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ORIGINAL PAPER

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Mapping of quantitative trait loci for partial resistance to *Mycosphaerella pinodes* in pea (*Pisum sativum* L.), at the seedling and adult plant stages

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Abstract The inheritance of resistance to Ascochyta blight, an economically important foliar disease of field pea (*Pisum sativum* L.) worldwide, was investigated. Breeding resistant pea varieties to this disease, caused by *Mycosphaerella pinodes*, is difficult due to the availability of only partial resistance. We mapped and characterized quantitative trait loci (QTLs) for resistance to *M. pinodes* in pea. A population of 135 recombinant inbred lines (RILs), derived from the cross between DP (partially resistant) and JI296 (susceptible), was genotyped with morphological, RAPD, SSR and STS markers. A genetic map was elaborated, comprising 206 markers distributed over eight linkage groups and covering 1,061 cM. The RILs were assessed under growth chamber and field conditions at the seedling and adult plant stages, respectively. Six QTLs were detected at the seedling stage, which together explained up to 74% of the variance. Ten QTLs were identified at the adult plant stage in the field, and together these explained 56.6–67.1% of the variance, depending on the resistance criteria and the organ considered. Four QTLs were detected under both growth chamber and field conditions, suggesting they were not plant-stage dependent. Three QTLs for flowering date and three QTLs for plant height were also identified in the RIL population, some of which co-located with QTLs for resistance. The relationship between QTLs for resistance to *M. pinodes*, plant height and flowering date is discussed.

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Introduction

Ascochyta blight, caused by *Mycosphaerella pinodes*, is the most economically important foliar disease of field pea (*Pisum sativum* L.) worldwide. It can be caused by three closely related pathogens—*Ascochyta pisi*, *Mycosphaerella pinodes* (Berk. and Blox.) and *Phoma medicaginis* var. *pinodella*—and is found in most of the pea-growing areas of the world almost every year. *M. pinodes* causes necrotic spots on all aerial parts of the pea plant and is responsible for important yield and seed quality losses (Allard et al. 1993; Garry 1996; Béasse et al. 1999). In France, *M. pinodes* is the most destructive of the three pathogens to crops (Tivoli et al. 1996).

Several cultural management practices have been recommended to prevent these losses (Allard et al. 1993); these include (1) the rapid destruction of crop residues following harvesting, (2) the use of 3- to 4-year rotations and the growing of nonhost crops between pea crops and (3) the choice of an appropriate sowing density fitting the variety, the sowing date and the local climate conditions. While chemical treatments can reduce disease severity and preserve pea yields, multiple sprays are often needed during the growing season. To decrease production costs and to better preserve the environment, the development of resistant cultivars is therefore an important component in an integrated strategy to control *M. pinodes*.

Breeding pea varieties with resistance to *M. pinodes* is difficult due to (1) the availability of only partial resistance (Ali-Khan et al. 1973; Ali et al. 1978; Bretag 1989, 1991; Clulow 1989; Clulow et al. 1991; Nasir et al. 1992; Xue et al. 1996; Kraft et al. 1998; Onfroy et al. 1999; Prioul et al. 2003), (2) the polygenic inheritance of the resistance (Zlamal 1984; Wroth 1999; Timmerman-Vaughan et al. 2002) and (3) the difficulty in evaluating resistance under field conditions because of interactions with agronomic (Bretag and Brouwer 1995; Tivoli et al. 1996; Wang et al. 1997), agricultural and/or environmental (Bretag 1991; Xue et al. 1997; Wroth and Khan 1999) factors. To date, no commercial varieties with an adequate level of resistance to *M. pinodes* are available. However, sources of partial resistance have been identified in non-adapted pea lines.

At the seedling stage, partial resistance to *M. pinodes* is expressed as a rate-reducing resistance (Zimmer and Sabourin 1986) that is characterized by a delay in both the appearance of the first symptoms and the development of the disease (Prioul et al. 2003). Different resistance mechanisms such as a decrease in the formation of infection vesicles, hypersensitive responses or limitation of the spread of the fungus in plant tissue have been reported under controlled conditions (Clulow 1989; Nasir et al. 1992; Wroth 1996). Various studies have sought to characterize the expression of partial resistance to Ascochyta blight at the adult plant stage in the field (Wroth and Khan 1999; Xue et al. 1997; Xue and Warkentin 2001), but it was difficult to draw conclusions from the results due to factors interacting with disease severity assessments.

The genetic control of *M. pinodes* resistance in pea is still a controversial issue. Using a qualitative approach under controlled conditions, Clulow et al. (1991) classified genotypes on the basis of disease scores: 0–2 indicated resistance, and genotypes with disease scores of 3–5 were susceptible. They identified four dominant genes for resistance at the seedling stage: *Rmp1* and *Rmp2* for stem resistance, and *Rmp3* and *Rmp4* for foliar resistance. Quantitative approaches based on diallel analysis, developed by Zlamal (1984) and Wroth (1999), have suggested that the inheritance of resistance to *M. pinodes* would be polygenic with additive effects. More recently, quantitative trait loci (QTLs) for resistance to natural epidemics of Ascochyta blight at the adult plant stage (Timmerman-Vaughan et al. 2002) have been identified. However, in this last study, specificity of the QTLs for resistance to the different pathogens of the Ascochyta complex (*M. pinodes, A. pisi* and *P. medicaginis*) was not determined. None of the above studies addressed the issue of the comparative control of partial resistance between the seedling and the adult stages.

The objective of the investigation reported here was to gain a better knowledge of the genetic factors controlling pea partial resistance to *M. pinodes* at two plant developmental stages and under two environmental conditions. We carried out QTLs mapping in a population of recombinant inbred lines (RILs) screened under controlled and field conditions, at the seedling and adult plant stage, respectively. Relationships between factors controlling resistance to *M. pinodes*, plant height and flowering time were also considered.

Materials and methods

Plant material

The segregating population used was a F_2-derived RIL population developed in the greenhouse by single seed descent from the cross JI296×DP. The line DP is a tall, purple-flowered, late-flowering fodder pea with normal leaves that is partially resistant to *M. pinodes* (Onfroy et al. 1999; Prioul et al. 2003). The susceptible parent, JI296, is a dwarf, white-flowered, early-flowering French garden pea cultivar with normal leaves (cv. Chemin Long).

Genetic map construction

A total of 135 $F_{2:6}$-derived RILs were genotyped using random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and sequence-tagged site (STS) markers. Three morphological traits were scored: *P1* for hilum color, *A* for anthocyanin production and *Np* for neoplastic pod. DNA was extracted from 4–5 g of leaf tissue, bulked from at least ten plants of the same line, using the CTAB method of Doyle and Doyle (1990). RAPD genotyping was carried out with Operon primer pairs (Operon Technologies, Alameda, Calif.) using the procedure described by Laucou et al. (1998). SSR markers, developed by Burstin et al. (2001) or by the pea Agrogène Consortium (Moissy Cramayel, France), were amplified following the protocol reported in Burstin et al. (2001). STS markers were generated using the primers developed from sequence-characterized pea cDNA by Gilpin et al. (1997) and from cloned genes by Weeden et al. (1999, 2001). PCR procedures for STS were performed as reported by the respective authors.

