

QTL Analysis of the Genetic Architecture Determining Resistance to Fire Blight in an Apple Progeny

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Keywords: *Malus x domestica*, *Erwinia amylovora*, quantitative resistance, digenic epistasis

Abstract

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is one of the most destructive diseases of apple (*Malus x domestica*). In order to analyse the genetic determinism of resistance to fire blight in apple, a quantitative trait analysis (QTL) approach was used. A F₁ progeny of 164 individuals derived from a cross between the apple cultivars 'Prima' and 'Fiesta' was inoculated in greenhouse conditions. Seven copies per genotype were used. The length of the necrosis observed on shoots was scored 7 and 14 days after inoculation. The MapQTL software was used for QTL analyses, using two previously built maps of the parents, and the symptoms scored on shoots. Digenic interactions between all pairwise combinations of genetic markers were tested using a two-way ANOVA model with the SAS software. QTL were detected at the same locations both 7 and 14 days after inoculation. Two weak effect QTL deriving from 'Prima' were detected on linkage groups (LG) 3 and LG16. One strong effect QTL deriving from 'Fiesta' was detected on LG7 that explained 46.6% of the phenotypic variation observed in the progeny. Two additional significant ($P < 10^{-4}$) digenic interactions were identified in the progeny between markers on LG9 and LG1, and markers on LG9 and LG5. The existence of a strong effect QTL in 'Fiesta' is of particular interest for breeding purposes.

INTRODUCTION

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is undoubtedly one of the most destructive diseases of apple (*Malus x domestica*), and the most destructive bacterial disease of apple. This disease was first observed in 1870 in New-York states, USA, and has now been reported in more than 40 countries in North-America, Europe, the Middle-East and the Pacific Rim (Bonn and van der Zwet, 2000). It can lead to serious economic losses if not controlled (Vanneste, 2000). The chemical control of this disease involves antibiotics, such as streptomycine and copper-based treatments (Vanneste, 2000). Unfortunately, the use of such products is forbidden in many countries, and strains resistant to streptomycine have already been reported in several countries. Therefore, it has become urgent to develop new genetically resistant apple cultivars.

There is a great variability for resistance to fire blight among apple genotypes (Le Lezec et al., 1985) but little is known about the genetics determining resistance to this disease in apple. No monogenic complete resistance to fire blight is known in apple. Nevertheless, some cultivars have a high level of resistance that might prove useful in breeding programs, especially once their genetic architecture is elucidated. The apple cultivar 'Prima' is known to possess a high level of resistance to fire blight in the field (Le Lezec et al., 1985) whereas 'Fiesta' has an intermediate level of resistance to this disease (Le Lezec et al., 1990). In this study, we tested a F₁ progeny derived from a cross between 'Prima' and 'Fiesta' for its resistance to fire blight in the greenhouse. We used a QTL analysis approach to identify its most important resistance genes. This progeny was

previously obtained and studied during the European project EAGMAP (King et al., 1991). To our knowledge, this study is the first to investigate the genetics underlying resistance to fire blight in an apple progeny.

MATERIALS AND METHODS

The apple F₁ progeny 'Prima' x 'Fiesta' was previously obtained during the EAGMAP European project. Genetic maps of 'Prima' and 'Fiesta' were constructed during this project (Maliepaard et al., 1998) and were recently improved (van de Weg, pers. commun.). This progeny was planted in 1997 at INRA, in Angers, France. Graftwood of each of the 164 genotypes was collected in early 2002 and grafted onto the apple rootstock 'MM106', giving 7 tree scions (replicates) per genotype. The young grafted trees were grown in the greenhouse for six to twelve weeks until their shoots reached at least 15 cm. Due to differences in growth rates, several successive inoculations had to be performed, so that each tree had reached 15 cm or more before being inoculated. Trees that did not reach 15 cm within 10 weeks were discarded. Inoculum was obtained as described in Brisset et al. (2000) using the strain CFBP 14-30 of *Erwinia amylovora* (Paulin and Samson, 1973), at a concentration of 10⁷ bacteria/ml. Trees were inoculated by cutting the two youngest nearly expanded leaves of each shoot with scissors soaked in the inoculum kept at 4°C. The mean length of necrosis was measured on stems, 7 and 14 days post inoculation (d.p.i.). Necroses reaching only veins of the inoculated leaves were scored as 0.5; those reaching the petioles of the inoculated leaves were scored as 1.0; necroses reaching the main stem were measured in cm, and 1.0 was added to the obtained value.

