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ORIGINAL PAPER

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Strain-specific and recessive QTLs involved in the control of partial resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in a recombinant inbred line population of melon

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Abstract *Fusarium oxysporum* f. sp. *melonis* (FOM) causes serious economic losses in melon (*Cucumis melo* L.). Two dominant resistance genes have been identified, *Fom*-1 and *Fom*-2, which provide resistance to races 0 and 2 and races 0 and 1, respectively, however FOM race 1.2 overcomes these resistance genes. A partial resistance to FOM race 1.2 that has been found in some Far East accessions is under polygenic control. A genetic map of melon was constructed to tag FOM race 1.2 resistance with DNA markers on a recombinant inbred line population derived from a cross between resistant (Isabelle) and susceptible (cv. Védrantais) lines. Artificial root inoculations on plantlets of this population using two strains, one that causes wilting (FOM 1.2w) and one that causes yellowing (FOM 1.2y), resulted in phenotypic and genotypic data that enabled the identification of nine quantitative trait loci (QTLs). These QTLs were detected on five linkage groups by composite interval mapping and explained between 41.9% and 66.4% of the total variation. Four digenic epistatic interactions involving seven loci were detected and increased the total phenotypic variation that was explained. Co-localizations between QTLs and resistance gene homologs or resistance genes, such as *Fom*-2 and *Vat*, were observed. A strain-specific QTL was detected, and some QTLs appeared to be recessive.

Introduction

Vascular wilt of melon (*Cucumis melo* L.) caused by *Fusarium oxysporum* Schlechtend f. sp. *melonis* Sny. & Hans. (FOM) is an economically important disease. Risser et al. (1976) defined four races of FOM (0, 1, 2 and 1.2) based on the interaction between two major resistance genes of the host and variants of the pathogen. The dominant resistance genes *Fom*-1 and *Fom-2*, provide resistance to FOM races 0 and 2 and to races 0 and 1, respectively. Race 1.2, which is able to overcome these two resistance genes, can be separated into race 1.2 yellowing (1.2y), which induces yellowing symptoms before the death of the plants, and 1.2 wilting (1.2w), which produces wilting and death without the yellowing symptoms. The yellowing symptoms caused by race 1.2y are more commonly observed in France than the wilting symptoms caused by race 1.2w. A number of Far East melon accessions, such as Ogon 9, show partial resistance to FOM race 1.2 (Risser and Rode 1973). We recently used a segregating recombinant inbred line population (RIL) to show that this partial resistance to FOM race 1.2 is under polygenic control (Perchepied and Pitrat 2004).

The inheritance of complex traits such as disease resistance often can be more precisely explained by studying their association with linked molecular markers than by classic quantitative genetics. Genetic variation involved in the expression of traits can be localized as quantitative trait loci (QTLs) on the basis of a molecular map, and QTL analyses have been applied to study partial resistance to numerous fungi and oomycetes. For example, quantitative resistance to *Stagonospora* glume blotch in Swiss winter wheat involves seven QTLs (Schnurbusch et al. 2003). QTL analyses of partial resistances to *Plasmopara viticola* and *Uncinula necator* in European grapevine cultivars (Fischer et al. 2004), to Fusarium head blight affecting barley (Dahleen et al. 2004) and to Fusarium wilt of common bean (Fall et al. 2001) revealed the number of factors involved in the control of resistance, the magnitude of their effect, their action and their specificity. In melon, some QTL analyses have been performed to study fruit quality traits or Cucumber mosaic virus resistance (Dogimont et al. 2000; Monforte et al. 2003).

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However, to date, no QTLs for any fungal resistance, in particular FOM race 1.2 resistance, have been described.

The construction of a linkage map with molecular markers is a key step in the linkage analysis of biologically important traits. Several linkage maps have been published for melon (Baudracco-Arnas and Pitrat 1996; Wang et al. 1997; Liou et al. 1998; Brotman et al. 2000, 2002; Oliver et al. 2001; Danin-Poleg et al. 2002; Silberstein et al. 2003), and these have enabled the localization of the dominant resistance genes, *Fom-1* and *Fom-2*. However, it is necessary to build a new genetic map based on a new segregating population in order to study the partial resistance to FOM race 1.2.

A genetic analysis of partial or quantitative resistance is difficult to achieve without conducting numerous tests. The development of doubled-haploid (DH) lines and RILs that are each highly homozygous in which the variation among lines is immortalized allows complex resistance to be studied because it is possible to conduct replicated tests in time and space and to use different strains of the pathogen.

In a previous study, we described a method for assessing partial resistance to FOM race 1.2 under conditions of artificial inoculation (Perchepied and Pitrat 2004). Our evaluation system of plant resistance carried out in the growth chamber enabled us to control and assess the development of the disease better than was possible in the field (Geiger and Heun 1989). In the investigation reported here, we used QTL analysis to study the genetic determinant underlying the partial resistance to FOM race 1.2. A RIL population was derived from a cross between the partially resistant accession Isabelle and the susceptible cultivar Védrantais and challenged with two strains of FOM race 1.2, corresponding to the two types of symptoms. Our objectives were: (1) to estimate the number and genomic positions of FOM race 1.2 resistance QTLs and (2) to determine the spectrum of action of each QTL to the two strains tested.

