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ORIGINAL PAPER

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Phenotypic and molecular evaluation of a recurrent selection program for a polygenic resistance to *Phytophthora capsici* in pepper

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Abstract 'Criollo de Morelos 334' (CM334) is one of the most promising sources of resistance to *Phytophthora capsici* in pepper. This Mexican accession is distantly related to bell pepper and its resistance displays a complex inheritance. The QTLs involved in resistance to *P. capsici* were previously mapped. In order to transfer the resistance factors from CM334 into a bell pepper genetic background, a modified, recurrent breeding scheme was initiated. The breeding population was divided into three sub-populations which were screened by distinct phenotypic tests of increasing severity. The plants from the first sub-population were screened with low-severity tests and backcrossed to the susceptible bell pepper; the plants from the second and third sub-populations were screened by more severe resistance tests and crossed with the plants from the first and second sub-populations, respectively. In this study, the phenotypic data for the three sub-populations during five screening/intermating cycles were analysed. In parallel, the changes in allelic frequencies at molecular markers linked to the resistance QTLs were reported. The resistance phenotype and allelic frequencies strongly depended on the sub-population and screening severity. Regarding allelic frequency changes across the selection cycles, a loss of resistant QTL alleles was observed in the first sub-population, particularly for the low-effect QTLs, whereas a better conservation of the resistant QTL alleles was observed in the two other sub-populations. The same trend was observed in the phenotypic data with an increasing resistance level from the first to the third sub-populations. The changes in the allelic frequencies of loci not linked to resistance QTLs and for horticultural traits across the breeding process indicated that the recovery of the recipient parent genome was not significantly affected by the selection for resistance.

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Introduction

Phytophthora capsici Leon., the oomycete causing pepper root rot, is a major threat to pepper production and ranked as the second most devastating disease worldwide (Yoon et al. 1991). Furthermore, this soil-borne pathogen is rather difficult to eradicate. Soil sterilisation with methyl bromide was the primary method to control the disease in western countries. However, such treatments are now banned in the European Community because of their negative impact on the environment. Alternative strategies such as breeding for resistant cultivars could be an efficient strategy to reduce crop losses. Several sources of resistance have been reported (Pochard et al. 1983; Barksdale et al. 1984), but all exhibited a partial effect with polygenic inheritance and were found in exotic germplasm. One of these accessions, 'Criollo de Morelos 334' (CM334), displayed a high level of resistance towards the most aggressive strains. This quantitative resistance was evaluated using two distinct phenotypic tests (one performed on roots and the other on stem) assessing four resistance components: root rot index, receptivity, inducibility and stability (Pochard and Daubèze 1980; Palloix et al. 1988a). The dissection of the plant-pathogen interaction into resistance components facilitated phenotypic selection because the heritability of each component was higher than the heritability of the resistance evaluated globally. More recently, the QTL analysis of the resistance from CM334 was performed using a genetic map of the F_2 progeny from the cross between CM334 and a *P. capsici*- susceptible bell pepper line 'Yolo Wonder' (YW) (Lefebvre et al. 2002). Six major chromosomal regions were reported to be involved in one or several resistance components to *P. capsici* (Thabuis et al. 2003). This confirmed the complex inheritance of resistance, the occurrence of component-specific and non-specific QTLs (Lefebvre and Palloix

1996) and provided tools to evaluate the efficiency of the breeding scheme.

In pepper, all the *P. capsici*-resistant accessions produce small, pungent fruits and are distantly related to large and sweet fruited cultivars. Thus, recovering an elite genetic background was required through selection. The backcross strategy proved to be efficient to rapidly recover an elite genetic background but is unsuitable when too many genes have to be transferred from the donor to the recipient parent. This was experienced with polygenic resistance to *P. capsici*. A significant decrease of the resistance level in the advanced backcross progenies was observed. It was probably due to the loss of some resistance factors over backcrossing cycles, so that a recurrent selection strategy was required (Palloix et al. 1990). In most published studies, the recovery of the recurrent-parent genetic background and the combination of resistance factors are performed in successive but separate programs so that only improved genotypes are included in the recurrent selection steps (Vear et al. 1984; Walker and Schitthenner 1984; Parlevliett and van Ommeren 1988; Poulos et al. 1991; Veillet et al. 1996). The breeding scheme presented in this study is an attempt to perform both processes together, namely (1) the transfer of resistance factors from exotic accessions into an elite genetic background and (2) the retention of the useful resistance factors in the breeding population. It consisted of dividing the whole breeding population into sub-populations that were subjected to differential selection pressures, i.e. resistance screens with different severity. Intermating with the susceptible cultivar and between the sub-populations was performed with the intention of releasing elite lines with an intermediate resistance in the short term and higher resistance in the long term (Palloix et al. 1997). This breeding scheme was performed on a genetic narrow-based population ('Morelos' population) including CM334 as the resistant donor parent and YW as the recipient parent. As the population was biallelic at all the marker loci (only two homozygous parental lines CM334 and YW), this material was highly suitable for the molecular analysis of the efficiency of this breeding scheme.

The present work presents the a posteriori analysis of this breeding scheme by taking into account phenotypic data from selection and molecular genotyping analyses of the population. The objectives of our study were (1) to validate the resistance QTLs previously mapped; (2) to evaluate the allelic frequency changes of the population with respect to the combination of the resistance QTLs and the genetic background; and (3) to check whether both the objectives of the scheme were fulfilled, i.e. improving resistance and maintaining horticultural traits.