For each segregating marker, a chi-square analysis (α=0.01) was used to test for deviations from the expected (1:1) segregation ratio in the RILs. Linkage analyses were performed using MAPMAKER/EXP ver. 3.1 (Lander and Botstein 1989; Lincoln et al. 1992). A stringent minimum LOD score of 6.0 was used to construct linkage groups and prevent false positive assignations of markers to linkage groups. Subsequently, a LOD score of 2.0 and a maximum distance of 30 cM were chosen for establishing marker order on each linkage group. The Kosambi function (Kosambi 1944) was used to calculate genetic distances in centiMorgans. The chromosomal assignment of linkage groups was deduced from the mapping of loci common to other published pea genetic maps (Gilpin et al. 1997; Laucou et al. 1998; Weeden et al. 1998, 2001; Pilet-Nayel et al. 2002).

Seedling-stage resistance in the growth chamber

A population of 131 $F_{2:7}$-derived RILs and the two parental lines as controls were used in this experiment. Seeds were sown in 9-cm (diameter) pots (three seeds/pot) of unsterilized soil/compost mixture (1:1:1; soil:sand:peat). The experiment was designed according to a completely randomized block design with four replicates (total of 12 plants per line). Growth chamber environmental conditions, inoculum preparation and plant inoculation procedures were as described in Prioul et al. (2003). As there is no evidence for the existence of virulence-based pathotype groups among French *M. pinodes* isolates (Onfroy et al. 1999), we therefore used a single monosporic isolate (Mp94.01.3) that had been collected in France in 1994.

Disease severity was assessed separately on stipules and stems, on the inoculated part of each plant, namely the first three stipules and the first three internodes. Disease severity on leaves and stems was scored on a 0–5 scale (Tivoli et al. 1996), as described in Prioul et al. (2003). Disease assessments were performed at 3, 5, 7, 10, 13, 17 and 20 days following inoculation. The area under the disease progress curve (AUDPC) was calculated by plotting mean disease severities against time according to the formula proposed by Shaner and Finney (1977). Two traits were used for QTL analysis, i.e. AUDPC on stipules and AUDPC on stems.

Adult plant resistance in the field

A population of 119 $F_{2:7}$-derived RILs and the two parental lines as controls were evaluated in the field during the spring of 2001, at INRA Rennes, France. One hundred and twenty plants per line were sown in a completely randomized block design with three replicates. Each plot in each replication consisted of 2-m-long twin rows with 20 plants per row. Wires were placed between rows so as to stake plants and prevent interaction between disease development and lodging. RILs were inoculated at the five-leaf stage by depositing between rows contaminated barley grains colonized by *M. pinodes.* Inoculum preparation and plant inoculation procedures are described in Roger and Tivoli (1996). Briefly, barley grain lots were infected separately with four single-spore isolates of *M. pinodes* (Mp94.14.6, Mp94.76.3, Mp94.56.1, Mp96.35.1) that had been isolated in France in 1994 and 1996. These isolates differ only in their aggressiveness and show no virulence effects under controlled conditions (C. Onfroy, personal communication). After 1 month of contamination, the four infected barley grain lots were mixed in equal amounts and the barley grains deposited on the soil along the plant rows. The field was then regularly irrigated to ensure plant contamination and disease development through splashing.

Disease severity was assessed on four plants per RIL per replication, on stipules and stems separately, on the first 15 internodes, using the same 0–5 scale (Tivoli et al. 1996) as the one for the seedlings. Disease assessments were performed at 452, 641 and 886°C day thermal times following inoculation. Mean disease severities over internodes were calculated for all assessment dates and the AUDPC were calculated on stipules and stems, as previously described. At the third scoring date, diseased stem height until the score 3 (HT3), diseased stipule height until the score 4 (HS4) and height of the 15th node (H15) were also measured, and the proportion of height-injured ratios were calculated as: %HT3=HT3/H15 and %HS4=HS4/H15. Flowering dates (Dflo) were also scored, as the number of days to 50% bloom. Four traits were used for resistance QTL analysis: AUDPC on stipules, AUDPC on stems, %HS4 and %HT3.

Statistical analyses

Statistical analyses were performed using the SAS ver. 6.12 package (SAS Institute, Cary, N.C.). For each resistance criterion, a one-way analysis of variance (ANOVA) was performed using a generalized linear model (PROC GLM). Normality of residual distribution ($P>0.05$) and homogeneity of variances ($P>0.05$) were checked using the Shapiro and Wilk test (Shapiro and Wilk 1965) and the Bartlett's test (Snedecor and Cochran 1957), respectively. Original data were used for analyses because angular or logarithmic data transformations did not improve normality. Heritability (h^2) was estimated from ANOVA using the formula $h^2 = \sigma_g^2/\left[\sigma_g^2+\left(\sigma_{gb}^2/n\right)+\left(\sigma_e^2/nr\right)\right]$ with σ_g^2 the genetic variance, σ_{gb}^2 the genotype × replicate interaction variance, σ_e^2 the residual variance, n the number of replicates and r the number of plants per line. For each resistance criterion × evaluation condition combination, adjusted means of the RILs were calculated from ANOVA and used as experimental units for QTL analysis. Pearson correlation coefficients between resistance criteria were estimated from adjusted mean data using correlation analysis (PROC CORR).

QTL analysis

QTL mapping was conducted by composite interval mapping (CIM) (Zeng 1993, 1994) using the computer program QTL CARTOGRAPHER WINDOWS V1.30 (Basten et al. 1994, 2001). A forward-backward stepwise regression was run to select background markers. Ten markers with the highest *F*-values ($P<0.05$) were considered as co-factors in the standard model (model 6 of the program). A window size of 10 cM was chosen for all analyses. Permutation tests were performed to estimate mean LOD thresholds. After 1,000 permutations, mean LOD thresholds of 2.9, and 2.8 were chosen to declare a putative QTL significant corresponding to approximately an overall 5% α-type I error (Lander and Botstein 1989), for traits observed under controlled conditions and in the field, respectively. For each QTL, estimates of phenotypic variance (r^2) and additive effect were obtained from the program. Markers associated to QTLs for resistance detected by CIM were checked by one-way ANOVA ($P\leq0.01$) and by the Kruskal-Wallis non-parametric test ($P=0.0001$, PROC NPAR1WAY of SAS). QTLs were named as '*mp*' (*Mycosphaerella pinodes*) followed by the linkage group (Roman number) and QTL number (Arabic number).

Results

Genetic map

The RILs were genotyped using 206 markers, including 122 RAPDs, 71 SSRs, ten STSs and three morphological trait genes. The average percentage of residual heterozygosity, calculated for each line as the percentage of heterozygote loci over all codominant loci, was 2%.