Materials and methods

Plant material

The *Cucumis melo* (L.) population used in this study, Vedisa, consisted of 120 $F_6/F_7/F_8$ RILs derived by single seed descent from a cross between cv. Védrantais (*Fom-1*) and Isabelle (*Fom-1*, *Fom-2*, and partial resistance to FOM race 1.2). The resistance to FOM race 1.2 in Isabelle is derived from the Chinese accession Ogon 9 after the F_1 and one backcross by Charentais type, selfing and phenotypic selection (Perchepied and Pitrat 2004). Ogon 9 and two commercial F_1 hybrid cultivars, Manta and Lunasol (*Fom-1*, *Fom-2*, and partial resistance to FOM race 1.2 derived from Isabelle), were used as controls in the experiments.

F_1 progenies between cv. Védrantais and a subset of 12 RILs that possessed one to four QTLs (see below) were produced.

Disease evaluation

The 120 RILs, the parental lines (cv. Védrantais and Isabelle) and the controls were evaluated for resistance to FOM race 1.2 following artificial inoculation with *Fusarium oxysporum* Schlechtend f. sp. *melonis* Sny. & Hans. strain TST (yellowing) and strain D'Oléon 8 (wilting) (Perchepied and Pitrat 2004).

The plants were evaluated independently by five seed companies and INRA at different locations. The D'Oléon 8 strain was used at two locations (A and B) and the TST strain at four locations (C, D, E and F). Two to six independent experiments were performed at each location, and 10 (one block) or 20 plants (two blocks) of each RIL were evaluated in each experiment in a complete randomized design. The growing conditions (i.e. soil substrate, fertilization, temperature, watering) were slightly different between locations, but the presence of controls (parental lines, cvs. Manta and Lunasol) allowed a good comparison between the locations.

Symptoms typically developed within 10–14 days post-inoculation in susceptible plants. As soon as symptoms appeared on a single plant (either RIL or control) the severity of symptoms was assessed on infected leaves using a semi-quantitative rating scale from 1 to 5 (1 = no symptoms, 5 = death of plant). Plants were examined at 3- to 4-day intervals for 3 weeks following the initial appearance of the symptoms.

Twelve RILs selected for QTLs and the 12 corresponding F_1 plants were inoculated with the two race 1.2 strains, TST and D'Oléon 8, at location A. For each strain, 30 plants of each RIL and F_1 population were evaluated in a complete randomized design with three blocks. The severity of the symptoms was assessed for 3 weeks using the same rating scale as described above.

The 120 RILs, the parental lines and the controls were artificially inoculated with strain FOM26 of FOM race 1 as described by Risser and Mas (1965) to evaluate the presence of gene *Fom-2*.

Statistical analyzes of the phenotypic data

Four variables were analyzed: the disease scores at the second evaluation date (T2), an intermediate evaluation date (TI) and the final evaluation date (TF) and the area under the disease progress curve (AUDPC) (Perchepied and Pitrat 2004). Data were analyzed by location ($j = 1$–6), by strain (W or Y for the D'Oléon 8 strain or the TST strain, respectively) and for the two strains (WY). Adjusted means of disease scores (lsmeans) of RILs on experiments and blocks (T2j, T2W, T2Y, T2WY, TIj, TIW, TIY, TIWY, TFj, TFW, TFY and TFWY) and adjusted values of AUDPC of RILs (AUDPCj,

AUDPCW, AUDPCY and AUDPCWY) were estimated from variance analysis (ANOVA).

Data were analyzed with using SAS software (SAS Institute, Raleigh, N.C.). Variance analysis of disease scores and AUDPC was performed using PROC GLM of SAS with nested (block effect for the locations B, C, D and F; location effect for W and Y; location and strain effects for WY) and random (RIL effect and interaction with the RIL effect) effects.

Genetic map construction

Genomic DNA of the RILs and parental lines was extracted from leaf tissue as described by Baudracco-Arnas (1995). This set of RILs was screened with 39 amplified fragment length polymorphism (AFLP) primer combinations, 45 simple sequence repeat (SSR) primers (Katzir et al. 1996; Danin-Poleg et al. 2000, 2001), 46 inter-microsatellite (IMA) primers, two PCR-specific markers, AM and FM (Wang et al. 2000) and one phenotypic marker (*Fom-2*). Twenty-three AFLPs, 44 SSR primers, and 46 IMA primer combinations were the same as those used to build the reference linkage map (Périn et al. 2002). An AFLP primer combination (E43/M50) and a SSR primer (CSCT335) (Danin-Poleg et al. 2000) were mapped on the reference genetic map to assign a linkage group (LG). Fifteen other AFLP primer combinations were used to add previously unmapped markers (Table 1). The AFLP, SSR and IMA protocols were as described in Périn et al. (2002).

Each AFLP fragment segregating on the RIL population Vedisa was identified by the primer combination followed by a number indicating the relative position of the band, starting from 151 for the least migrating band. The IMA markers were denoted using a letter code followed by the approximate weight of the band. All markers were scored by two persons independently. Segregation of the markers among the RIL progeny was analyzed by the χ^2 -test. Markers with a strongly distorted segregation from the expected 1:1 ($P < 0.01$) were eliminated. Since the Vedisa population had a common parent (cv. Védrantais) with the reference map, we assumed that the comigrating AFLPs (i.e. amplified with the same primer combination and giving a band with the same mobility) between the Vedisa map and the reference map correspond to the same locus, and therefore the name of the reference map for this locus was used. AFLPs, IMA and SSRs common to both maps were used to assign the LGs of the Vedisa map to the reference map.