Statistical analyses of the phenotypic data were conducted using the SAS software (SAS Institute, Inc., 1989). Analyses of variance (ANOVA) were performed using the GLM procedure of SAS, to test the significance of effects due to genotypes, date of inoculations, and interactions between these two factors. Data were adjusted according to the « date of inoculation » effects using the MIXED procedure of SAS. Broad-sense heritability was calculated using the following formula: $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/n)$, where n is the mean number of replicates per genotype, σ_g^2 is the genetic variance and σ_e^2 is the residual error variance.

QTL were detected using the rMQM (restricted multiple QTL mapping) procedure of the MapQTL software (van Ooijen and Maliepaard, 1996), using the markers closest to QTL peaks as cofactors. Both parental maps were used in the same analysis. This type of analysis in an F₁ progeny with parental maps only allows the detection of additive QTL, and not the detection of dominant or epistatic QTL. QTL with a maximum LOD (logarithm of odds ratio) score greater or equal to 2.5 were declared significant. Epistatic QTL were detected by testing simple effects and interactions of all pair wise combinations of molecular markers of each parental map in two ways ANOVA. Interactions detected with a probability less than 10⁻⁴ were declared significant. Only interactions involving several molecular markers per genomic region were taken into account. The percentage of phenotypic variation (R²) explained by all the QTL (both additive and epistatic) detected was calculated by an analysis of variance, in which each QTL was estimated by the marker nearest to its peak.

RESULTS

Twenty-four individuals out of 164 could not be inoculated because; either the scions could not be grafted, or none of their replicates reached 15 cm during the experiment. Analyses of variance showed strong « genotype » and « date of inoculation » effects. Data were adjusted according to the « date of inoculation » effects, before calculating the mean length of necrosis over the replicates. The distribution of individuals according to their mean length of necrosis at 7 and 14 d.p.i. (Fig. 1) reflects the progression of the disease in the progeny: more less-resistant individuals were observed at 14 d.p.i.. The broad-sense heritability of the mean length necrosis at 14 d.p.i. was 0.85, as opposed to 0.86 at 7 d.p.i. For further analyses, only results obtained with data collected at 14 d.p.i. are shown.

Two minor QTL and one major QTL could be detected with MapQTL (Fig. 2). The two minor QTL were detected on the linkage group 3 of 'Prima' (LG P3) with a LOD score of 4.6, and on LG P16 with a LOD score of 2.6. The last QTL was detected on LG F7 with a LOD score of 19.1. This last QTL was by far the strongest, with a percentage of phenotypic variation (R^2) of 46.6%. Five epistatic QTL involved in three digenic interactions were detected. Two of these QTL were the QTL F7 and P3 already detected using the usual additive model with MapQTL. An interaction between these QTL associated with a probability of 6.4×10^{-6} was found, using an ANOVA model without simple effects of the QTL. This interaction is clearly illustrated by the comparison between the mean lengths of necrosis associated with the different possible allele combinations at these QTL (Fig. 3). It appears that the QTL on LG P3 only has effects when the strong QTL on LG F7 is absent. Another interaction was found with a probability of 5.4×10^{-5} between a new region at the bottom of LG F1 and a new region at the bottom of LG P9. A third interaction was found with a probability of 6.3×10^{-5} between the same last region at the bottom of LG F9 (in 'Fiesta', instead of 'Prima'), and a new region at the bottom of LG F5.