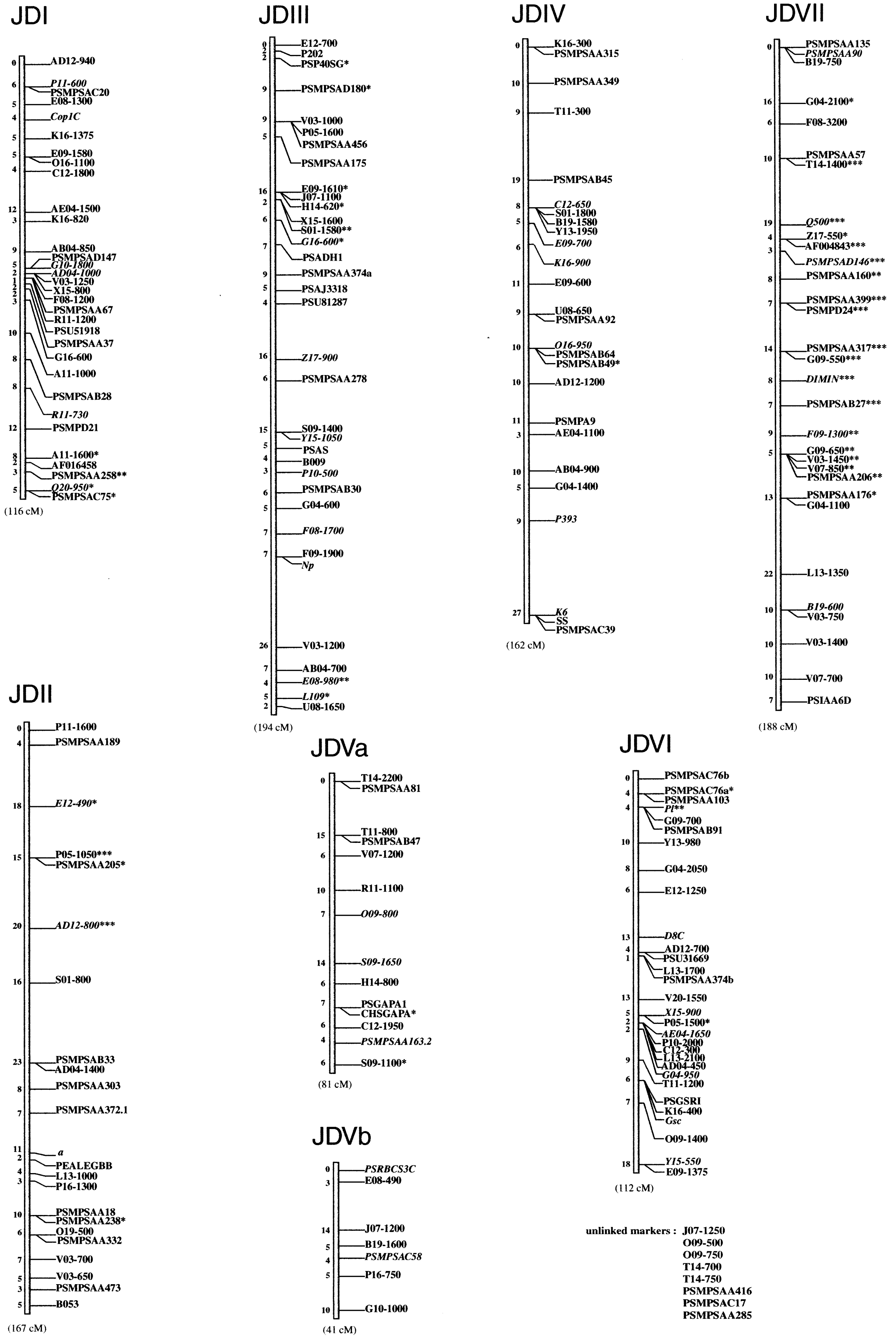
Of these 206 markers, eight markers remained unlinked and 198 were distributed over eight linkage groups, covering 1,061 cM (Fig. 1). Linkage groups were assigned to the international pea consensus genetic map (Weeden et al. 1998) using 43 markers common to other pea genetic maps previously described in the literature. No linkage group remained unassigned. The map was longer than the expected approximately 800 cM based on cytogenetic studies (Hall et al. 1997). To our knowledge, this is the first pea genetic map comprising so many (71) SSR markers.

Among the 206 markers, 42 (20.4%) did not segregate according to the expected Mendelian ratio (1:1). Both dominant and codominant markers were affected by segregation distortion. Markers showing segregation distortion were found on all linkage groups, except Vb. Deviations towards the susceptible parent (JI296) alleles were found on linkage groups I, III, Va and VI, while those favoring the resistant parent (DP) alleles were observed on linkage groups II and VII. A large cluster of markers with extreme distortion was found on linkage group VII (Figure 1).

Resistance tests

Under both controlled conditions and in the field, analyses of variance showed significant genotype and replicate effects ($P\leq0.0001$) on both the stipules and stems for all resistance scoring criteria. Genotype × replicate interactions were statistically significant, but the contribution of these interactions to the total variation was much lower than that of the main genotype effect

Fig. 1 Genetic linkage map constructed from 135 $F_{2:6}$-derived RILs from the cross JI296×DP. The size of each linkage group is indicated in Kosambi centiMorgans (cM) *below* each group. Markers displayed in ***bold italics*** are anchored to other published pea genetic maps (Gilpin et al. 1997; Laucou et al. 1998; Weeden et al. 1998, 2001; Pilet-Nayel et al. 2002). Markers with segregation distortion at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$ are indicated by *, ** and ***, respectively


JDI
AD12-940
P11-600
PSMPSAC20
E08-1300
CopIC
K16-1375
E09-1580
O16-1100
C12-1800
AE04-1500
K16-820
AB04-850
PSMPSAD147
G10-1800
AD04-1000
V03-1250
X15-800
F08-1200
PSMPSAA67
R11-1200
PSU51918
PSMPSAA37
G16-600
A11-1000
PSMPSAB28
R11-730
PSMPD21
A11-1600*
AF016458
PSMPSAA258**
Q20-950*
PSMPSAC75*
(116 cM)
JDIII
E12-700
P202
PSP40SG*
PSMPSAD180*
V03-1000
P05-1600
PSMPSAA456
PSMPSAA175
E09-1610*
J07-1100
H14-620*
X15-1600
S01-1580**
G16-600*
PSADH1
PSMPSAA374a
PSAJ3318
PSU81287
Z17-900
PSMPSAA278
S09-1400
Y15-1050
PSAS
B009
P10-500
PSMPSAB30
G04-600
F08-1700
F09-1900
Np
V03-1200
AB04-700
E08-980**
L109*
U08-1650
(194 cM)
JDIV
K16-300
PSMPSAA315
PSMPSAA349
T11-300
PSMPSAB45
C12-650
S01-1800
B19-1580
Y13-1950
E09-700
K16-900
E09-600
U08-650
PSMPSAA92
O16-950
PSMPSAB64
PSMPSAB49*
AD12-1200
PSMPA9
AE04-1100
AB04-900
G04-1400
P393
K6
SS
PSMPSAC39
(162 cM)
JDVII
PSMPSAA135
PSMPSAA90
B19-750
G04-2100*
F08-3200
PSMPSAA57
T14-1400***
Q500***
Z17-550*
AF004843***
PSMPSAD146***
PSMPSAA160**
PSMPSAA399***
PSMPD24***
PSMPSAA317***
G09-550***
DIMIN***
PSMPSAB27***
F09-1300**
G09-650**
V03-1450**
V07-850**
PSMPSAA206**
PSMPSAA176*
G04-1100
L13-1350
B19-600
V03-750
V03-1400
V07-700
PSIAA6D
(188 cM)
JDII
P11-1600
PSMPSAA189
E12-490*
P05-1050***
PSMPSAA205*
AD12-800***
S01-800
PSMPSAB33
AD04-1400
PSMPSAA303
PSMPSAA372.1
a
PEALEGBB
L13-1000
P16-1300
PSMPSAA18
PSMPSAA238*
O19-500
PSMPSAA332
V03-700
V03-650
PSMPSAA473
B053
(167 cM)
JDVa
T14-2200
PSMPSAA81
T11-800
PSMPSAB47
V07-1200
R11-1100
O09-800
S09-1650
H14-800
PSGAPA1
CHSGAPA*
C12-1950
PSMPSAA163.2
S09-1100*
(81 cM)
JDVb
PSRBCS3C
E08-490
J07-1200
B19-1600
PSMPSAC58
P16-750
G10-1000
(41 cM)
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PSMPSAC76b
PSMPSAC76a*
PSMPSAA103
Pl**
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PSMPSAB91
Y13-980
G04-2050
E12-1250
D8C
AD12-700
PSU31669
L13-1700
PSMPSAA374b
V20-1550
X15-900
P05-1500*
AE04-1650
P10-2000
C12-300
L13-2100
AD04-450
G04-950
T11-1200
PSGSRI
K16-400
Gsc
O09-1400
Y15-550
E09-1375
(112 cM)
unlinked markers :
J07-1250
O09-500
O09-750
T14-700
T14-750
PSMPSAA416
PSMPSAC17
PSMPSAA285

according to the highly significant *F* test (data not shown). Figure 2 shows the distributions of the RIL adjusted means: there is a continuous variation of the traits, but these do not fit normal distributions according to the W statistics of Shapiro-Wilk ($P \leq 0.05$) except for AUDPC on stipules assessed in the field. Transgressive lines with increased resistance and susceptibility were observed for all field resistance traits.