Table 1 Sequences of AFLPs primers used

Primers
E31 = 5′-GACTGCGTACCAATTC AAA
E32 = 5′-GACTGCGTACCAATTC AAC
E41 = 5′-GACTGCGTACCAATTC AAG
M47 = 5′-GATGAGTCCTGAGTAA CAA
M48 = 5′-GATGAGTCCTGAGTAA CAC
M49 = 5′-GATGAGTCCTGAGTAA CAG
M50 = 5′-GATGAGTCCTGAGTAA CAT
M51 = 5′-GATGAGTCCTGAGTAA CCA
M52 = 5′-GATGAGTCCTGAGTAA CCC
M53 = 5’-GATGAGTCCTGAGTAA CCG
M54 = 5′-GATGAGTCCTGAGTAA CCT
M58 = 5′-GATGAGTCCTGAGTAA CGT
M59 = 5′-GATGAGTCCTGAGTAA CTA
M60 = 5′-GATGAGTCCTGAGTAA CTC
M62 = 5′-GATGAGTCCTGAGTAA CTT

The genetic map was constructed with MAPMAKER ver. 3.0 software (Lander et al. 1987). A LOD (logarithm of odds ratio) threshold of six was used to define the LGs. Marker ordering was carried out using the command ORDER. Markers that could not be confidently ordered were placed using the TRY command. Candidate orders were confirmed with the command RIPPLE. The Kosambi function was used to calculate genetic distances (centiMorgans) (Kosambi 1944). When several markers clustered at the same position, the marker of best segregation was conserved in order to construct a framework linkage map.

As the polymorphism between cv. Védrantais and Isabelle was quite low, the polymorphism between cv. Védrantais and Ogon 9 (from which the resistance was introgressed into Isabelle) was evaluated for five AFLP primer combinations, namely E35/M35, E46/M35, E43/M44, E42/M31 and E40/M34. In comparison with the reference map, the polymorphism between cv. Védrantais and Ogon 9 was assigned to the different LGs.

QTLs detection

QTLs were detected for 36 traits corresponding to the lsmeans of four variables (the three disease scores and AUDPC) for the six locations, the wilting strain (W), the yellowing strain (Y) and both strains (WY). Variance analysis (LR), interval mapping (IM) and composite interval mapping (CIM) were performed using QTL CARTOGRAPHER ver. 1.17 software (Basten et al. 2003) for each location and for W, Y and WY. After performing 1,000 permutations with LR, we used a LOD threshold of 2.73 to declare a putative QTL significant. For CIM, the three to seven most informative markers per trait were chosen as cofactors. The same significance threshold was used for IM and CIM. For each significant QTL, a confidence interval corresponding to a LOD score drop of 1 on either side of the likelihood peaks was estimated. The QTLs detected for several traits were interpreted to be the same QTL when the confidence interval of their position overlapped. For each trait, a multiway ANOVA was performed with molecular markers near the QTL peaks to estimate the total percentage of phenotypic variation (R^2) explained by the significant QTLs.

The QTLs were denoted by three letters indicating the pathogen, followed by the number of the linkage group and a number to distinguish several QTLs on the same linkage group.

In addition to additive effects, digenic epistatic interaction was tested with a two-factor ANOVA model with an interaction between pairs of markers. Using the PROC GLM of SAS software, we performed 7,140 interaction tests, and a significance level of $P < 0.0001$ (to detect only 0.7 false positive) was chosen for detecting digenic interaction.

Results

Genetic mapping

Among the 46 IMA primer combinations tested, 30.4% were polymorphic between the two parents, cv. Védrantais and Isabelle. Of the 45 SSRs, 15.6% were polymorphic between the two parents, while only 8.6% of the AFLP bands were polymorphic between the parents. This latter value was significantly lower than the 28.9% polymorphism reported by Périn et al. (2002) between cv. Védrantais and PI 161375.

A genetic map was obtained following a linkage analysis of 165 AFLP, 28 IMA, one phenotypic (*Fom-2*), seven SSR and two PCR-specific (AM and FM) markers. Sixteen LGs were ordered and six markers were not linked (LOD = 6). Among the 16 ordered groups, 14 were assigned to nine LGs on the melon reference map (Périn et al. 2002). As cv. Védrantais is a parent in both the reference map and the Vedisa map, we were able to align the LGs (Périn et al. 2002) using common AFLP, IMA and SSR markers and to assign between 1 and 13 common markers to each LG (markers in bold in Fig. 1). This unsaturated map spanned 641 cM. While the mean marker interval was 4.9 cM, the largest interval between two markers was 22.3 cM. When a LOD threshold of 4 was used, two minor groups joined together on LGXII.

LGIII is covered equally well in both the Vedisa and the reference map (183.1 cM). Other LGs of the reference map, such as LGIV and LGV, are quite well covered but correspond to two sub-groups in the Vedisa map, with a gap of 30–40 cM between them. Still other LGs of the reference map were covered by several groups of the Vedisa map. Of the 12 LGs observed in melon, nine were assigned and each new marker added to one of these LGs. Three LGs were not represented at all—LGI, LGVII and LGs. In each LG, the order of the common markers was highly conserved between the Vedisa genetic map and the reference map.

The level of resistance in Isabelle was as high as in Ogon 9, but the molecular polymorphism evaluated for a few AFLP primer combinations was much lower between cv. Védrantais and Isabelle than between cv. Védrantais and Ogon 9 (Table 2). This decrease in polymorphism was not evenly distributed over the 12 LGs: it was not significantly different for LGs III, V and IX but was significantly different ($P < 0.001$) for the nine other LGs which were barely covered or not represented at all on the Vedisa map.

QTL mapping

The detection of QTLs did not differ significantly using the three methods, LR, IM and CIM. Because CIM provides a more accurate estimation of R^2 values and additive effects (Zeng 1994), we present only CIM results.