Materials and methods

The breeding population

The breeding population was obtained from crosses between the recipient *Phytophthora* root rot-susceptible bell pepper line 'Yolo Wonder' (YW) and the resistant donor line 'Criollo de Morelos 334' (CM334) (Fig. 1). Three sub-populations were obtained: L1 was the susceptible BC_1 progeny (YW × CM334) × YW (designated BC_{1s}), L2 was the resistant BC_1 progeny (YW × CM334) × CM334 (designated BC_{1r}) and L3 was obtained by selfing the resistant L2 plants recovered from the first resistance screening test. L1, L2 and L3 were submitted to screening tests differing in severity using a moderately aggressive *P. capsici* strain in L1, a highly aggressive strain in L2 and a highly aggressive strain under high temperature (32°C) that increased the severity of the test in L3. This differential screening procedure was aimed at maintaining increased resistance levels from L1 to L3. In each sub-population, 300 to 500 plants were submitted to disease screening with a selection intensity from 5% to 3%. After each screening test, an average of 15 most resistant plants were selected. The surviving plants from L1 were backcrossed to the susceptible bell pepper

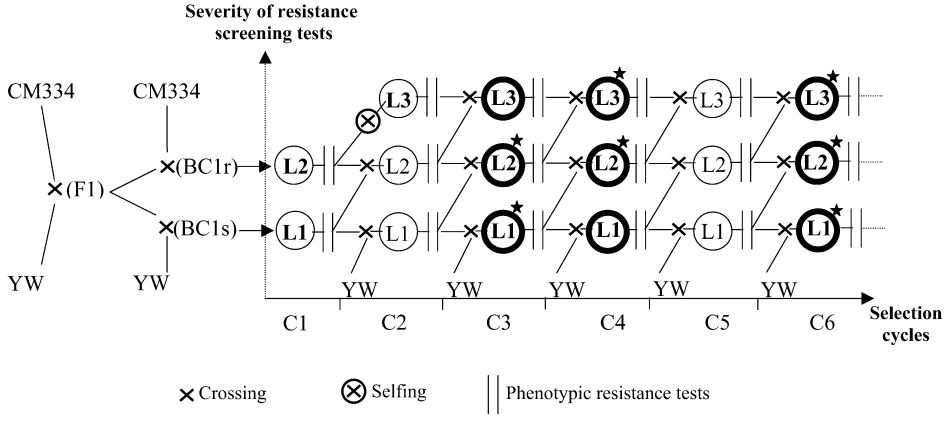

Fig. 1 Schematic presentation of the 'Morelos' population breeding scheme. 'Criollo de Morelos 334' (CM334) is the resistant parent and 'Yolo Wonder' (YW) is the susceptible bell pepper cultivated parent. The *horizontal axis* represents the cycles of selection. Each sub-population at each cycle is represented by the *circles*. At every cycle, the L1 sub-population is backcrossed by susceptible YW and the L2 and L3 sub-populations are crossed by L1 and L2 (pollen mixtures), respectively. The *bold circles* indicate the sub-populations and cycles that were sampled for molecular genotyping. The *stars* indicate the sub-populations from which doubled haploid or recombinant inbred lines were produced for horticultural traits evaluation. The *vertical axis* represents the severity of resistance screening tests applied to the sub-populations

YW; the surviving plants from L2 and L3 were crossed to the surviving plants from L1 and L2, respectively, using pollen mixtures, so that 15 half-sib families were obtained in each sub-population. One cycle of selection consisted of one backcross (L1 sub-population) or intermating (L2 and L3 sub-populations), followed by a resistance screening of the three sub-populations. Six cycles of selection were performed (C1 to C6). This crossing plan was aimed at progressively introducing the genetic background of YW in the population while progressively transferring the resistance factors from CM334 by continuous gene flow from YW and L1 to L3. Dividing the population into three sub-populations also permitted reduction of the global population size required for breeding a multigenic trait.

Resistance evaluation and screening

The stem inoculation test was performed as described by Pochard and Daubèze (1980). When the plants were at the six/seven-leaf stage, they were decapitated, and a mycelium plug was placed on the fresh section. The inoculated plants were placed in growth chamber under controlled conditions. The length of stem necrosis induced by the pathogen was measured (in millimeters) 3, 7, 10, 14, 17 and 21 days post inoculation (DPI). The speed of the necrosis spread (in millimeters per day) was calculated for each scoring date (S_3, S_7, S_{10}, S_{14}, S_{17} and S_{21}). For the analyses, three resistance components were computed: receptivity (REC=S_3), the speed of necrosis spread at 10 DPI (S_{10}) and stability [STA=(S_{14}+S_{17}+S_{21})/3]. The *P.capsici* strains S101 and S197 were isolated from pepper and maintained as described in Clerjeau et al. (1976). S101 was chosen for its moderate aggressiveness and S197 for its high aggressiveness, measured as the speed of fungal growth and the extent of root necrosis in a set of pepper genotypes (Clerjeau et al. 1976; Palloix et al. 1988b). The L1 plants were tested at 22°C with isolate S101. The L2 plants were tested at 22°C with isolate S197 and the L3 plants at 32°C with isolate S197. In all the tests, the controls varieties YW (susceptible), CM334 (resistant) and 'PI201234' (intermediate) were used and displayed the expected phenotypes with variation coefficients (ratio mean/standard deviation) lower than 0.25 for the different resistance components.