At the seedling stage under controlled conditions, high heritabilities were found for AUDPC on stipules (h^2=0.91) and stems (h^2=0.90). AUDPC on stipules and AUDPC on stems were highly correlated (Table 1).

At the adult plant stage in the field, high heritabilities were found for AUDPC on stipules (h^2=0.79), %HS4 (h^2=0.85) and %HT3 (h^2=0.78). A lower heritability (h^2=0.53) was associated with AUDPC assessed on the stems. Except for correlations between AUDPC on stems and %HS4 or %HT3, all other scoring criteria were significantly correlated (Table 1).

Correlations between both conditions (growth chamber, field) were highly significant for both stipules and stems (Table 1).

QTL mapping

A sub-set of 143 evenly-spaced markers (average interval of 6.7 cM) was kept for QTL analysis, including 90 RAPDs, 44 SSRs, eight STSs and one morphological trait gene.

Seedling-stage resistance

Six QTLs associated with AUDPC were localized on linkage groups III, Va, VI and VII. Genomic positions of each QTL, individual additive effects and LOD scores are summarized in Table 2 and Fig. 3. The QTLs explained individually from 5% to 20% of the total phenotypic variation, depending on the organ concerned. Altogether, the QTLs accounted for 73% and 74% of the total phenotypic variance on the stipules and stems, respectively. Among these QTLs, five were common to both stipules and stems. All of the resistance alleles associated with QTLs for AUDPC were derived from the resistant parent DP, except for the *mpIII-2* QTL located on the distal part of the linkage group III (Table 2).

Adult-stage resistance

Ten QTLs, distributed over linkage groups II, III, Va and VII, were detected either on stipules or stems or on both organs using the parameters AUDPC, %HS4 and %HT3 (Table 2). These QTLs explained individually from 6% to 42% of the total phenotypic variation, depending on the criterion and the organ concerned. Altogether, the QTLs detected with AUDPC explained 63.7% and 56.6% of the total phenotypic variance on the stipules and stems, respectively. The QTLs identified with %HS4 collectively explained 58.1% of the total phenotypic variance, and those identified with %HT3 collectively explained 67.1% of the variance. Among these ten QTLs, three were common to stipules and stems (*mpII-2*, *mpIII-1* and *mpIII-3*), three were identified only on stipules (*mpII-1, mpVII-1* and *mpVII-2*), and four were specific to the criteria measured on stems (*mpIII-4*, *mpIII-5, mpVa-1* and *mpVa-2*). For those QTLs detected on the stipules, resistance alleles originated from the resistant parent, except for the QTLs *mpVa-1* and *mpVII-1*; on the stems, resistance alleles were donated by the susceptible parent for QTLs *mpIII-5* and *mpVa-2*.

Comparison between conditions

Four genomic regions identified from AUDPC data obtained under controlled conditions co-located with QTLs identified from the field data (Table 2, Fig. 3), and these were identified on linkage groups III (*mpIII-1* and *mpIII-3*), Va (*mpVa-1*) and VII (*mpVII-1*). QTL *mpIII-1* was detected with five of the six scoring traits used in this study and accounted for 18–40% of the variation, depending on the criterion concerned. QTL *mpIII-3* was detected from both stem AUDPC data obtained under controlled conditions and from field-scored %HS4 and %HT3. QTLs *mpVa-1* and *mpVII-1* were both detected with AUDPC on stipules and stems under controlled conditions and also with AUDPC on stipules (*mpVII-1*) and on stem (*mpVa-1*) in the field. The remaining QTLs were detected specifically in one or the other condition (*mpIII-2* and *mpVI-1* at the seedling stage, *mpII-1, mpII-2*, *mpIII-4*, *mpIII-5*, *mpVa-2* and *mpVII-2* at the adult stage) (Table 2, Fig. 3).

Mapping of QTL controlling morphological traits

Correlation analyses showed that flowering date (Dflo) and plant height were both significantly negatively correlated with the resistance criteria in the field (Table 1), except for the correlation between Dflo and AUDPC on stems. The effects of plant height and Dflo on resistance were further examined by comparing the genomic location of QTLs detected for those traits with the QTLs for resistance to *M. pinodes* previously described.

For adult plants grown in the field, three QTLs located on linkage groups II, III and VII (Table 3, Fig. 3) accounted for up to 86.4% of the total variation for plant height. Of these three QTLs, *ht2* explained a major part of the variation (63%). QTLs *ht1* and *ht2* mapped approximately to the same regions as the resistance QTLs *mpII-1* and *mpIII-1*, respectively.

Three genomic regions controlling Dflo were identified on linkage groups II, III and VI (Table 3, Fig. 3), explaining together 62.3% of the variation. The QTL *flo1* mapped on linkage group II and contributed to a major part of the variation observed (38%). QTL *flo2* was

