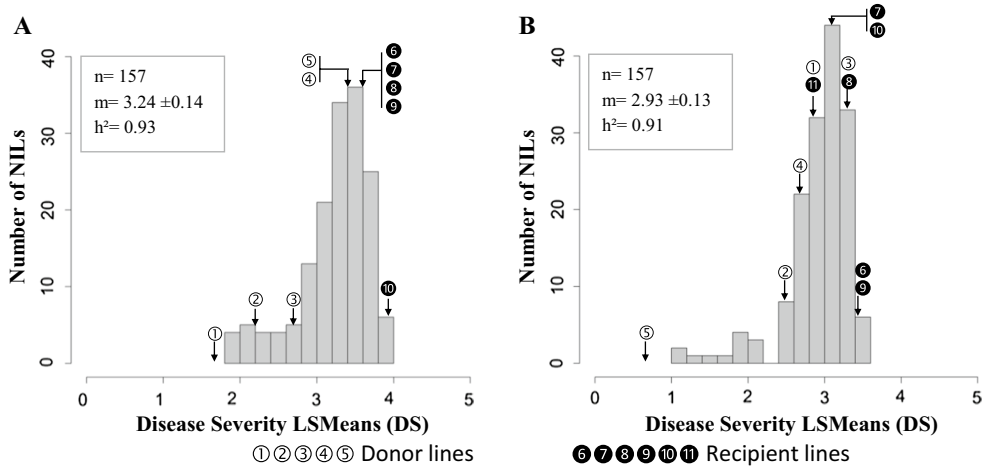


Fig. 3 Frequency distribution of disease severity (DS) of the 157 NILs produced by MAB, for resistance to two strains of *A. euteiches*, **A** RB84 strain, **B** Ae109 strain. DS LSMeans values of the donor lines are shown in *white*: 1-RIL 847.50, 2-RIL BAP8.195, 3-RIL BAP8.70, 4-552 and 5-RIL 831.08, respectively. The values of the recipient lines are shown in *black*: 6-Eden, 7-Isard, 8-DSP, 9-Puget, 10-Baccara and 11-Enduro, respectively. *n* total number of NILs assessed, *m* mean ± standard error of the total NILs, h^2 heritability estimate