Horticultural traits evaluation

For evaluation of the horticultural traits in the breeding population, inbred lines were derived from the plants selected at different cycles: 40 to 60 doubled haploid lines were obtained through in vitro androgenesis (method of Dumas de Vaulx et al. 1981) from each of the sub-populations L1 and L2 at cycle C3 and from L2 and L3 at cycle C4. At the C6 cycle, 45 to 48 S_6-inbred lines were derived by single-seed descent from each of the L1, L2 and L3 sub-populations. The horticultural traits were evaluated in a single field that included all the inbred lines and the parental lines YW and CM334. The plants were cultivated in Montfavet (France) from May to October 2001 under drip irrigation in a row design with 1.5 m between rows and 0.35 m between plants in the row. The experimental design was composed of three randomised blocks of two plants per inbred line. In each block, the two controls (YW and CM334) were included. Ten fruits were harvested from the two plants of each plot, bulked and weighed together to calculate the mean fruit weight (FW). Two additional traits were measured: the primary stem length from cotyledons to first flower (axis length, AL) and the number of leaves on this axis (NL) to compute the mean internode length (IL=AL/NL).

Molecular assays

For molecular genotyping analyses, 50 plants were sampled from the L1, L2 and L3 sub-populations at cycles C3, C4 and C6, so that 450 plants were analysed (50 plants × three sub-populations × three cycles). A total of 36 markers were used, including 34 AFLP loci and two specific PCR markers (Fig. 2). Those markers were chosen on the basis of the QTL mapping results from Lefebvre et al. (2002) and Thabuis et al. (2003). Six resistance QTLs were tagged using 14 markers located in their confidence intervals defined as (LOD_{max}−1) interval. Three markers were located on the chromosomes containing at least one resistance QTL (i.e., carrier chromosomes) but outside the confidence intervals of the resistance QTLs, and 19 additional markers mapped on five chromosomes without previously mapped resistance QTLs (i.e. non-carrier chromosomes).

DNA extraction

At the two-leaf stage, the two youngest leaves from the plants sampled were cut and frozen. Total genomic DNA was extracted according to the microprep protocol from Fulton et al. (1995). One CAPS marker (ASC012 located in the vicinity of the resistance QTL on chromosome P6) and one SCAR marker (ASC014 located on chromosome P9b) were assessed. Both markers were derived from RAPD markers: ASC012 originated from OPERON-A07_0.5c and ASC014 from D11_0.8c. AFLP reactions were performed as described by Vos et al. (1995) using *Eco*RI (+3), and *Mse*I (+3) primer combination (PC). Three PCs (E31M53, E38M61 and E41M54) were used, delivering 13 markers for the six resistance QTLs (on chromosomes P4, P5, P6, P11 and P12, see Fig. 2) and additional loci in the genetic background.

Data analyses

All statistical analyses were performed with SAS package (SAS Institute 1989). Homoscedasticity of the phenotypic data from resistance screenings and from horticultural traits was checked by comparing the variances of the data sets between cycles, between sub-populations and between families using the Bartlett test (P=0.05). Homogeneity of variance was accepted at P=0.05, except between the sub-populations at C6, due to the significantly higher variance of L3 compare to L2 for the S_{10} resistance component (P=0.011). The phenotypic data collected from the resistance screening tests of the C2 to C6 selection cycles were converted to a 0 to 1 scale using linear regression (PROC REG) with respect to the controls CM334 arbitrarily converted to 0 (resistant) and YW converted to 1 (susceptible) in order to allow comparison to each other. The three resistance components were analysed for the main factors affecting the resistance phenotypes in the population. This was performed using a nested ANOVA model (PROC GLM), $P_{ijkl}=\mu+C_i+L_j(C_i)+F_k(L_jC_i)+R_{ijkl}$ where μ is the mean of the data, C_i the 'cycle' factor, $L_j(C_i)$ the 'sub-population' factor (within the cycle), $F_k(L_jC_i)$ the 'family' factor (within the cycle and the sub-population) and R_{ijkl} the residual effect. Means and phenotypic variances were computed for each sub-population for the three resistance components REC, S_{10} and STA using PROC UNIVARIATE. For each sub-population, the Pearson correlation coefficients among the three resistance components were estimated using PROC CORR.

Three horticultural traits were analysed: AL, IL and FW. Raw data were used for AL and IL, but for FW data were transformed using the 'ln' function as it improved normality. The main factors affecting the horticultural traits of the population were studied with the same nested ANOVA model used for the resistance data. For the three traits, the adjusted means ('lsmeans' option) by cycle and by sub-population were computed and compared using a multiple mean comparison test ('tdiff' option, Duncan multiple-range test, P=0.05).

For comparing observed values of horticultural traits and observed allelic frequencies to the expected values, the theoretical allelic frequencies of the sub-populations (in the absence of selection) were calculated, assuming that the frequency of an allele in a progeny is the mean of the parental allelic frequencies: if $F_{i,j}$ is the frequency of YW alleles in the sub-population 'i' at the selection cycle 'j': $F_{i,j}=(F_{i,j-1}+F_{i-1,j-1})/2$. With YW as a recurrent