The QTLs for the AUDPC variable were detected on six LGs (Fig. 1). On the basis of the confidence intervals estimated for each location or the grouping of locations by strains (W, Y and WY), we attempted to determine if only one or two linked QTLs were detected in these linkage groups. Almost all of the confidence intervals of the QTLs detected on the bottom end of the LGV, on the middle of LGVI and on LGXI overlapped on a common segment of LGs (Fig. 1), and we concluded that only one QTL was involved in each case. Conversely, confidence intervals of QTLs detected for each location or by grouping the locations by strain did not overlap on LGIII, at the top end of the LGV and on LGXII. As the top ends of LGIII and LGV and LGXII were not well covered by markers, we assumed there was only one QTL in each case—*fomIII.1*, *fomV.1* and *fomXII.1*, respectively. Finally, at the bottom end of the LGIII, two independent QTLs were identified for each location or by grouping the locations by strain, except in location F. Although the confidence intervals of QTLs detected for locations B and E and for W overlapped, we considered there were two QTLs in this area, namely *fomIII.2* and *fomIII.3*. Thus, a total number of nine QTLs were identified, most of which were located at the LG ends.

The analysis based on location of the QTLs indicated that the QTL *fomIII.1* was detected for locations C, D, E and F and explained from 8.1% to 12.3% of the phenotypic variance (Table 3). The percentage of phenotypic variance explained by the QTLs *fomIII.2* and *fomIII.3* ranged from 5.6% (location F) to 13.3% (location B), and from 6.1% (location A) to 14.3% (location B), respectively. On LGV, the R^2 of QTL *fomV.1* ranged from 9.8% (location E) to 21.2% (location B), and the QTL *fomV.2* was detected only for the location A. On LGVI, the QTL *fomVI.1* was a major QTL (R^2 from 25.2% to 35.6%) and was detected for the locations C, D, E, and F, while the QTL *fomVI.2* was detected only for location A. The QTL *fomVI.2* had a negative additive effect, indicating that the cv. Védrantais allele improves the resistance to FOM race1.2. On LGXI, one QTL was consistently identified among the different locations, *fomXI.1*, with R^2 ranging from 5.3% to 17.8%. On LGXII, the QTL *fomXII.1* was identified only for location F, and its additive effect was as negative as that of *fomVI.2*.

For the three evaluation dates (T2, TI and TF), the same QTLs were detected as with AUDPC (Fig. 2). The effect of five of these QTLs, namely *fomIII.1*, *fomIII.2*, *fomV.1*, *fomVI.2* and *fomXI.1*, decreased during the infection, with *fomVI.2* being no more detected at TF, the last evaluation date. On the contrary, the effect of QTLs *fomV.2* and *fomVI.1* increased with time post-inoculation.

Four digenic epistatic interactions were found to have a significant effect on FOM race 1.2 resistance evaluated with the variable AUDPC (Fig 1). One digenic interaction occurred between the markers E43/M44-28 (LGIII) and E31/M48-153 (LGVI), which have no additive effect and accounted for 19% of the variation for the location A ($P = 9.4\times10^{-5}$). Two other interactions occurred between the QTL *fomV.2* and the marker E39/M42-154 (LGXI) and between the QTL *fomIII.3* and the SSR marker CSCCT571 (LGIV). These explained 26.2% ($P = 1.3\times10^{-5}$) and 25.5% of the variation for the location E, respectively. The last digenic interaction occurred between E41/M35-154 (LGV) and E32/M62-161f (LGIII) and explained 27.1% of the resistance for location B ($P = 2.6\times10^{-5}$). The same genomic region of LGIII between E43/M44-28 and E32/M62-161f was implicated in two digenic interactions.

The total phenotypic variance explained by all QTLs ranged from 41.9% to 66.4%. When the epistatic interactions are included in the calculations, the total R^2 increased by 6–9%.

QTL specificity

In order to detect QTLs which could be strain-specific, we grouped the evaluations carried out with the yellowing strain (Y) or with the wilting strain (W) separately, or we considered all of the evaluations together (WY), when calculating the AUDPC variable (Fig 1). The QTL *fomIII.1* was detected for Y ($R^2 = 14.8\%$). The QTL *fomVI.1* was identified for Y and WY and explained 36.7% and 15.3% of the variation, respectively. The QTLs *fomV.2* ($R^2 = 6.9\%$) and *fomXII.1* ($R^2 = 5.2\%$) were detected for W only. On the contrary,