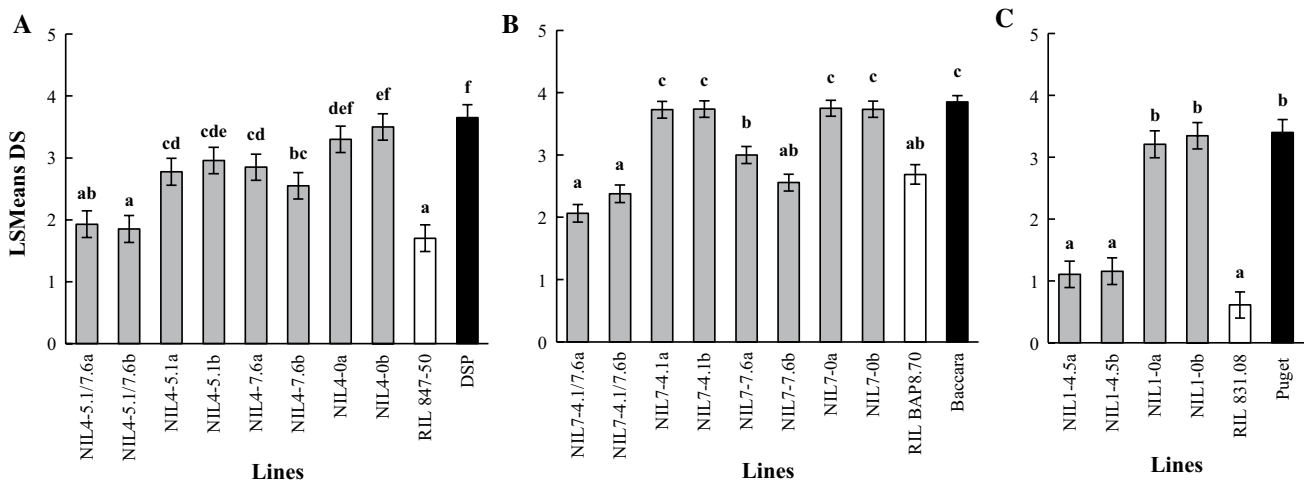


Fig. 4 Comparison of disease severity (DS) between NILs and parental lines, in three NIL sets, produced in reference genetic backgrounds **A** DSP × RIL 847.50, **B** Baccara × RIL BAP8.70 and **C** Puget × RIL 831.08. *White*, *black*, and *gray bars* indicate DS LSMeans values with the RB84 strain (**A**, **B**) or Ae109 strain (**C**) of the donor parent, the recipient parent and the NIL set, respectively. *Bars* standard errors. LSMeans labeled with *different letters* differ significantly ($P < 0.05$, Tukey HSD)

lines. QTL × genetic background interactions were identified mainly for QTL *Ae-Ps7.6* and to a lesser extent for QTL *Ae-Ps4.1, Ae-Ps4.5* and *Ae-Ps5.1*, by analyzing the data in the NIL sets showing the highest effects, from the three donor lines RIL 847.50, RIL BAP8.70 (RB84 strain) and RIL 831.08 (Ae109 strain) (Fig. 6). QTL × genetic background interactions were also noted for QTL *Ae-Ps2.2* from RIL BAP8.195 and QTL *Ae-Ps7.6* from 552, depending on the strain (Supplementary data file 3). The three alleles (PI180693, 90-2131 and 552) introgressed at QTL *Ae-Ps7.6* conferred significantly different levels of effects in each agronomic recipient line.

Introgressions of the 90-2131 resistance alleles at QTL *Ae-Ps5.1* and *Ae-Ps7.6* from RIL 847.50 had different effects on resistance to RB84, depending on the recipient line (Fig. 6A). The effect of QTL *Ae-Ps7.6* alone was significantly higher in Isard than in DSP and Eden, with differences in the DS LSMeans values between the NILs carrying and not carrying the QTL at 1.3, 0.7 and 0.8, respectively. The QTL *Ae-Ps5.1* only had an effect in DSP. The effect of the two combined QTL was similar in Isard and DSP, and significantly higher than in Eden (LSMeans differences of 1.2, 1.5 and 0.7, respectively, between NILs with and without the two QTL). However, the two QTL in DSP provided the same level of resistance as the single QTL *Ae-Ps7.6* in Isard.

Interactions between the PI180693 resistance allele at QTL *Ae-Ps7.6* from RIL BAP8.70 and recipient lines were also observed for RB84, with a significantly greater effect in Isard than in Baccara and Eden (LSMeans differences between NILs with or without the QTL were 1.0, 1.0 and 0.4, respectively) (Fig. 6B). The PI180693 allele at QTL *Ae-Ps4.1* did not confer resistance when introgressed individually into

Fig. 5 Comparison of Root Rot symptoms between two NILs from the DSP × RIL 847.50 NIL set, either carrying donor introgressions or not at the two QTL *Ae-Ps7.6* and *Ae-Ps5.1*. Symptoms are shown at seven days after inoculation with the RB84 strain

the three recipient lines. When used in combination with QTL *Ae-Ps7.6,* QTL *Ae-Ps4.1* increased significantly the level of resistance in the spring recipient lines Baccara and Eden whereas it decreased resistance in Isard, compared with