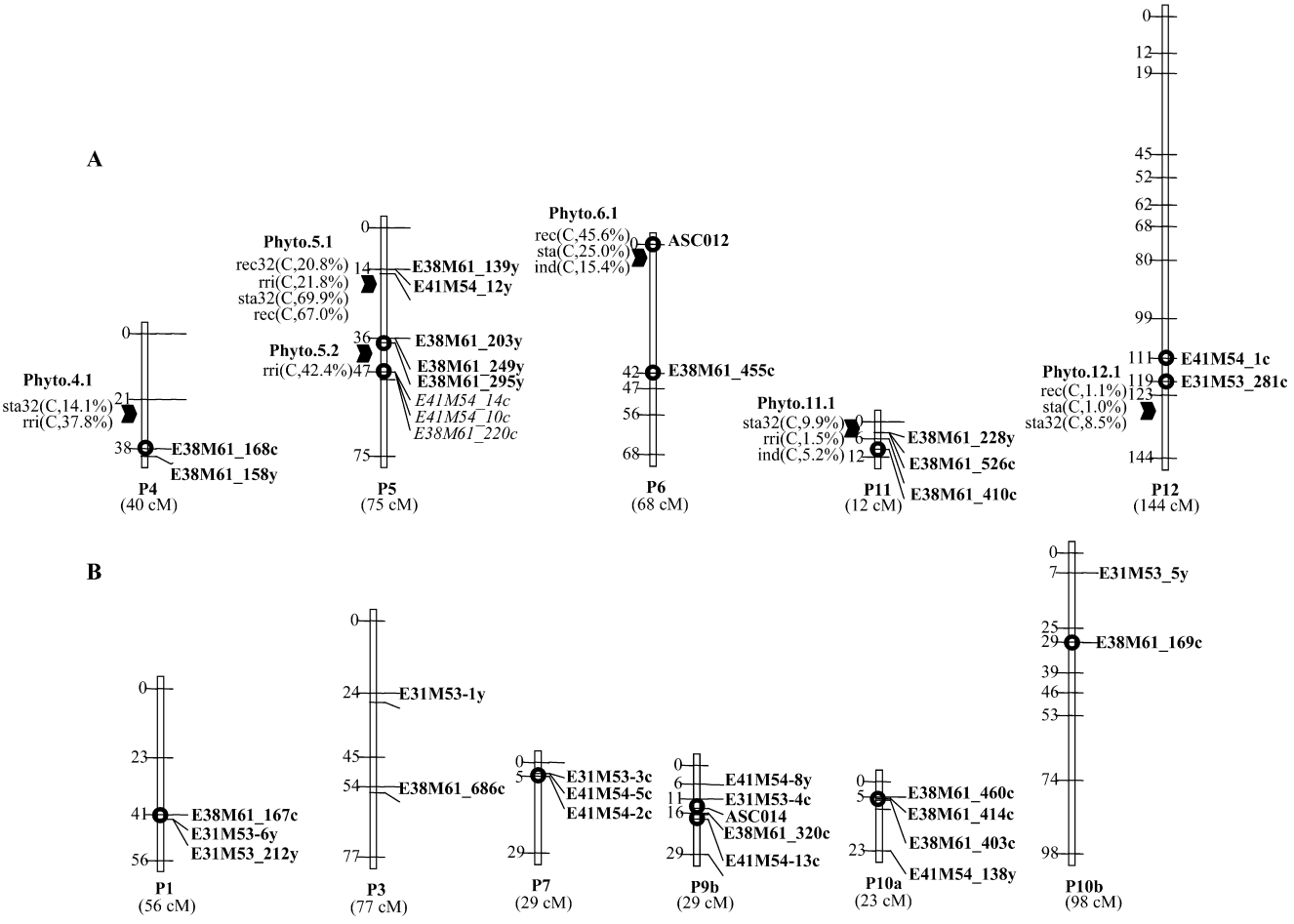

Fig. 2A, B Chromosomal regions tagged for the molecular genotype analyses of the 'Morelos' population based on a previous study. Chromosomes carrying resistance QTLs to *Phytophthora capsici* (**A**) and chromosomes not carrying *P. capsici* resistance QTLs (**B**). The *double vertical lines* represent the chromosome for which the name and the length (in Haldane centiMorgans) is written at the *bottom*. The *horizontal lines* represent the location of the markers composing the framework map defined by Lefebvre et al. (2002). The markers indicated *in bold* on the QTL carrier chromosomes were used for calculating the allelic frequencies. Those markers indicated *in italics* were located outside the confidence intervals of the QTLs. For each resistance QTL, the name was designated by "Phyto" followed by QTL carrier chromosome number and a second number identifying the QTL on the chromosome (e.g. Phyto.5.2). A *thick black arrow* indicates the most likely location of the QTL based on the highest LOD score in CIM. The spectrum of action of the QTL is given by the resistance components (*rec*, *ind*, *sta*) and, in *parentheses*, the QTL parental allele increasing the resistance level (*C* CM334) and the percentage of variation accounted for by each component. All these results were based on composite interval mapping (Thabuis et al. 2003). The *bold circles* on each chromosome frame indicate the QTLs re-detected in the breeding population. *Circles* cover the position of the most significant marker (ANOVA)

parent, the theoretical allelic frequencies in L1 vary from 75% in C1 (=BC_{1s}) to 99.22 in C6 (=BC_6) (Table 3).

The QTL validation was performed with the 36 markers on the 450 plants that were sampled for molecular analyses. These plants were evaluated for their resistance to *P. capsici* and a QTL analysis was performed using a single factor ANOVA (PROC GLM). Because those markers did not define large linkage groups but only single intervals, IM and CIM methods were not used. The QTL analyses were performed independently in the three sub-populations with 150 plants per sub-population (50 plants × three cycles). With 108 ANOVAs performed per sub-population (36 markers × three resistance components), a QTL was empirically declared significant if $P<5.10^{-3}$, minimising the detection of false-positive QTLs to 0.5 per sub-population.