The complete ANOVA model used to estimate the total R^2 explained by the detected QTL was as follows:

$$Y = F7 + P3 + P16 + F7*P3 + F5*F9 + F1*P9 + E$$

where Y is the phenotypic value of each individual; F7, P3, etc... are the QTL detected on LG F7, P3, etc...; and E is the residual error. This model explained up to 63% of the phenotypic variation in the progeny. F7, P3 and P16 respectively explained 37.5%, 4.2% and 2.1% of the phenotypic variation in this model. The interactions F7*P3, F5*F9, F1*P9 respectively explained 5.1%, 7.3% and 6.8% of the phenotypic variation in this model.

DISCUSSION

In order to inoculate the individuals of the 'Prima' x 'Fiesta' progeny at similar growth stages, we chose to inoculate trees only once their shoot height reached at least 15 cm. As all the trees did not reach this height at the same time, we had to perform several successive inoculations. The date of inoculation was found to have effects on the mean length of necrosis of the individuals in the progeny. Although environmental conditions were controlled, slight changes in temperatures, photoperiod or humidity might have occurred. The concentration and aggressiveness of the inoculum might also have changed. Three additive QTL and three digenic interactions between epistatic QTL were found, that together explained 63% of the phenotypic variation encountered in the progeny. This is less than the broad sense heritability of 85%, which means that not all of the genes underlying resistance to fire blight in this progeny were found. Epistatic interactions between more than two genes might occur in this progeny. Some remaining weak effect QTL might also not have been detected during this experiment.

A major QTL derived from 'Fiesta' was found on LG7, with a R^2 of 46.6%. This QTL might behave like a major gene in a susceptible background. It might prove very useful in breeding programs once its efficiency in different genetic backgrounds is confirmed. It would be very interesting to find out what the precise function of the gene underlying this QTL is. Is it involved in the recognition process of the bacteria, like many cloned R-genes, or is it involved in more general defence pathways? The absence of major genes for resistance to *Erwinia amylovora* in apple might lead to the conclusion that polymorphic genes underlying resistance to this pathogen in this species are different from the plant R-genes usually detected. Until now, a candidate gene strategy carried out in our laboratory with both R-gene-like sequences and genes involved in general defence pathways failed to identify a putative function for this gene (unpublished results).

Only minor QTL derived from 'Prima' were found. This result may seem surprising, since 'Prima' was previously proved to be more resistant than 'Fiesta' in the

field (Le Lezec et al., 1985; Le Lezec et al., 1990). But this experiment was carried out in the greenhouse, where environmental conditions, inoculation techniques and physiological growth stages of the trees are different from those found in the field. In addition, 'Prima', as well as 'Fiesta', might possess fixed, homozygous genes that cannot be identified in a F₁ progeny. Dominance and epistatic interactions between resistance genes might also occur in 'Prima', which could have been lost after crossing with 'Fiesta'.

Several digenic interactions were found in the progeny, which together explain more phenotypic variation than the minor, additive QTL derived from 'Prima'. These results mean that epistatic interactions (between two or more genes) might explain a significant part of the phenotypic variation encountered in the progeny, which could make it difficult to manage in breeding programs using marker-assisted selection (MAS).

CONCLUSION

This study is a first step toward a better understanding of the genetics determining resistance to fire blight in apple. We found one major and two minor additive QTL, and three epistatic interactions. Before handling some of these QTL in breeding programs with MAS, their efficiency in other genetic backgrounds will be checked. It would also be interesting to identify the function of the gene underlying the major QTL on LG F7, which might be done through the candidate gene analysis already underway in our laboratory. If the expression of the major QTL on LG F7 in different genetic backgrounds is confirmed, it will be necessary to develop breeder-friendly markers for MAS.