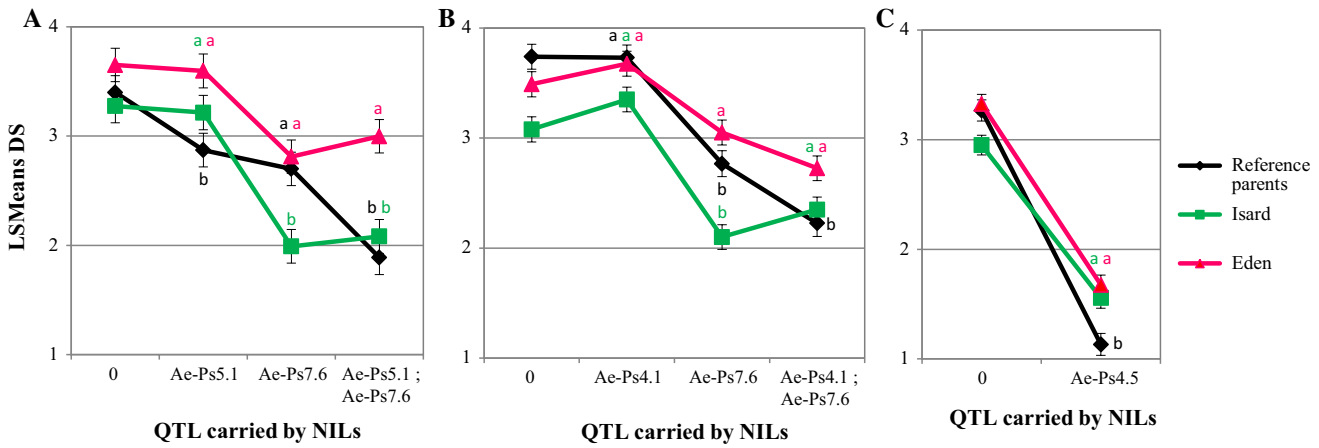


Fig. 6 Effects of individual or a combination of Aphanomyces resistance QTL, in NILs carrying introgressions from the donor lines, **A** RIL 847.50, **B** RIL BAP8.70 and **C** RIL 831.08, in different recipient lines. Each value is the average of DS LSMeans obtained for the two sister lines of each NIL type, with the RB84 strain (**A**, **B**) or Ae109 strain (**C**). *Bars* standard errors. LSMeans differences between NILs with or without QTL that are labeled with *different letters* differ significantly in the three recipient lines ($P < 0.05$, Tukey). *Black*, *green*, and *pink lines* indicate the reference parents (DSP or Baccara or Puget) and the agronomic recipient parents Isard and Eden, respectively

the effect of the single QTL *Ae-Ps7.6* (LSMeans differences between NILs with the single QTL *Ae-Ps7.6* and NILs with the two QTL are 0.2 in Isard, 0.5 in Baccara and 0.3 in Eden).

Introgressions of the 90-2079 resistance allele at QTL *Ae-Ps4.5* from RIL 831.08 had a strong effect in the three genetic backgrounds on resistance to the Ae109 strain, with a slightly but significantly higher effect in Puget than in Eden and Isard (Fig. 6C).

Discussion

Validation of QTL for resistance to *A. euteiches* in different pea genetic backgrounds is essential for further genetic research and integration of QTL in MAS. We used MAB to generate a unique plant resource of sixteen pea NIL sets carrying resistance alleles at one to three of the seven main Aphanomyces resistance QTL, in different genetic backgrounds including two elite agronomic cultivars (Eden and Isard). NILs allow individual or combinations of QTL to be isolated in a given genetic background. Consequently, phenotypic differences between the recipient parent and the NILs are most likely attributable to the targeted QTL. We evaluated the NIL sets for resistance to two strains of *A. euteiches* under controlled conditions. The effects of several QTL were validated, depending on the genetic background, and agronomically important NILs, with improved resistance to *A. euteiches* compared to recipient parents, were identified. Our findings validate previous QTL mapping studies (Hamon et al. 2011, 2013; Pilet-Nayel et al. 2002, 2005), and also show the feasibility of using MAS for improving resistance to *A. euteiches* in pea.

A unique plant resource of pea NILs at Aphanomyces resistance QTL

Our MAB approach produced a total of 157 NILs homozygous for resistance alleles at one to three main resistance QTL from five introgression schemes into three recipient lines. We conducted an extensive program for NIL creation, which generated more than 28,000 SSR genotyping data points and 1300 manual flower inter-crosses per generation. Some examples of extensive MAS programs have been reported in the literature (Fujita et al. 2010; Thabuis et al. 2004; Yi et al. 2009; Zhou et al. 2005).