Results

Validation of QTLs

Five of the six chromosomal regions previously reported by Thabuis et al. (2003) as involved in resistance using an F_2 mapping population (noted on Fig. 2) were significantly involved in *P. capsici* resistance in the breeding population, but new marker-QTL associations were also detected (Table 1). Only QTL Phyto.5.1 was not detected again. The effect on the resistance of the P5 and P11 chromosomal regions were confirmed in all three sub-populations L1, L2 and L3. The effect of the P4, P6, and P12 chromosomal regions was confirmed in the L2 and L3 sub-populations. However, the position of the QTLs

Table 1 Marker-QTL associations detected in the 'Morelos' breeding population. REC Receptivity, *S_{10}* speed of necrosis spread at 10 days post inoculation, *STA* stability

Sub-population	Resistance component	Marker	Chromosome	QTLs detected in the mapping population[a]	*P*-value	R^2 (%)
L1	REC	E38M61_220c	P5	Phyto.5.2	0.00326	6.18
	S_{10}	E41M54_14c	P5	Phyto.5.2	0.00387	6.33
	STA	E38M61_410c	P11	Phyto11.1	0.00311	6.42
L2	S_{10}	E38M61_167c	P1	-	0.00166	7.80
		E38M61_168c	P4	Phyto.4.1	6.0×10^{-7}	18.33
		E38M61_249y	P5	Phyto.5.2	0.00215	7.23
		E38M61_220c	P5	Phyto.5.2	8.83×10^{-5}	12.67
		E38M61_455c	P6	Phyto.6.1	0.00162	8.10
		E41M54_5c	P7	-	2.53×10^{-5}	13.79
		E38M61_320c	P9b	-	0.00561	6.22
		E38M61_169c	P10b	-	0.00408	12.54
		E38M61_410c	P11	Phyto.11.1	7.0×10^{-4}	8.64
		E41M54_1c	P12	Phyto.12.1	0.00184	8.40
	STA	E38M61_167c	P1	-	2.0×10^{-7}	20.07
		E38M61_168c	P4	Phyto.4.1	1.0×10^{-7}	20.42
		E38M61_249y	P5	Phyto.5.2	3.8×10^{-6}	15.65
		E38M61_220c	P5	Phyto.5.2	2.59×10^{-4}	11.09
		ASC012	P6	Phyto.6.1	0.00123	7.41
		E41M54_5c	P7	-	6.0×10^{-7}	18.93
		E38M61_320c	P9b	-	1.78×10^{-5}	14.28
		E38M61_169c	P10b	-	6.8×10^{-6}	16.47
		E41M54_1c	P12	Phyto.12.1	2.9×10^{-6}	16.49
		E31M53_281c	P12	Phyto.12.1	0.00161	7.74
L3	REC	E41M54_13c	P9b	-	0.00169	7.80
		E38M61_414c	P10a	-	0.00077	8.96
	S_{10}	E38M61_167c	P1	-	0.000966	8.71
		E38M61_168c	P4	Phyto.4.1	3.15×10^{-5}	13.19
		E38M61_249y	P5	Phyto.5.2	0.00451	6.37
		E31M53_3c	P7	-	2.53×10^{-5}	16.64
		ASC014	P9b	-	7.78×10^{-4}	10.98
		E38M61_414c	P10a	-	$<1\times10^{-7}$	22.74
		E41M54_1c	P12	Phyto.12.1	6.1×10^{-6}	6.37
	STA	E38M61_167c	P1	-	9.6×10^{-4}	8.71
		E38M61_168c	P4	Phyto.4.1	3.15×10^{-5}	13.19
		E38M61_455c	P6	Phyto.6.1	0.00365	06.93
		E31M53_3c	P7	-	$<1\times10^{-7}$	34.84
		ASC014	P9b	-	0.00131	10.30
		E38M61_169c	P10b	-	5.4×10^{-5}	12.56
		E38M61_526c	P11	Phyto.11.1	1.76×10^{-4}	10.85

[a] QTLs detected previously in the F_2 mapping population YW × CM334 were described in detail in Thabuis et al. (2003). A *dash* indicates that marker was not significantly associated with a QTL in the previous study

detected in this study slightly differed from the one detected in the F_2 mapping population, and the distinction between the two linked QTLs on P5 was not observed. The new QTL detected on P9b may correspond to a putative QTL previously detected in the mapping population only with the interval mapping method. Four other chromosomal regions on P1, P7, P10a and P10b that were not previously reported as harbouring resistance QTLs were found to be significantly associated with resistance in this study.

Changes in resistance phenotype and in QTL allele frequencies between the three sub-populations

An analysis of variance was performed on the adjusted data for traits REC, S_{10} and STA from L1, L2 and L3 across five cycles of phenotypic selection (C2 to C6). The 'cycle' factor, the 'sub-population' factor and the 'family' factor were all highly significant ($P<0.0001$). The predominant effect was observed for the 'sub-population' factor ranging from 8% to 18% of the total phenotypic variation, depending on the resistance component, followed by the 'cycle' factor that accounted for 10% of the phenotypic variation, then the 'family' factor that accounted for 8% of the phenotypic variation.

Regarding the changes between the sub-population means, L1 consistently displayed a large difference from L2 and L3 for the three resistance components (Fig. 3). A significant increase in resistance was detected from L1 to L2 sub-populations for all the cycles except at C4, where the high S_{10} and STA values of L2 have to be considered carefully, since unusually young plants were inoculated at this cycle. Comparing L2 and L3, the observed differences were less clear and depended on the resistance component or on the selection cycle. L3 remained more