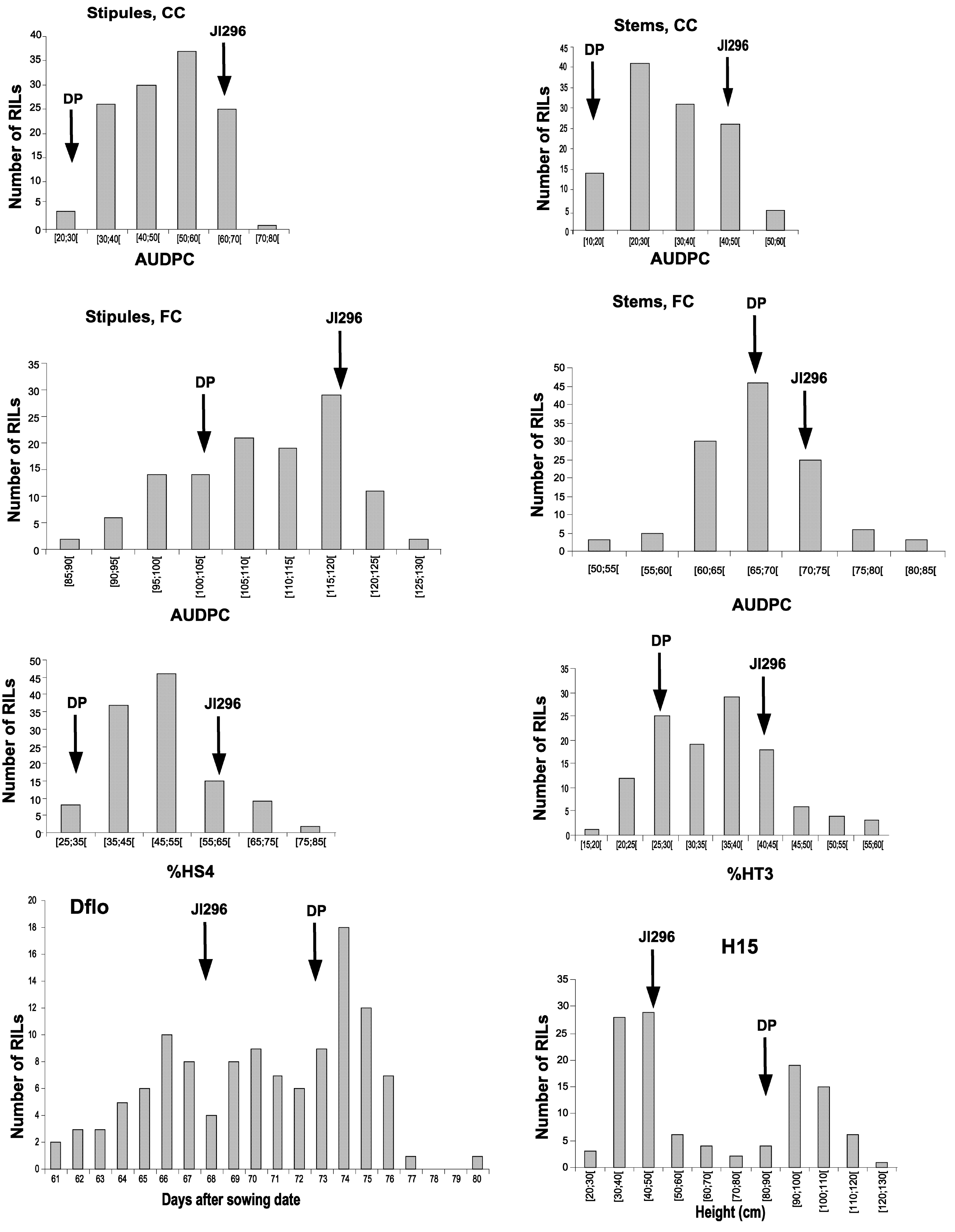
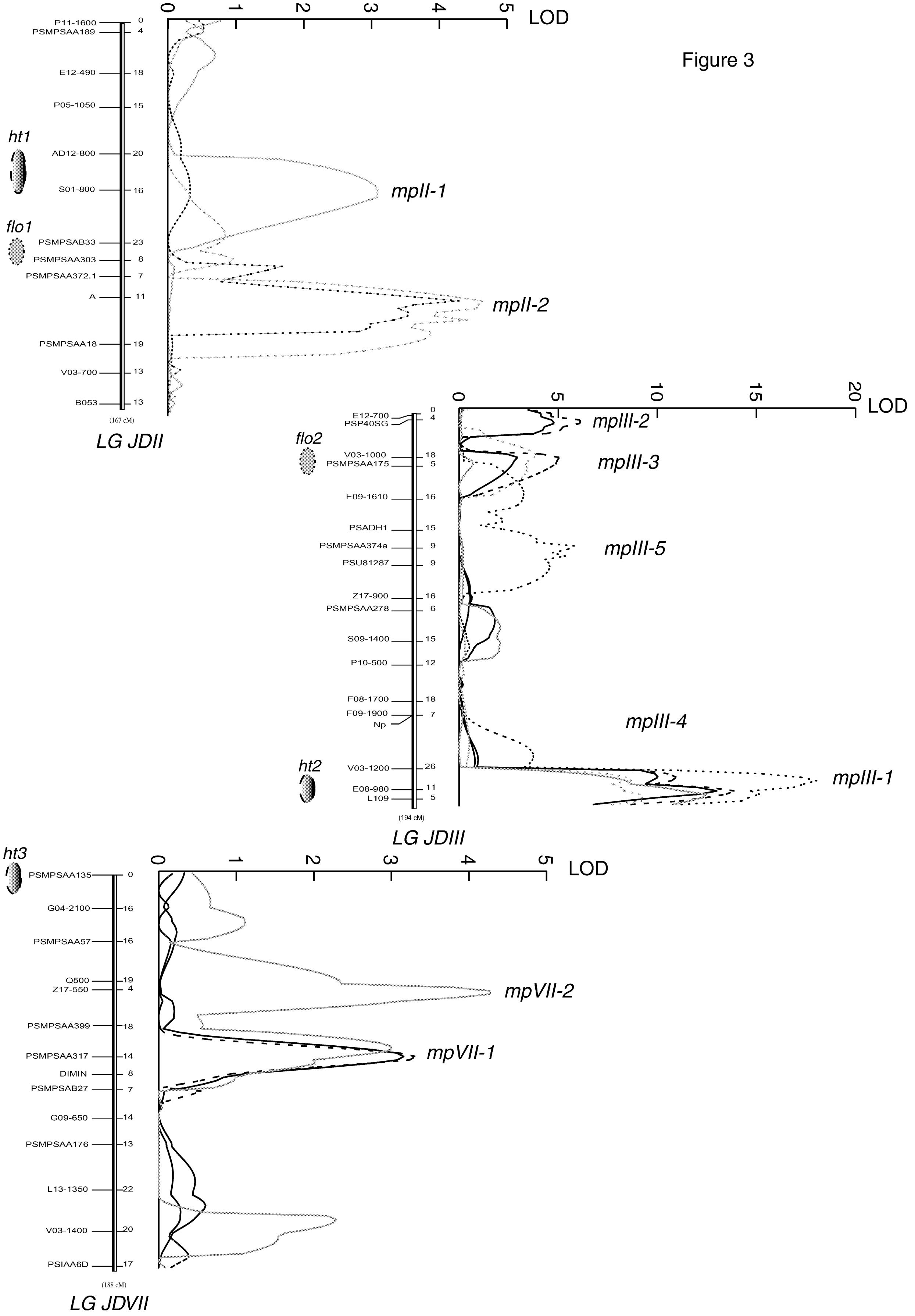


Fig. 2 Adjusted mean frequency distributions of the JI296×DP RIL population for AUDPC on stipules and stems under controlled conditions (*CC*) and in the field (*FC*) for the proportion of height-injured ratios (*%HT3*, *%HS4*), for the height of the 15th node (*HN15*) and for the flowering date (*Dflo*). *Arrows* indicate the means of the resistant (*DP*) and susceptible (*JI296*) parents

Figure 3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1
2
3
4
5
LOD
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E12-490 18
P05-1050 15
ht1
AD12-800 20
S01-800 16
flo1
PSMPSAB33 23
PSMPSAA303 8
PSMPSAA372.1 7
A 11
PSMPSAA18 19
V03-700 13
B053 13
(167 cM)
LG JDII
mpII-1
mpII-2
0
5
10
15
20
LOD
E12-700 0
PSP40SG 4
flo2
V03-1000 18
PSMPSAA175 5
E09-1610 16
PSADH1 15
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PSU81287 9
Z17-900 16
PSMPSAA278 6
S09-1400 15
P10-500 12
F08-1700 18
F09-1900 7
Np
ht2
V03-1200 26
E08-980 11
L109 5
(194 cM)
LG JDIII
mpIII-2
mpIII-3
mpIII-5
mpIII-4
mpIII-1
ht3
0
1
2
3
4
5
LOD
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G04-2100 16
PSMPSAA57 16
Q500 19
Z17-550 4
PSMPSAA399 18
PSMPSAA317 14
DIMIN 8
PSMPSAB27 7
G09-650 14
PSMPSAA176 13
L13-1350 22
V03-1400 20
PSIAA6D 17
(188 cM)
LG JDVII
mpVII-2
mpVII-1