The introgression fragments were chosen based on their level and consistency of effect on resistance in previous QTL detection programs (Hamon et al. 2013). Resistance alleles from 552 at QTL *Ae-Ps1.2* and *Ae-Ps4.1* were not targeted in the present introgression program despite their interest (Hamon et al. 2013) but are being considered in an ongoing additional MAB program (Pilet-Nayel Pers. Comm.). Resistance alleles from several donors at identical QTL, specifically QTL *Ae-Ps7.6*, were targeted in the MAB program, since there was no information on whether common or different linked genes underlie the different alleles at this QTL. Common genes may be expected to confer resistance from the PI180693 and 90-2131 alleles at QTL *Ae-Ps7.6* since these two resistant germplasms are related (Kraft 1992).

The strategy developed in the MAB program successfully allowed 93 % of the target introgressions to be obtained in NILs comprising more than 98 % of the recipient genome. However, several factors may have influenced MAS efficiency. First, the number of plants screened at each BC and

first selfing generation (45–91 and 91–273, respectively) was adapted to the number of simultaneously introgressed QTL and to technical constraints (entire or half of a 96-well DNA plate). The plant number was usually adequate or overestimated for introgressing one or two QTL or for selecting different allelic combinations at target QTL in BC5/6F2/3. It could have been underestimated in some crosses for simultaneous introgression of three QTL (91 lines per BC generation), according to theoretical estimates (Hospital and Charcosset 1997), leading to no or low plant selection outside QTL regions. Second, the number of markers to control introgressions (≥3 for 84 % of the introgressed fragments) was mostly adequate to prevent QTL loss from double recombination events in the moderate to large sized QTL fragments (9–37.5 cM), even though marker positions were not always optimized due to the lack of polymorphic markers available. Undesirable alleles (normal leaves, colored flowers), which were previously reported to be linked to PI180693 resistance alleles at *Ae-Ps1.2* and *Ae-Ps2.2* (Hamon et al. 2013), were introduced into the NILs, as a result of unreduced introgression sizes (Supplementary data file 1). Such negative associations between resistance and morphological alleles were frequently reported (St Clair 2010; Tanksley and Nelson 1996). Third, the number of markers for controlling the return to the recipient genome was reasonable on each QTL non-carrier linkage group (one to three markers), since 50 % of the recipient genome is returned without markers at each BC generation. On QTL carrier linkage groups, few flanking markers to control and reduce introgression sizes were available, sometimes resulting in larger introgressed fragments than initially targeted. Five or six BC generations were conducted to ensure a higher level of return to recipient genome and overcome the overall low number of markers for MAS. Recently, massive numbers of SNP markers were developed from next-generation sequencing programs in pea (Boutet et al. (2015) submitted; Duarte et al. 2014; Sindhu et al. 2014), which will be a valuable tool for improved optimization of future MAB programs.

Major- and some minor-effect QTL validated in NILs for resistance to *A. euteiches* under controlled conditions

In the NIL sets which showed the highest QTL effects, individual QTL effects observed in reference recipient backgrounds were detected with similar levels as previously described for RIL populations, i.e., high for QTL *Ae-Ps7.6* and *Ae-Ps4.5*, low for QTL *Ae-Ps5.1* and null for QTL *Ae-Ps4.1* under controlled conditions. Bi-QTL combinations increased resistance compared to individual QTL, suggesting additive or epistatic QTL effects when combined.

The QTL *Ae-Ps7.6* had a major effect on resistance in most of the NILs evaluated with the RB84 strain. Effects of introgressions from the different donor lines at QTL *Ae-Ps7.6* were different in the NILs produced, either high or null for resistance alleles derived from 90-2131 or PI180693 and from 552, respectively, in accordance with previous QTL results. The QTL *Ae-Ps7.6* was not detected previously in the Baccara × 552 RIL population with the RB84 strain but it was found with other strains, while the 90-2131 and PI180693 alleles at QTL *Ae-Ps7.6* were detected and explained up to 60 and 14 % of the phenotypic variation (R^2) in the DSP × 90-2131 and Baccara × PI180693 RIL populations, respectively (Hamon et al. 2011, 2013). Differences in effects were previously reported between the 90-2131 and PI180693 alleles at QTL *Ae-Ps7.6* which were not detected in the same genetic background.