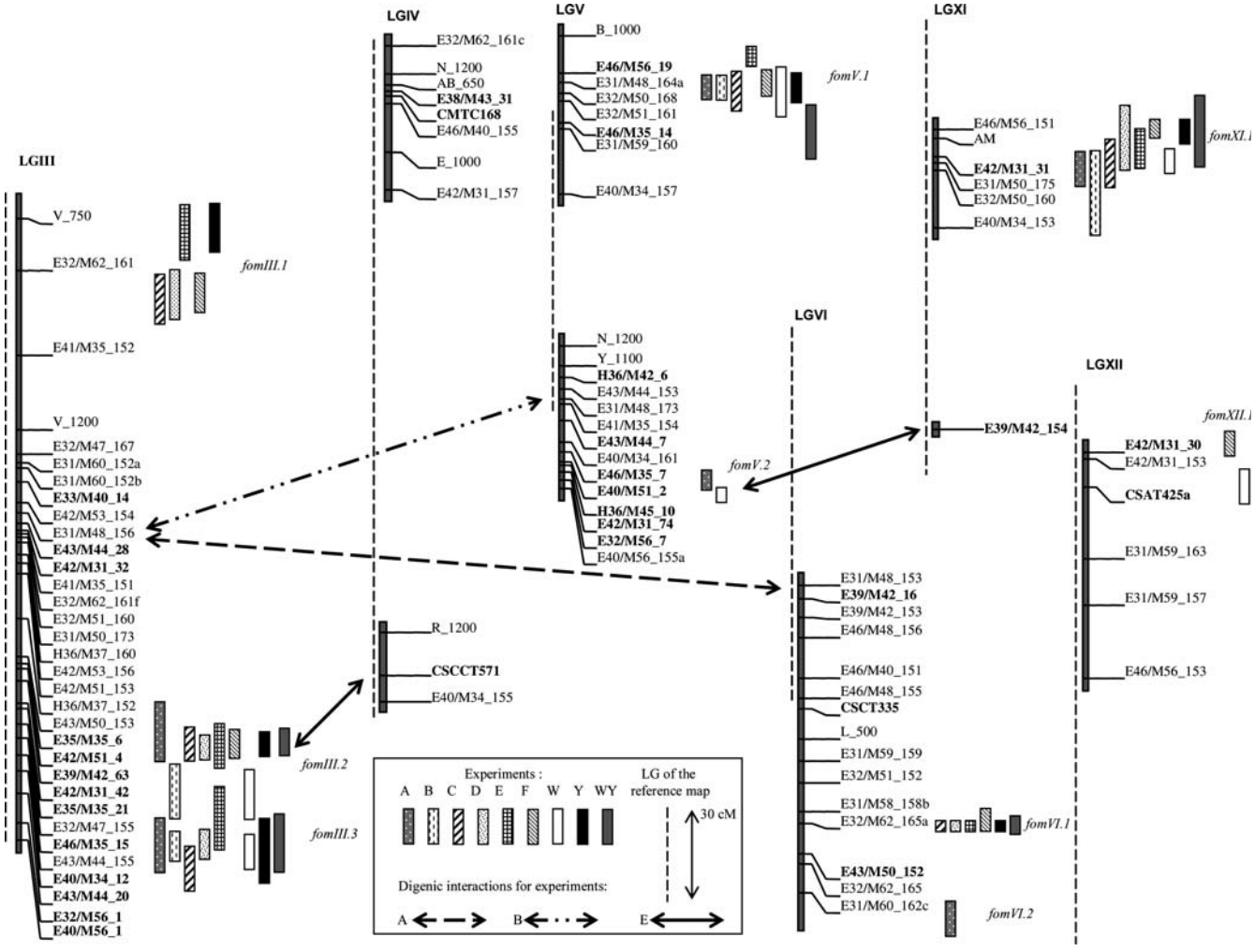

Fig. 1 *Fusarium oxysporum* f. sp. *melonis* race 1.2 resistance QTLs detected on the RIL progeny derived from the cross between cv. Védrantais and Isabelle (Vedisa) for the AUDPC. Only those linkage groups (*LG*) on which some QTLs and digenic interactions were detected are represented. The QTL length represents the confidence interval: the distance equivalent to a LOD decrease of 1 on each side of the position of the maximal LOD value. Loci (AFLP and SSR markers) common to both the Vedisa linkage map and the reference map (Périn et al. 2002) are indicated in *bold type*

Table 2 Polymorphism[a] between cv. Védrantais and Isabelle, and cv. Védrantais and Ogon 9 for five AFLP combinations (E35/M35, E46/M35, E43/M44, E42/M31 and E40/M34) on 12 linkage groups (LGs)

LG polymorphism	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Védrantais/Isabelle	0	0	12	0	2	3	0	3	2	0	1	0
Védrantais/Ogon 9	9**	7**	12	9**	5	11**	4*	11**	4	6**	6*	4*

[a]Number of polymorphic bands are indicated for each LG. Significantly different rates of polymorphism between cv. Védrantais and Isabelle and between cv. Védrantais and Ogon 9 are indicated by * ($P < 0.001$) or ** ($P < 0.0001$)

Table 3 QTLs[a] associated with the resistance of melon to *Fusarium oxysporum* f. sp. *melonis* race 1.2

Locations	QTL name	Position (cM)	LOD score	R^2	Total R^2 explained by all QTLs	Total R^2 explained by QTLs and digenic interaction
A	*fomIII.2*	144	3.51	5.9	54.7	60.8
	fomIII.3	163	3.63	6.1		
	fomV.1	27	8.74	16.9		
	fomV.2	43	3.37	5.9		
	fomVI.2	95	4.43	10.6		
	fomXI.1	13	5.75	10.0		
B	*fomIII.2*	161	5.38	13.3	41.9	49.4
	fomIII.3	176	5.51	14.3		
	fomV.1	27	7.97	21.2		
	fomXI.1	13	4.46	10.7		
C	*fomIII.1*	29	3.36	8.1	66.4	No interaction
	fomIII.2	147	5.75	10.8		
	fomIII.3	176	4.09	7.9		
	fomV.1	28	6.85	14.6		
	fomVI.1	71	14.33	35.6		
	fomXI.1	13	3.68	7.4		
D	*fomIII.1*	27	4.81	10.9	55.0	No interaction
	fomIII.2	149	3.76	8.8		
	fomIII.3	165	4.13	11.1		
	fomVI.1	71	10.24	26.3		
	fomXI.1	4	4.88	11.2		
E	*fomIII.1*	9	4.46	12.3	56.8	66.1
	fomIII.2	149	5.08	10.4		
	fomIII.3	167	4.90	10.8		
	fomV.1	18	4.99	9.8		
	fomVI.1	71	11.30	25.2		
	fomXI.1	9	2.82	5.3		
F	*fomIII.1*	27	5.57	10.2	59.5	No interaction
	fomIII.2	147	3.16	5.6		
	fomV.1	25	8.62	16.0		
	fomVI.1	71	16.14	31.4		
	fomXI.1	3	10.11	17.8		
	fomXII.1	2	3.16	4.8		