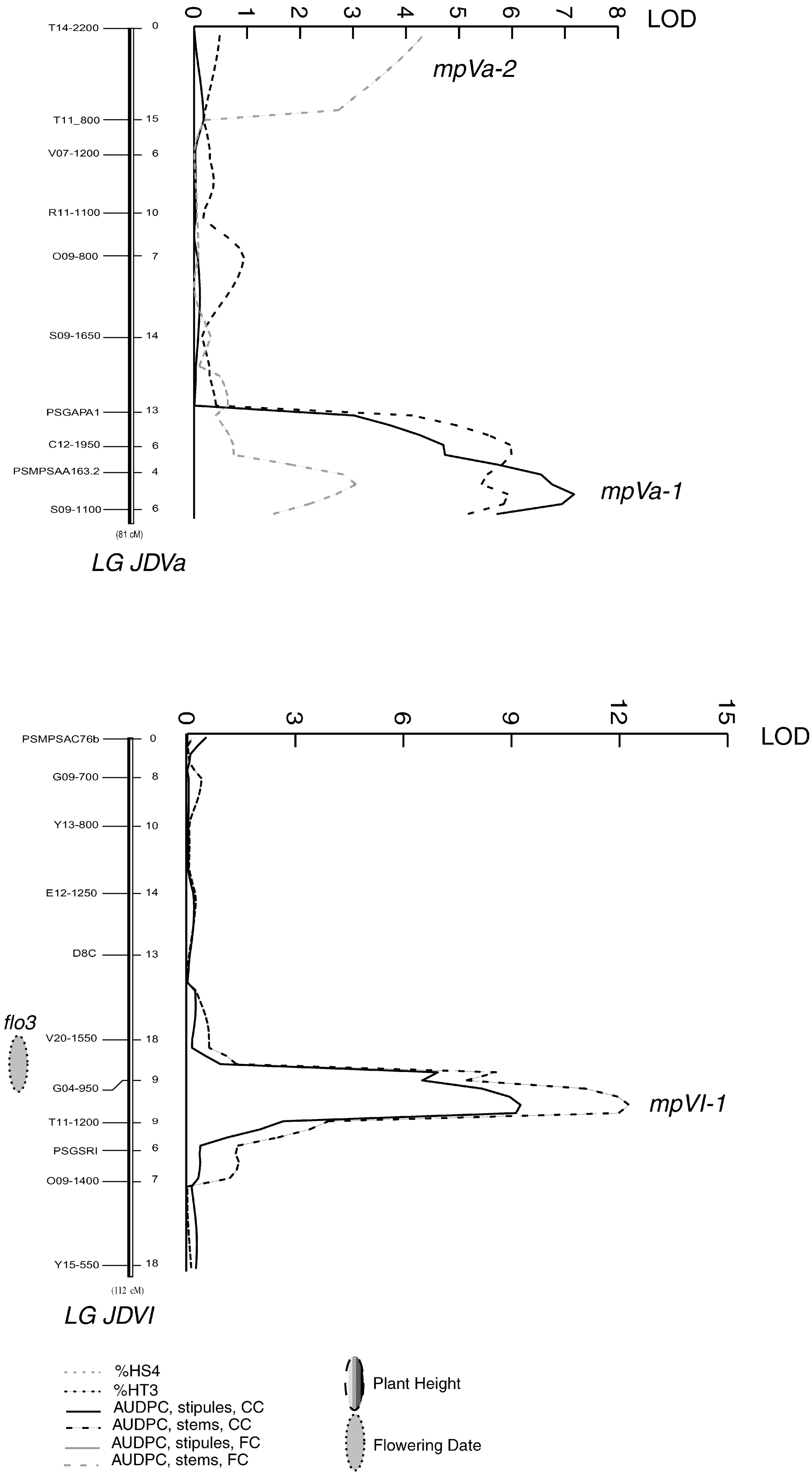

Fig. 3 LOD score curves on linkage groups (*LG*) II, III, Va, VI and VII for QTLs associated with resistance to *Mycosphaerella pinodes* at the seedling stage under controlled conditions (*CC*) and at the adult plant stage in field conditions (*FC*). LOD plots were obtained from different traits measured on the stipules and stems and detected by composite interval mapping in the cross JI296×DP (*JD*). The positions and the names of molecular markers are shown on the linkage groups *along the horizontal axis*. The genomic localizations of QTLs detected for flowering date and plant height at the adult plant stage are also indicated. *Ellipse length* corresponds to the confidence interval of the QTL defined within tenfold (1 LOD) of its peak LOD

Table 1 Pearson correlation coefficients between scoring criteria for partial resistance to *Mycosphaerella pinodes* on stipules and stems (AUDPC, %HS4 and %HT3), flowering date and plant height in a pea (*Pisum sativum* L.) RIL population derived from the cross JI296×DP. Assessments were made at the seedling stage under controlled conditions (CC) and/or at the adult plant stage in the field (FC; Rennes, France)

	Stipules[a]		Stems[a*]		
	AUDPC (FC)	%HS4	AUDPC (CC)	AUDPC (FC)	%HT3
Stipules					
AUDPC CC	0.52***	0.59***	0.94***	–0.03ns	0.60***
AUDPC FC		0.59***	0.48***	0.46***	0.58***
%HS4			0.60***	0.03ns	0.90***
Stems					
AUDPC CC				–0.48***	0.60***
AUDPC FC					0.16ns
%HT3					
Flowering date	–0.28**	–0.70***	–	0.23*	–0.51***
Plant height	–0.72***	–0.62***	–	–0.26**	–0.71***

[a] The significance of the *F*-values:*0.05>*P*>0.01,**0.01>*P*>0.001,****P*<0.001; ns, not significant
* Stipules: AUDPC (FC)+%HS4
Stems: AUDPC (CC)+AUDPC (FC)+%HT3

Table 2 QTLs for resistance to *M. pinodes* detected by composite interval mapping in the RIL population derived from the cross JI296×DP using three different scoring criteria (AUDPC, %HT3 and %HS4). Assessments were made on stipules and stems at the seedling stage under controlled conditions (CC) and/or at the adult plant stage in the field (FC)