QTL *Ae-Ps4.5* showed a highly significant effect on resistance to the Ae109 strain in all NIL sets from the RIL 831.08 donor line. This result validates those obtained in the Puget × 90-2079 RIL population (Pilet-Nayel et al. 2002, 2005), which showed a contribution of QTL *Ae-Ps4.5* to the resistance up to 44 % depending on the environments tested. Furthermore, the QTL were recently detected in this population with a very strong effect on resistance to Ae109 and a lower effect on resistance to RB84 (Pilet-Nayel, unpublished data). These results explained the high and low levels of resistance to Ae109 and RB84, respectively, of the subsequent selected RIL 831.08, used as donor parent in this study.

Other QTL with significantly smaller individual effects on resistance in the NILs (*Ae-Ps5.1* from RIL 847.50, *Ae-Ps2.2* from RIL BAP8.195) were also previously detected in RIL populations with lower effects than QTL *Ae-Ps7.6* and *Ae-Ps4.5* (Hamon et al. 2013), and not consistently with the RB84 and Ae109 strains. Alleles from 552 at QTL *Ae-Ps2.2* and *Ae-Ps3.1,* which did not have any significant individual effects in NILs, were previously detected in RILs with low R^2 (<10 %) for resistance to the RB84 and Ae109 strains. Minor-effect QTL are difficult to demonstrate in pathological tests using semi-quantitative scoring scales, all the more so when transferred into new genetic backgrounds in NILs (Falke and Frisch 2011). The scoring scale used for partial resistance evaluation did not allow very large variation to be revealed between donor and recipient lines, limiting the detection of minor differences between NILs. Finer phenotyping methodologies will be required to validate minor-effect QTL.

The effects of several of the QTL could not be validated in the NIL sets produced. In most cases, these QTL were not previously detected in RIL populations with the RB84 or Ae109 strain data under controlled conditions (Hamon et al. 2013). In particular, the effects of PI180693 alleles at QTL *Ae-Ps1.2, Ae-Ps3.1* and *Ae-Ps4.1*, not detected in the NILs, were previously only identified for field resistance.

Little variability in the effects of the QTL between sister NILs was observed, as suggested by the highly similar genome composition of each sister NIL pair revealed by the SSR markers tested. Nevertheless, some phenotypic differences were sometimes observed between sister NILs, which were either consistent with their genomic differences (introgression sizes, NIL15-2.2/7.6a-b; residual donor fragments outside target QTL, NIL12-1.2/2.2/3.1a-b) or unrelated to their apparent identical genomic composition (NIL5-7.6a-b; NIL14-7.6a-b). High-density SNP fingerprinting of NILs is of high interest to confirm their genome content (Pea et al. 2013) and is in progress for all the NILs produced in this study (Tayeh et al. 2015).

QTL effects on resistance to *A. euteiches* depending on pea genetic backgrounds

Different QTL × genetic background interactions were observed for validated QTL in reference genetic backgrounds. The interactions could be attributed to remaining donor fragments in unlinked or flanking QTL regions during the NILs construction which biased the real QTL effect. They also could be due to removed or new epistatic interactions when QTL were transferred into other genetic backgrounds.

In the agronomic genetic backgrounds, the QTL had three different types of effects, when compared to reference backgrounds. No or few QTL × recipient line interactions were identified for QTL *Ae-Ps4.5*, which may be related to its pronounced effect on resistance to Ae109, as previously observed (Pilet-Nayel Pers. Comm.). Positive QTL × recipient line interactions were found for QTL *Ae-Ps7.6* from RIL 847.50 or RIL BAP8.70, resulting in an increased effect on resistance in the winter pea cultivar Isard compared to the spring pea cultivars Eden, Baccara or DSP. QTL *Ae-Ps5.1* and *Ae-Ps4.1* showed negative QTL × recipient line interactions, and displayed, individually or in combination with QTL *Ae-Ps7.6*, no effect on resistance (QTL *Ae-Ps5.1*) or an even increased effect on susceptibility (QTL *Ae-Ps4.1*) in both the winter Isard and spring Eden cultivars compared to the reference backgrounds.