[a]The analysis was conducted on 120 RILs derived from the cross cv. Védrantais × Isabelle in six locations and with two strains. The D'Oléon 8 wilting strain was evaluated in locations A and B; the TST yellowing strain was evaluated in locations C–F. The QTL names, the position of the peak of LOD scores on the map built on this population, the significant peak values of LOD scores, the percentage of phenotypic variance explained (R^2), the total phenotypic variance explained by the QTLs and by QTLs and digenic interactions based on CIM analysis are summarized

the QTLs *fomIII.2* (R^2 from 8.2% to 14.7%), *fomIII.3* (R^2 from 7.1% to 13%), *fomV.1* (R^2 from 12.5% to 25%) and *fomXI.1* (R^2 from 11.1% to 14.4%) were identified for W, Y and WY.

QTL dominance

As the RILs are homozygous, we were unable to determine the dominance or recessivity of the QTLs (Table 4). The F_1 between cv. Védrantais and Isabelle was susceptible, indicating a general recessivity of the resistance. Nevertheless, a major QTL could be recessive, and one or several minor QTLs could be dominant. In order to test this hypothesis, 12 RILs were selected on the basis that they possessed one to four QTLs. These were less resistant than Isabelle, thereby confirming the complex inheritance of the resistance. Assessed with the TST yellowing strain, the 12 F_1 lines—between cv. Védrantais and the selected RILs—were all susceptible. The F_1s from RILs 15, 17, 34, 36, 61, 62 or 106 were significantly more susceptible than the corresponding parental RILs, which indicated the recessivity of most of the QTLs. The phenotypic evaluation performed with the D'Oléon 8 wilting strain was severe, and almost all of the RILs were as susceptible as cv. Védrantais, with the exception of RILs 34, 61 and 106. The F_1 (Védrantais × RIL 61) was more

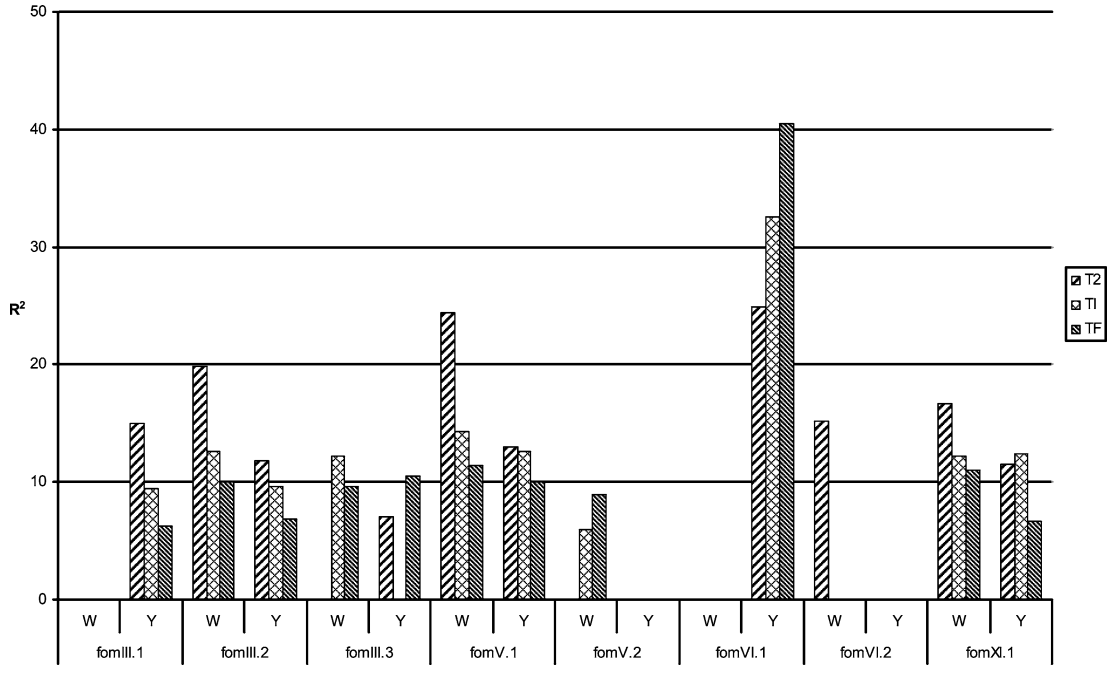

Fig. 2 Percentage of phenotypic variance explained by QTLs for resistance to *F. oxysporum* f. sp. *melonis* race 1.2 (*FOM*) as a variable of time post-infection. Resistance to FOM of a RIL population derived from the cross between cv. Védrantais and Isabelle was evaluated in six locations. *W* Estimate based on the two locations where the D'Oléon 8 wilting strain was tested, *Y* estimate based on the four locations where the TST yellowing strain was tested. *T2* Disease score at the second evaluation date, *TI* disease score at the intermediate evaluation date, *TF* disease score at the final evaluation date

Table 4 Study of the dominance/recessivity of the resistance[a] to *F. oxysporum* f. sp. *melonis* race 1.2 (FOM 1.2)