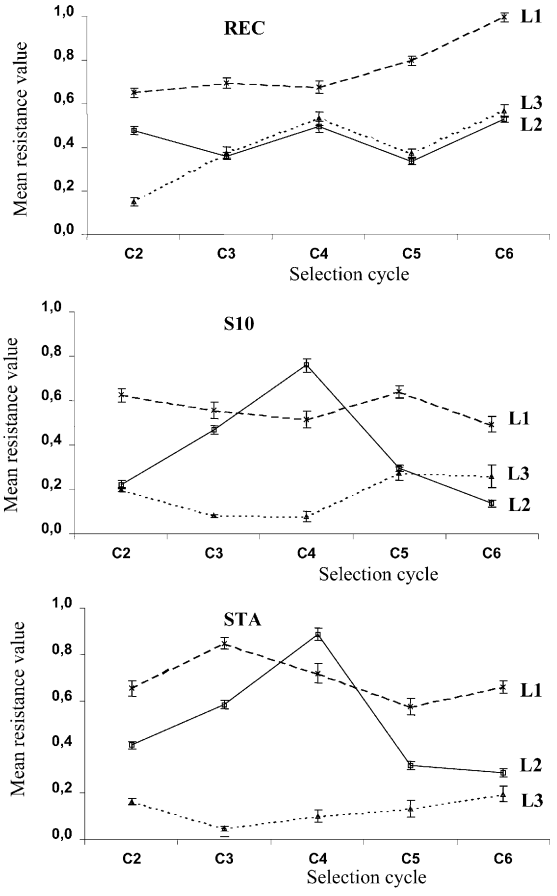

Fig. 3 Changes in the resistance phenotype of the sub-populations across the selection cycles. Average resistance values of the three components of resistance to *P. capsici*: receptivity (*REC*), the speed of the necrosis spread at 10 DPI (S_{10}) and stability (*STA*). For all the estimations, the standard error was computed (*vertical bars*): 0 represents the resistant parent value and 1 the susceptible parent value as defined in 'Materials and methods'

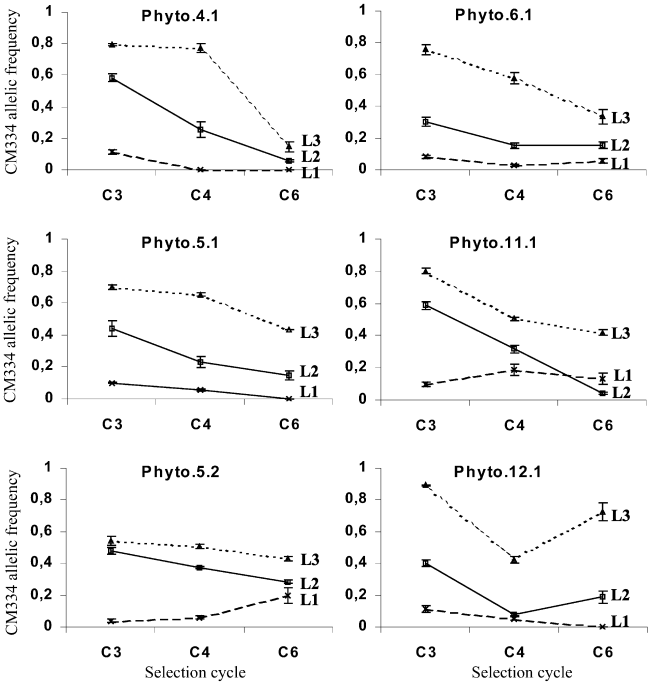

Fig. 4 Changes in CM334 allelic frequencies for the markers of the six resistance QTLs. For all the estimations, the standard error was computed (*vertical bars*)

resistant than L2 for S_{10} and STA, but no difference occurred for REC.

The analyses of molecular genotype data confirmed the phenotypic differences between the sub-populations L1, L2 and L3 were due to differences in CM334-resistant allele frequencies and combinations. Considering the allelic frequencies at the resistance QTL markers, the sub-population effect was highly significant and accounted for most of the observed variance ($P<0.0001$, $R^2=50\%$). For all the QTL markers (Fig. 4), the CM334 allelic frequencies were generally higher in L3 than in L2 and in L2 than in L1. In L1, very low frequencies at some QTL markers indicated that some QTL alleles were not selected in this sub-population.

Changes in resistance phenotypes and QTL allele frequencies across the selection cycles

The phenotypic data obtained in the L2 and L3 sub-populations displayed normal distributions for all the selection cycles with resistance values distributed between the parent means, indicating the polygenic segregation of resistance factors. In L1, a normal-like distribution was observed in C2, then progressively moved towards bimodal distributions between C3 and C6 for all the resistance components, indicating the selection of major resistance factors during the breeding process (data not shown).

Considering the change in sub-population means during the selection cycles, two main trends were observed, depending on the resistance component and sub-population (Fig. 3). Firstly, sub-population means varied with cycles, but with weak amplitude, so that the resistance levels remained globally constant within the sub-populations over the selection cycles such as S_{10} and STA in L1, L2 (except at C4) and L3 and for REC in L2. Secondly, the sub-population means displayed a progressive shift towards susceptibility with the selection cycles for REC in L1 and L3.

The correlation coefficients among the three resistance components were highly significant and generally increased across the five selection cycles for L1, indicating a favourable response to selection of the three resistance components (Table 2). In L2 and L3, S_{10} and STA remained highly correlated, whereas REC was less correlated to the other two at all the cycles.

CM334 allelic frequencies generally decreased with selection cycles at most of the QTL-linked markers except for Phyto.5.2 (all the sub-populations) and for Phyto.12.1 in L2 and L3 (Fig. 4). Depending on the QTL alleles and sub-populations, the frequency decrease was

Table 2 Changes in Pearson correlation coefficients among the three resistance components across the cycles of selection for the three resistance levels

Selection cycle		L1		L2		L3	
		REC	S_{10}	REC	S_{10}	REC	S_{10}
C2	S_{10}	0.478**	–	0.392**	–	0.009	–
	STA	0.489**	0.490**	0.295*	0.552**	–0.045	0.807***
C3	S_{10}	0.672***	–	0.527**	–	0.373**	–
	STA	0.564***	0.566***	0.379**	0.683***	0.233**	0.550***
C4	S_{10}	0.554***	–	0.360**	–	0.300**	–
	STA	0.558***	0.522***	–0.122	0.319**	0.221**	0.892***
C5	S_{10}	0.657***	–	0.282*	–	0.539***	–
	STA	0.759***	0.709***	0.315**	0.428**	0.214**	0.721***
C6	S_{10}	0.742***	–	0.464**	–	0.094	–
	STA	0.752***	0.805***	0.529***	0.705***	-0.049	0.647***