	QTLs	Linkage group	Closest markers[a]	Stipules				Stems			
				Criteria	LOD[b]	R[2c]	Additivity[d]	Criteria	LOD[b]	R[2c]	Additivity[d]
CC	*mpIII-1*	III	*E08-980*	AUDPC	13.0	0.18	–5.374	AUDPC	13.9	0.20	–4.90
	mpIII-2	III	*PSP40SG*	AUDPC	4.8	0.07	3.556	AUDPC	6.1	0.09	3.583
	mpIII-3	III	*V03-1000*					AUDPC	4.9	0.06	–2.889
	mpVa-1	Va	*PSMPSAA163.2*	AUDPC	7.2	0.10	–3.759	AUDPC	5.9	0.08	–2.983
	mpVI-1	VI	*G04-950*	AUDPC	9.3	0.15	–4.530	AUDPC	12.3	0.20	–4.814
	mpVII-1	VII	*PSMPSAA399*	AUDPC	3.2	0.05	–3.819	AUDPC	3.3	0.06	–4.164
FC	*mpII-1*	II	*AD12-800*	AUDPC	3.1	0.06	–2.265				
	mpII-2	II	*a*	%HS4	4.6	0.09	–3.184	%HT3	4.3	0.06	–2.207
	mpIII-1	III	*E08-980*	AUDPC	12.5	0.26	–5.204				
	mpIII-1	III	*E08-980*	%HS4	9.3	0.20	–5.187				
	mpIII-1	III	*V03-1200*					%HT3	18	0.42	–5.839
	mpIII-3	III	*V03-1000*	%HS4	3.9	0.07	–2.845				
	mpIII-3	III	*PSMPSAA175*					%HT3	3.2	0.06	–2.297
	mpIII-4	III	*F09-1900*					AUDPC	6.8	0.29	–3.075
	mpIII-5	III	*PSMPSAA374a*					%HT3	5.8	0.11	3.188
	mpVa-1	Va	*PSMPSAA163.2*					AUDPC	3.1	0.07	1.483
	mpVa-2	Va	*T14-2200*					AUDPC	4.4	0.16	2.255
	mpVII-1	VII	*PSMPSAA399*	AUDPC	3.0	0.09	3.548				
	mpVII-2	VII	*Z17-550*	AUDPC	4.3	0.08	–2.871				

[a] The most closely associated marker locus with the LOD peak of the QTL is indicated
[b] LOD is the log-likelihood ratio at the QTL position
[c] R^2 is the percentage of phenotypic variation individually explained by each QTL
[d] Effect of substituting DP alleles for JI296 alleles at the LOD peak of the QTL. A negative sign reflects that the QTL alleles which increased resistance were contributed by the resistant parent, whereas a positive value means that resistance alleles were donated by the susceptible parent

Table 3 QTLs controlling plant height and flowering date detected by composite interval mapping in the RIL population derived from the cross JI296×DP. Assessments were carried out at the adult plant stage in the field

QTLs	Linkage group	Closest markers[a]	LOD[b]	R[2c]	Additivity[d]
Plant height					
ht1	II	*AD12-800*	4.4	0.04	6.181
ht2	III	*U08-1650*	39.9	0.63	26.891
ht3	VII	*PSMPSAA135*	4.5	0.03	–5.4
Flowering date					
flo1	II	*PSMPSAB33*	13.0	0.38	2.691
flo2	III	*V03-1000*	10.4	0.16	1.761
flo3	VI	*G04-950*	4.1	0.09	1.285

[a] The most closely associated marker locus with the trait is indicated
[b] LOD is the log-likelihood at the position
[c] R^2 is the percentage of phenotypic variation explained by the QTL
[d] Effect of substituting DP alleles for JI296 alleles at the LOD peak of the QTL. A positive value indicates that the QTL alleles which increased the trait were contributed by the resistant parent, whereas a negative sign means that alleles increasing the trait were donated by the susceptible parent

localized on linkage group III and mapped to the same region as the resistance QTL *mpIII-3*. QTL *flo3*, localized on linkage group VI, mapped to the same region as the resistance QTL *mpVI*-1 identified at the seedling stage under controlled conditions.

In all cases, resistance alleles at the resistance QTLs were associated with alleles that increased plant height and delayed the flowering date.

Discussion

Genetic dissection of partial resistance to *M. pinodes*

Using a genetic map with 143 markers and a QTL analysis of the RIL population derived from the cross between JI296 and DP, we (1) confirmed the polygenic control of resistance to *M. pinodes* at the adult plant stage as well as at the seedling stage and (2) identified six and ten genomic regions affecting pea partial resistance to *M. pinodes* at the seedling and adult plant developmental stages, respectively, with four being common to both stages. These QTLs collectively accounted for a high proportion of the total phenotypic variation (73–74% at the seedling stage, 56.6–67.1% at the adult stage). However, part of the phenotypic variation still remains unexplained. This may be due to (1) epistatic effects (Lefebvre and Palloix 1996; Manzanares-Dauleux et al. 2000) and/or (2) sensitivity limitations in QTL detection, leading to the non-detection of other QTLs with minor individual effects. A better coverage of the genetic map could lead to the identification of additional QTLs and also improve the accuracy of localizing QTLs, for instance on the distal parts of linkage group Va.

Seedling-stage resistance

This study is the first report of QTL analysis for pea resistance to *M. pinodes* at the seedling stage. The major part of the variation observed for seedling resistance was explained by QTLs identified from scorings on both stipules and stems. Only *mpIII-3* was specific to stems and explained only 6% of phenotypic variation. The two QTLs *mpIII-1* and *mpVI-1* explained a major part of the variation. Other QTLs showed minor effects. Previous results on a set of independent genotypes had shown a highly significant correlation between resistance observed on stipules and resistance observed on stems (Prioul et al. 2003). We have confirmed these results here on a segregating population. Our results strongly suggest that the genetic control of resistance to *M. pinodes* at the seedling stage is polygenic and may be common to stipules and stems, whereas Clulow et al. (1991) identified four independently inherited dominant genes involved in stem (*Rmp1* and *Rmp2*) and foliar (*Rmp3* and *Rmp4*) resistance to *M. pinodes* in pea seedlings. Discrepancies between results in the number of genetic factors involved and their organ specificity may be due to the use of different plant lines but also to differences in methodological approaches (inoculation and disease scoring procedures, interpretation of the data, pathogen isolates used). Although the French *M. pinodes* populations are not structured into pathotypes (Onfroy et al. 1999), we plan to test other isolates of the pathogen to control the stability of detected QTLs towards pathogen variation. As the *Rmp* genes have not been mapped, comparison between the *Rmp* genes and QTL localizations is impossible, except for the *Rmp4* gene, which has been found to be linked to the *Np* (Neoplastic pod) gene (Clulow et al. 1991). In this study we mapped *Np* onto linkage group III, as previously reported on the pea consensus genetic map (Weeden et al. 1998). The *Rmp4* gene (involved in foliar resistance) and the QTL *mpIII-1* (involved both in stipule and stem resistance) could correspond to the same locus.

Adult-stage resistance

In the literature, there is only one report on the localization of genomic regions involved in resistance to Ascochyta blight field epidemics: in western Australia, Timmerman-Vaughan et al. (2002) used $F_{2:3}$ and $F_{2:4}$ populations derived from the cross A88×Rovar. In the study reported here, ten QTLs for partial resistance to *M. pinodes* were identified at the adult plant stage in the field. Only three of these QTLs were common to both stipules and stems, namely *mpII-2*, *mpIII-1* and *mpIII-3*, while the remaining QTLs were identified either for resistance on stipules or for resistance on stems. The low correlations between criteria assessed on stipules and stems and the small number of QTLs common to both organs suggest a different genetic control of leaf and stem resistance at the adult plant stage, which is contrary to the observation at the seedling stage. A similar hypothesis was suggested by Xue and Warkentin (2001). In A88×Rovar, among the 13 QTLs detected, only two QTLs (*Asc2.1* and *Asc7.1)* were mapped using disease scores on both the stems and the leaves (Timmerman-Vaughan et al. 2002).

Comparison of the locations of QTLs on the A88×Rovar and the JI296×DP derived maps is difficult due to the lack of common markers between the two maps. Nevertheless, potentially common QTLs could be identified by comparative mapping using other published genetic maps, such as the QTLs *Asc2.1* and *mpII-1* and/or *mpII-2*, the QTL *Asc3.1* and the two QTLs *mpIII-1* and/or *mpIII-4*, the QTL *Asc5.1* and the two QTLs *mpVa-1* and *mpVa-2*, and the QTLs *Asc7.1* and *mpVII-2*.