Strain	TST		D'Oléon 8	
Genotype	RIL	F_1	RIL	F_1
15 (*VI.2, XI.1*)	3.7	4.5*	4.2	4.9
16 (*VI.1*)	4.7	4.8	4.9	4.8
17 (*III.1, VI.1*)	2.9	4.5*	4.7	4.7
24 (*V.2, XI.1*)	4.6	4.5	4.8	5.0
34 (*III.2, III.3, V.1, VI.2*)	3.4	4.7***	3.7	3.8
35 (*III.2, III.3, XII.1*)	4.8	4.7	4.7	4.8
36 (*III.2, VI.1, XII.1*)	3.2	4.6**	5.0	4.9
61 (*V.2, VI.1*)	3.3	4.5**	3.9	4.6*
62 (*III.2, III.3, VI.1, XI.1*)	3.0	4.5***	4.1	4.8
75 (*III.2, III.3, V.1, VI.2*)	4.4	4.7	4.6	4.9
85 (*VI.2, XII.1*)	5.0	4.9	4.5	4.8
106 (*III.1, V.1, VI.1, XI.1*)	2.7	4.2***	3.7	3.6
Isabelle	2.0		1.8	
Védrantais	4.8		4.4	
F_1 (Védrantais × Isabelle)		3.7		4.8

*, **, *** Indicates that the RIL and corresponding F_1 were significantly different at $P < 0.05$, $P < 0.001$, $P < 0.0001$, respectively

[a]Values are the adjusted means of the disease scores (TF) of the RILs with one to four QTLs (QTLs given in parenthesis) and the corresponding F_1 (cv. Védrantais × RIL). The evaluations for resistance to FOM 1.2 were performed with strains TST and D'Oléon 8

susceptible than the corresponding RIL 61 (as with the TST strain). The F_1s with the RILs 34 and 106 were as resistant as the corresponding RILs, showing dominance. RILs 34 and 106 possessed four QTLs each, having only QTL *fomV.1* in common. This QTL was also present in RIL 75; The F_1 (Védrantais × RIL 75) was fully susceptible. Thus, we were unable to come to any conclusion with respect to the recessivity or the dominance of the QTLs detected with the D'Oléon 8 wilting strain.

Discussion

We report here for the first time the identification of QTLs involved in FOM race 1.2 resistance in melon.

A partial linkage map of melon was constructed based on the Vedisa RIL population. This map included only a few molecular markers relative to the reference map of melon, and the genome coverage (641 cM) was below the 1,654 cM of the latter. The common parental

line, cv. Védrantais, and the use of common markers, such as AFLP and SSR markers, allowed us to align nine LGs of the Vedisa map with those of the reference map, with only two minor groups remaining unassigned. The SSR markers provided codominant, locus-specific markers that were effective anchor points for map merging. AFLPs are dominant fingerprinting markers, and these are quite well distributed on our Vedisa map as well as being transportable between crosses (Périn et al. 2002).

The low rate of polymorphism between the parental lines and the low genome coverage of the Vedisa map are likely due to cv. Védrantais and Isabelle being closely related. Ogon 9, the source of partial resistance to FOM race1.2, belongs to the "makuwa" type of melon with small seeds and not very vigorous plants. F_2 or RIL progenies between Ogon 9 and cv. Védrantais would have segregated not only for FOM 1.2 resistance but also for vigor, and the existence of interactions between vigor and expression of resistance is well known. As the partial resistance of Ogon 9 had been transmitted to Isabelle, we preferred the morphological homogeneity of the Vedisa RIL population to the molecular polymorphism between cv. Védrantais and Ogon 9 to further our evaluations of the quantitative resistance to FOM race 1.2. The lack of polymorphism in certain genomic regions for cv. Védrantais and Isabelle was confirmed by the comparison of molecular polymorphism between cv. Védrantais and Isabelle and between cv. Védrantais and Ogon 9. After two crosses (F_1 and BC_1) with lines of the Charentais cultigroup, 25% of the Ogon 9 polymorphism remained between cv. Védrantais and Isabelle as expected (Table 2; 88 polymorphic bands between cv. Védrantais and Ogon 9 compared to the 23 polymorphic bands between cv. Védrantais and Isabelle). As the loss of molecular polymorphism between Ogon 9 and Isabelle was not accompanied by a loss of level of resistance, we can assume that no important FOM 1.2 resistance QTLs are likely located in areas showing no molecular polymorphism (for instance, LGI, LGVII or LGX).

In spite of the fact that our map was unsaturated, nine QTLs were identified for resistance to FOM race 1.2 on five LGs. The resistance alleles of seven QTLs originated from the partially resistant parent Isabelle, whereas resistance alleles of two QTLs originated from the susceptible line, cv. Védrantais. This was in accordance with the presence of significant transgressions towards susceptibility shown in a previous study (Perchepied and Pitrat 2004). Favorable alleles for disease resistance originating from the susceptible parents have been reported for several host-pathogen interactions (Arahana et al. 2001; Al-Chaarani et al. 2002; Foulongne et al. 2003).