Significance of Pearson correlation coefficients: *P>0.05, **P>0.01, ***P>0.001

Table 3 Evaluation of the return to the recipient parent 'Yolo Wonder' (YW) regarding allelic frequencies and horticultural traits in the three sub-populations L1, L2 and L3 across the selection cycles. The expected percentage of YW alleles was deduced from the crossing plan and in the absence of selection (see Materials and methods). The observed percentage of YW alleles is based on molecular observation for the QTL non-carrier chromosomes. For horticultural traits, the estimation of return to YW was based on fruit weight (*FW*) data. None was significantly different from the expected percentage (P=0.05). For every sub-population (L1 to L3) and selection cycle (C3, C4, C6), average values are given with standard errors (*SE*). *n*d Not determined

		C3 (SE)	C4 (SE)	C6 (SE)
L3	Expected percentage	37.50	53.12	78.12
	YW allele percentage	34.8 (8.1)	43.0 (7.54)	71.8 (5.31)
	YW FW percentage	nd	62.7 (9.2)	71.5 (7.3)
L2	Expected percentage	68.75	81.25	93.75
	YW allele percentage	69.2 (5.6)	86.5 (3.7)	94.1 (2.4)
	YW FW percentage	73.2 (6.2)	79.8 (9.1)	92.8 (4.1)
L1	Expected percentage	93.75	96.87	99.22
	YW allele percentage	91.3 (2.9)	94.4 (2.37)	98.0 (2.1)
	YW FW percentage	94.9 (3.3)	nd	100.3 (2.8)

slow, suggesting the conservation of the QTLs at the heterozygous state in most of the selected plants (Phyto.5.1 and Phyto.6.1), or the decrease was steep, indicating the probable loss of some resistant QTL alleles with selection cycles, particularly for Phyto.4.1 and in L1.

Recovery of horticultural traits

During the breeding process, the 'Morelos' population was screened for *P. capsici* resistance but not for horticultural performance. In this study, we aimed to evaluate the effect of selection for resistance on horticultural traits. The evaluation of horticultural traits was done on the inbred lines derived from the *P. capsici*-resistant plants selected at C3, C4 and C6 (Fig. 1) to estimate an accurate line value of the selected genotypes. The trial displayed no significant block effect. According to the ANOVA results, the sub-population factor was the main source of variation for FW, whereas AL and IL were equally affected by the selection cycle and by the sub-population (data not shown). As expected, the means for the three horticultural traits in L1 were closer to the recipient parent values than the means in L2 and in L3. For L1, L2 and L3 analysed independently, the means were significantly different among the selection cycles and indicated a significant change towards the recipient parent, particularly in L1 where the IL values reached that of YW by the fourth cycle.

The observed and the expected frequencies of YW alleles (recipient parent) in the three sub-populations were computed (Table 3). For the three sub-populations, the data obtained from molecular markers and from the phenotypic measurements (FW) fitted closely the theoretical data and the results were not significantly different (at α=5%). This indicated that selection for *P. capsici* resistance had no significant impact on the recovery of horticultural traits nor on the recovery of the recipient genome in non-carrier chromosomes. However, in L3, slight deviations were observed: (1) at C4 where both phenotypic and marker data displayed, respectively, a higher or a lower value than the expected one, probably resulting from sampling effect and (2) at C6, where both molecular and phenotypic data showed lower values than expected (significant at α=10%), suggesting a slight slowing down of the recovery of the recipient parent genome when screening for a very high resistance level.

Discussion

Validation of QTLs

This study validated the QTLs detected previously in the F_2 mapping population (Thabuis et al. 2003) with five out of six chromosome regions involved in resistance detected again. However, the percentage of phenotypic variation explained by the markers was smaller and the QTL positions were slightly different. This might be due to the fact that QTL mapping with one factor ANOVA is less precise for estimating QTL effect and position than composite interval mapping (Zeng 1994); however, the reduced set of markers analysed did not permit construction of linkage groups for IM nor CIM analysis. The reduced set of markers, the unbalanced allelic frequencies and use of ANOVA weaken the QTL detection and position and affected the distinction between the two linked QTLs

Phyto.5.1 and Phyto.5.2 which were probably redetected as a single one. Another major reason explaining these differences was the occurrence of genetic recombination between markers and QTL during the cycles. Four chromosomal regions on P1, P7 and P10a and P10b were significantly involved in resistance in this study. Although they were not reported by Thabuis et al. (2003), the P10a and P10b QTLs were detected before in another resistant accession (Lefebvre and Palloix 1996). The stringent threshold used in the ANOVA ($P<5.10^{-3}$) should avoid false positive detection with the 36 markers used, but the unbalanced allelic frequencies resulting from the recovery of the recipient genome might have lead to false positives. Other studies have reported the validation of QTLs in breeding populations, such as De Koeyer et al. (2001), who found 13 common marker-QTL associations between an oat breeding population and a mapping population for yield traits. Using breeding populations for QTL mapping could enable the breeder to detect QTLs in multi-allelic situations, which is not the case in typical mapping populations, and then set up a molecular breeding program. This would save time and avoids disconnecting QTL mapping from breeding. However, breeding populations do not maximise allelic differences between parents for QTL detection in contrast to mapping populations because they typically involve crosses among elite and non-distant parents. In our study, phenotypic selection did not ensure to maintain allele segregation at QTL markers, and recombination between marker and QTL impeded QTL detection. Theoretical studies showed that unless an ultra-dense map is available, using breeding populations for QTL detection is less powerful than using specific mapping populations where linkage disequilibrium was only due to physical linkage (Kim and Stephan 1999). Practical results confirmed this trend. Enjalbert et al. (1999) proposed to use the variation of allelic frequency for detecting QTLs in multi-allelic situations. They tested their strategy by trying to re-detect the effect of two major genes with linked markers in a wheat composite population and reported inconclusive results.