Possible variations in genome content between the different recipient lines introgressed with the same fragments could explain the QTL × genetic background interactions observed. Particularly, slightly shorter introgression sizes for QTL *Ae-Ps5.1* in Isard and Eden compared to DSP were observed, which is consistent with the absence of an effect of QTL *Ae-Ps5.1* individually or in combination with QTL *Ae-Ps7.6*. In contrast, a larger introgression size for QTL *Ae-Ps4.5* was identified in Isard and Eden than in Puget, which may cause the slightly lower effect of this QTL in the agronomic lines.

Epistatic interactions between the target QTL and other QTL or genomic regions, as identified in Hamon et al. (2011), could also explain the QTL × genetic background interactions observed. During the generation of the NILs, the loss of parental donor or reference recipient alleles all over the genome, which may interact with the target QTL, would result in a decrease or loss of the QTL effect when transferred into other genetic backgrounds. In our study, QTL *Ae-Ps5.1* lost its effect when introgressed into either Eden or Isard genetic backgrounds. Effect of the PI180693 resistance allele at QTL *Ae-Ps7.6*, which was previously reported to interact with the Baccara allele at QTL *Ae-Ps2.2* for increasing resistance to RB84 strain [Ae-PsE5, $R^2 = 10.6$ % (Hamon et al. 2013)], was confirmed in the Baccara reference recipient line and was stronger than in Eden. Similar examples of lost QTL effects in introgression lines were reported (Reyna and Sneller 2001; Xue et al. 2009). On the other hand, during NIL construction, new epistatic interactions can also be created between the introgressed QTL fragment and the new genetic background, which may result in higher QTL effects. In our study, the effect of 90-2131 and PI180693 resistance alleles at QTL *Ae-Ps7.6* was enhanced in the winter pea cultivar Isard and confirmed in the winter cultivar Enduro, compared to the spring pea genetic backgrounds. In wheat, Salameh et al. (2011) found that two QTL controlling fusarium head blight resistance had variable effects when transferred into different European winter varieties. Winter plants most likely contain genes involved in tolerance to abiotic stress such as winter frost (Yadav 2010), which may interact with and/or be the same as resistance loci to pathogens. Several studies reported positive interactions between frost and pathogens at the phenotypic level, involving mechanisms based on modification of photosynthetic activity and sugar content (Pociecha et al. 2010) and induction of PR proteins with antifreeze activity (Yu et al. 2001). In pea, common loci were identified between partial resistance to ascochyta and frost tolerance (Giorgetti, unpublished data). Analyzing genetic relationships between Aphanomyces resistance and frost tolerance loci in winter pea genetic backgrounds would lead to an understanding of the QTL × winter vs. spring genetic background interactions observed.

Conclusion

In this study, a novel and valuable plant material resource was developed for further research on QTL mechanisms and their application in breeding programs. We validated the effects of two major and several minor QTL and demonstrated interaction effects between QTL and recipient lines in the NILs created. QTL effects in the NILs will be validated for field resistance in infested conditions,

regarding finer genomic characterization. The NILs will be particularly useful for studying the effect of the QTL on the pathogen life cycle (Chung et al. 2010; Richardson et al. 2006). They will be also valuable for fine mapping of QTL (Balkunde et al. 2013; Ding et al. 2011; Zhong et al. 2014) from heterozygous NILs at each individual QTL that were also produced in this study. NILs will also be used to identify the best QTL combinations for increasing resistance in pyramided lines carrying more than three resistance QTL from different donor lines (Fujita et al. 2010).

Author Contribution statement CL generated the phenotypic and genotypic data, carried out all the statistical and genetic analysis and drafted the manuscript. AL coordinated and participated in the entire MAB program and attended in generating the phenotypic data. CPi and GB participated in PCR and analysis of microsatellite data of the MAB program and the resulted NILs. GLR participated in the pea growings and flower manual crosses in the MAB program. CPo produced all the genotyping data on the GENTYANE platform. AM participated in the first generations of the MAB program. RD co-supervised CL's PhD work and participated in drafting the manuscript. AB participated in elaborating the MAB program and drafting the manuscript. MLPN supervised the MAB and NIL evaluation program, the PhD work of CL and the manuscript writing. All authors approved the final draft of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards Authors declare that the described experiments comply with the French laws.

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