Among the nine QTLs detected, *fomXI.1* was consistently identified for all of the traits studied. Five QTLs, *fomIII.2*, *fomIII.3*, *fomV.1*, *fomXI.1*, and *fomXII.1*, were effective for strains (W, Y, WY) but had slightly smaller effects with the TST yellowing strain than with the D'Oléon 8 wilting strain. On the other hand, *fomVI.1* was a major QTL for resistance to the TST strain only. This analysis demonstrated that partial resistance to FOM race 1.2 is governed in part by shared loci as well as by a strain-specific locus. In a previous study, we reported the identification of strain-specific effects on the basis of significant RIL × strain interaction and the higher number of genetic factors involved in resistance to both strains (11–13) than for the wilting strain (7–10) or the yellowing strain (6–7) (Perchepied and Pitrat 2004). Isolate- or race-specific QTLs for quantitative resistance to several pathogens have already been identified (Parlevliet and Zadoks 1977; Leonards-Schippers et al. 1994; Caranta et al. 1997a; Qi et al. 1999; Arru et al. 2003; Talukder et al. 2004), thereby confirming the assumption of Parlevliet and Zadoks (1977) that minor-gene-for-minor-gene interactions occur in pathosystems.

The QTLs were generally detected early in the infection (T2) and their effects decreased over time (Fig. 2). However, the effect of the major QTL *fomVI.1* and also of the minor QTL *fomV.2* increased from T2 to TF, the last date of evaluation. To our knowledge, there is no statistical test that allows the R^2 of QTLs to be compared, but it seems that the expression of QTLs involved in the resistance to FOM race 1.2 may depend on the length of time following infection.

The QTLs detected explained from 41.9% to 66.4% of the phenotypic variance. Comparison with the narrow-sense heritabilities (from 0.72 to 0.96) suggests that not all of the genetic variance is explained by these QTLs (Perchepied and Pitrat 2004). This may result from the population size or from the choice of the significance threshold, which may have prevented the detection of minor QTLs. The involvement of epistatic interactions in the unexplained part of the genetic variance was hypothesized and verified with significant digenic epistatic interactions. These digenic interactions explained up to 27.1% of the phenotypic variation. These types of interactions have been identified and involved in resistances to gray leaf spot in maize (Saghai-Maroof et al. 1996), to rice yellow mottle virus (RYMV) in rice (Pressoir et al. 1998), to *Phytophthora capsici* (Lefebvre and Palloix 1996) and cucumber mosaic virus (Caranta et al. 1997b) in pepper and to clubroot in *Brassica napus* (Manzanares-Daulieux et al. 2000) and explained up to 37% of the phenotypic variance for the pathosystem rice/RYMV (Pressoir et al. 1998). The three digenic interactions identified for resistance to FOM race 1.2 were, however, not consistent at all the locations. Liao et al. (2001) detected different epistatic effects under two experimental conditions and suggested that the effects of environment are greater on epistatic loci than on QTLs. Even if the correlation coefficients between the different locations were highly significant, the evaluations were realized independently and their significant effect was assessed from ANOVA (Perchepied and Pitrat 2004). Thus, location effects could explain the different epistatic effects detected.

Two types of epistasis affected the resistance to FOM race 1.2. Two digenic interactions were detected between two QTLs with additive effects, *fomIII.3* and *fomV.3*, and two markers located on LGIV and LGXI. For two other digenic epistatic interactions between genomic regions with no detected additive effect, the same region on LGIII was implicated. These latter interactions occurred for the evaluations conducted at locations A and B in which the D'Oléon 8 wilting strain was used. This locus on LGIII could be strain-specific.

The QTL *fomV.2* co-localized with the resistance genes *Vat*, which confers resistance to aphid colonization and virus transmission, and *Pm-w* for powdery mildew resistance. It is localized within a cluster of resistance gene homologs (RGHs)—NBS2, NBS5, NBS46-7 (Brotman et al. 2002), MRGH4 and MRGH63 (Garcia-Mas et al. 2001), which have been cloned using degenerate primers designed from conserved motifs in the nucleotide binding site (NBS) domain of disease resistance genes. The QTL *fomXI.1* co-localized with the resistance gene *Fom-2* (specific marker AM), which confers resistance to FOM races 0 and 1, and with the RGHs NBS3 (Brotman et al. 2002). The loci implicated in resistance to FOM race 1.2 and races 0 and 1 may be different but tightly linked; for instance, one conferring complete resistance to FOM races 0 and 1 and another one conferring a partial resistance to FOM race 1.2. The presence of both quantitative and qualitative resistance genes in the same genomic regions also suggests also that QTLs may correspond to allelic variation of qualitative resistance genes with intermediate phenotypes (Robertson 1985; Robertson 1989). It has been demonstrated that genes sharing a common structure with R (resistance)-genes could confer a weak resistance. For instance, in rice, the gene *Xa21D* belonging to the *Xa21* gene family confers a partial resistance phenotype to bacterial blight (Wang et al. 1998).

Most of the QTLs involved in resistance to FOM race 1.2 appear to be recessive. Saghai-Maroof et al. (1996) demonstrated that two QTLs involved in resistance to gray leaf spot disease in maize were additive, one being dominant and the other recessive. A complex trait, such as resistance, can be controlled by genes with different effects of dominance/recessivity and additivity (Lefebvre and Chèvre 1995). A cluster of resistance genes can be composed of both recessive and dominant genes; for instance, in lettuce, the recessive resistance gene to *Plasmopara lactucae-radicis*, *plr*, has been localized in a cluster of dominant resistance genes to downy mildew and turnip mosaic virus (Kesseli et al. 1993). This report shows that dominant R-genes and RGHs co-localize with recessive QTLs.

The availability of molecular markers linked to the QTLs of FOM race 1.2 resistance will enhance marker-assisted selection in breeding programs. It would be informative to test markers associated with QTLs on hybrids resistant to FOM race 1.2 derived from Isabelle that have been improved for horticultural traits to test if the linkage between markers and QTLs has been maintained for cycles of phenotypic selection. Otherwise, marker-assisted selection schemes should be proposed from the parental line Isabelle.

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