Efficiency of selection for resistance

Both phenotypic and molecular genotype analyses showed that the sub-population was the main source of variation of the population. The increased resistance level from L1 to L2 and from L2 to L3 for the resistance components was also convergent with the increasing frequencies of the resistant-parent QTL alleles between these sub-populations. This confirmed that the increase in severity of the screening test resulted in the selection of increased sets of resistant alleles, since the selection intensities were not strongly different among the sub-populations.

The differences in the maintenance of the QTL alleles across the selection cycles delivered information on the efficiency of the phenotypic selection. In L1, the plants were directly backcrossed by YW, maintaining heterozygosity at the resistance QTLs. Because the markers were analysed in the backcross progenies of disease-tested (and heterozygous) plants, the maximum CM334 allelic frequency expected at one selected QTL is 0.25. The frequency of some CM334 alleles dropped below this threshold in L1, suggesting that resistant alleles at several QTLs were lost at least in part of L1 individuals, despite their dominant or co-dominant effect (Thabuis et al. 2003). Only the QTLs Phyto.5.2 and Phyto.11.1 were retained in L1 at the sixth selection cycle. However, phenotypic data indicated that the resistance level was maintained for S_{10} and STA but not for REC. Phyto.5.2 and Phyto.11.1 seemed sufficient for resistance expression in the low severity test, except for REC, which required different or additional QTLs. The bimodal segregation observed in L1 between the fourth and sixth selection cycles confirmed this loss of resistant alleles. The QTLs on the chromosome P6 also displayed a major effect in previous analyses (Lefebvre and Palloix 1996; Thabuis et al. 2003). This confirms that phenotypic selection preferably retained the genes or QTLs with major effect and higher heritability, as expected by Gallais (1990) and Kervella et al. (1998).

The other sub-populations L2 and L3 retained resistant alleles at additional QTLs; despite a trend of decrease, the CM334 allele frequencies remained higher than expected in the absence of selection (Table 3) except for Phyto.4.1 and Phyto.11.1 in L2, as can be deduced from frequencies in Fig. 4 compared to expected percentage in Table 3. In L3, all the alleles were retained as either heterozygous or homozygous as attested by the high frequency of Phyto.12.1. The severe testing conditions were efficient for additional allele selection. The accumulation of these alleles in a single genotype is necessary to maintain a resistant phenotype under severe artificial as well as natural infection conditions (Palloix et al. 1990). The successful selection of the whole QTL allele set in the population and the allele accumulation observed in the L3 sub-population validated the sub-division into sub-populations and the crossing method. This breeding scheme overcame the loss of resistance alleles during backcrossing and the use of unrealistically large populations when large numbers of alleles have to be transferred into a new genetic background. Using marker-assisted selection (MAS), Hospital and Charcosset (1997) showed that the selection of more than four QTLs simultaneously was unrealistic in a single-backcross population, particularly when the parents were genetically distant. In such cases, smaller sets of QTLs have to be selected separately, and accumulated in a second step. MAS selection would be more useful to screen specific sets of QTLs in the sub-populations and to increase the selection efficiency of resistance components like REC that showed a weak response to selection (Hospital et al. 1997; Moreau et al. 1998).

Efficiency of the return to the recipient parent

As a wide diversity of commercial standards exists in pepper, the change of genetic background is a frequent problem for the pepper breeder. Resistance factors mostly originate from exotic gene pools with small and pungent fruits and must be introgressed into large and sweet-fruited cultivars (Poulos 1994). In our case study, CM334 FW averaged 6 g, whereas commercial cultivars averaged 200 g to 300 g. This scheme was an attempt for recovering the recipient-parent phenotype while maintaining the resistant phenotype.

The observed frequency of return to YW genome estimated either from molecular genotype analysis or from phenotypic data fit well to the expected frequency for L1, L2 and L3 (Table 3). This indicates that selection for resistance did not drag unfavourable alleles for the horticultural traits considered. Mapping data from another population showed that fruit traits mapped in different chromosomes (Ben Chaim et al. 2001). The allelic frequencies also indicated that chromosomes that do not carry resistance alleles met expected allele frequencies without selection. Using MAS for the genetic background would also accelerate the recovery of the recipient parent genome as demonstrated by Hospital and Charcosset (1997) and Thabuis et al. (2004), and it would allow optimisation of the number of backcross or intermating cycles. These authors showed that once the genetic background is recovered, additional backcrosses will result in the loss of transferred QTLs. In our case, once the L1 plants had recovered the recipient genome, our selection effort could be shifted to the increase of L2 and L3 population size and increased selection efficiency.

Conclusion

On a practical basis, the breeding scheme presented is highly flexible, as the favourable genetic background can be easily changed for introducing new characters by changing the recipient line for L1. Considering cultivar release, short-term and long-term achievements can be managed together; rapid genetic gain for horticultural traits can promote release of partially resistant cultivars from L1, while highly resistant and improved cultivars can be further selected from the other sub-populations. The molecular and phenotypic evaluation of the breeding scheme showed that most of the objectives were achieved, but also suggested some improvements of the strategy. Based on the previous QTL-mapping data and QTL validation in the breeding population, markers are presently being used in the breeding process. The experimental results will allow future comparison of the performance of phenotypic and MAS breeding methods.

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