In the cross A88×Rovar, the major QTL was located on linkage group I and explained up to 35% of the phenotypic variation. This QTL was not identified in the JI296×DP population. It appears that at least one stable major QTL (*Asc1.1*) is specific to the A88×Rovar study and that probably several minor genes are specific to both studies. These specificities may be due to the different genetic backgrounds studied (different genes may confer resistance in DP and A88), the different generations of mapping populations used ($F_{2:3}$ and $F_{2:4}$ for A88×Rovar vs. $F_{2:7}$ for JI296×DP), the use of different inoculation methodologies and inoculum composition (Ascochyta blight complex natural epidemics vs. barley grain inoculation with *M. pinodes* controlled isolates), different traits scored or varying environmental interactions. Alignment of the genetic maps with common markers, a thorough check of the isolates used for inoculation, standardization of the traits scored together with cross screenings in both environments, including subsequent screenings for QTL analysis consistency across environments in JI296×DP, would be required before any conclusions could be drawn as to the identity of QTLs identified in A88×Rovar and JI296×DP.

Identification of common QTLs between both stages

Four genomic regions were involved in resistance observed at both the seedling and adult stages—namely *mpIII-1* and *mpIII-3* on linkage group III, *mpVa-1* on linkage group V and *mpVII-1* on linkage group VII. These QTLs seemed to be moderately affected by genotype×environment interactions and by the plant's physiological development. QTL *mpIII-1* had a major additive effect in both stages. This region on the distal part of linkage group III was reported to carry other pea disease resistance genes: the major gene *Rmp4*, involved in the stem resistance to *M. pinodes* in pea seedlings (Clulow et al. 1991) and the *Fw* gene, conferring resistance to *Fusarium oxysporum* f. sp. *pisi* race 1, initially mapped on linkage group IV (Dirlewanger et al. 1994) and then located on linkage group III on the pea consensus map (Weeden et al. 1998). More recently, Timmerman-Vaughan et al. (2002) mapped the QTL *Asc3.1* for resistance to Ascochyta blight on the same region of linkage group III. These results suggest that the distal part of linkage group III could play a key role in resistance to pea diseases. The QTLs *mpVa-1* and *mpVII-1* had minor effects (R^2<10%). At these QTLs, alleles conferring resistance under controlled conditions were donated by DP, while under field conditions the resistance alleles were brought by JI296. These contrary results may be due to errors in estimates of the effect of the QTL due to a lack of precision in scoring criteria and/or linkage coverage, or may be due to the existence of different linked genes.

QTLs specific either to the seedling stage or to the adult stage may result from different factors, including (1) the expression of genetic factors specific to a given stage; (2) interactions with environmental effects, such as climate effects; (3) differences in scoring methodologies such that under controlled conditions the focus is on resistance to infection only and under field conditions an overall response, including both resistance to infection and resistance to fungus progress upwards on the plant, is taken into account. Simulation studies have shown that in cases where numerous QTLs with minor effects are detected from small progeny numbers, the power of QTL detection may be biased and the explained variation may be overestimated (Melchinger et al. 1998). Further experiments are therefore needed on larger populations, under other environments in the field, to confirm that observed differences in QTL detection and contribution to phenotypic variation between stages or between organs are actual biological differences.

Co-localizations of QTLs for resistance with QTLs controlling plant height and flowering time

In the present study, we identified three QTLs for plant height and three QTLs for flowering time. Genes involved in plant height and flowering control have been mapped on other pea genetic maps (Weeden and Wolko 1989; Dirlewanger et al. 1994; Laucou et al. 1998; Weeden et al. 1998; Timmerman-Vaughan et al. 2002). Comparative analysis suggests similar chromosomal positions for the QTL *ht2* and the *Le* gene (controlling internode length) on linkage group III, for the QTL *flo1* and the QTL for reproductive stage *Mat2.1* (Timmerman-Vaughan et al. 2002) on linkage group II and for the QTL *flo2* and the *Hr* (high response to photoperiod) gene on linkage group III (Weeden et al. 1998).

We identified three co-localizations between QTLs for resistance to *M. pinodes* and QTLs for plant height and/or flowering date. In pea, associations have been observed between resistance to *Ascochyta pisi* race C and plant height measured in the field (Dirlewanger et al. 1994), between resistance to *Aphanomyces* root rot and the *Le* gene (Marx et al. 1972) and between a QTL affecting plant reproductive stage and the QTL *Asc2.1* for resistance to field epidemics of Ascochyta blight in the cross A88×Rovar (Timmerman-Vaughan et al. 2002). In our study, similar chromosomal positions were observed for the resistance QTLs *mpIII-1* and *mpIII-4* and the QTL *ht2* (possibly corresponding to *Le*), for QTL *mpIII-3* and the QTL *flo2* (possibly corresponding to *Hr*) on linkage group III. Putative co-localizations can also be inferred from comparative mapping for the QTL *mpVI-1* and the *E* (early flowering) and/or *na* (extremely shortened internodes) genes on linkage group VI according to the consensus map (Laucou et al. 1998; Weeden et al. 1998). These co-localizations may be due to (1) the direct effect of plant architecture, canopy structure or earliness on the epidemiology of the pathogen, (2) genetic linkages between genes controlling resistance to *M. pinodes* and plant height or flowering time and/or (3) pleiotropic effects of genes. Support for the first hypothesis was provided by Le May (2002), who showed that disease progress was affected by canopy structure: faster disease progress was observed on pea cultivars showing architectural features conducive to dense canopies (short stem height, branching, high leaf area index) that provided a favorable microclimate for fungal spread and disease development. Similarly, susceptibility to *M. pinodes* is known to increase with plant earliness (Bretag and

Ramsey 2001). Zimmer and Sabourin (1986) observed that the rate of disease increase was greater in older leaves than in younger ones, suggesting the existence of a correlation between the disease increase and a decrease of pisatin production in older leaves. The hypothesis is that the level of plant maturity could be involved in the level of observed partial resistance.

We can therefore not exclude that in our study plant architecture, canopy structure and/or earliness influenced the field epidemics and consequently QTL detection. However, *mpIII-1*, *mpIII-3* and *mpVI-1* were also detected at the seedling stage under controlled conditions, where interactions with plant maturity and plant height can be considered much lower. The three hypotheses listed above (architecture effects, genetic linkage, pleiotropic effects) may therefore be verified either individually or together. Further investigations are in progress to dissect these effects, including complementary studies with mapping populations not segregating for morphological traits, the mapping of genes involved in flowering date or plant height control and fine mapping on isogenic lines for detailed examination within the genomic regions showing co-localizations.

Conclusion

Four stable QTLs were identified for resistance to *M. pinodes* in pea common to seedling and adult stages. Further screenings for QTL analysis consistency at the adult stage across field environments are still needed. Work is therefore in progress to confirm the loci mapped in this investigation and to define QTL stability across different locations and genetic backgrounds, so as to identify the most interesting ones for their use in breeding programs.

Identified co-localizations between QTLs for resistance and QTLs for plant height and flowering time raise the need for a further genetic dissection of the resistance and architectural factors that decrease the epidemic rate in the field. Using the growth-chamber methodology at the seedling stage will be helpful for this purpose. Some QTLs already appeared to be independent of morphological traits, which suggests that the development of resistant pea cultivars can be considered independently of undesirable morphological or physiological traits.

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