Literature Cited

- Bonn, W.G. and van der Zwet, T. 2000. Distribution and economic importance of fire blight. p.37-53. In: J.L. Vanneste (ed.), Fire Blight: the Disease and its Causative Agent, *Erwinia amylovora*. CAB International.
- Brisset, M.-N., Cesbron, S., Thomson, S.V and Paulin, J.P. 2000. Acibenzolar-S-methyl induces the accumulation of defense-related enzymes in apple and protects from fire blight. *Eur. J. Plant Pathol.* 106:529-536.
- King, G.J., Alston, F.H., Batlle, I., Chevreau, E., Gessler, C., Janse, J., Lindhout, P., Manganaris, A.G., Sansavini, S., Schmidt, H. and Tobutt, K.R. 1991. 'The European Apple Genome Mapping Project' - developing a strategy for mapping genes coding for agronomic characters in tree species. *Euphytica* 56:89-94.
- Le Lezec, M., Babin, J. and Lecomte, P. 1990. Le feu bactérien - Sensibilité de nouvelles variétés de pommes, premiers essais. *L'arboriculture fruitière* 434:24-32.
- Le Lezec, M., Thibault, B., Balavoine, P. and Paulin, J.-P. 1985. Sensibilité variétale du pommier et du poirier au feu bactérien. *Phytoma*, february: 37-44.
- Maliapaard, C., Alston, F. van Arkel, G., Brown, L., Chevreau, E., Dunemann, F., Evans, K.M., Gardiner, S., Guilford, P., van Heusden, A.W., Janse, J., Laurens, F., Lynn, J.R., Manganaris, A.G., den Nijs, A.P.M., Periam, N., Rikkerink, E., Roche, P., Ryder, C., Sansavini, S., Schmidt, H., Tartarini, S., Verhaegh, J.J., Vrieling-van Ginkel, M. and King, G.J. 1998. Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. *Theor. Appl. Genet.* 97:60-73.
- Paulin, J.-P. and Samson, R. 1973. Le feu bactérien en France: II. Caractères des souches d'*Erwinia amylovora* (Burill) Winslow et al., 1920, isolées du foyer franco-belge. *Annu. Phytopathol.* 5:389-397
- Van Ooijen, J.W. and Maliapaard, C. 1996. MapQTL version 3.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen.
- Vanneste, J.L. 2000. What is fire blight ? Who is *Erwinia amylovora* ? How to control it ? p.1-6. In: J.L. Vanneste (ed.), Fire Blight: the Disease and its Causative Agent, *Erwinia amylovora*. CAB International.

Figures

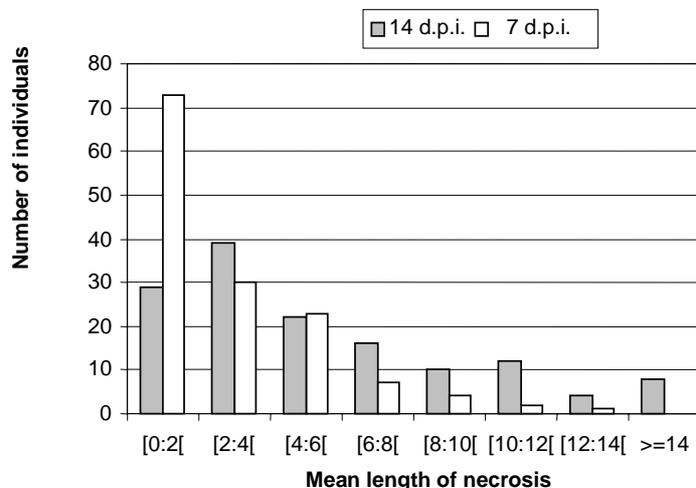


Fig. 1. Distribution of the individuals of the 'Prima' x 'Fiesta' progeny, according to their mean length of necrosis measured 7 and 14 days post inoculation (d.p.i.) and adjusted according to the « date of inoculation » effects calculated.

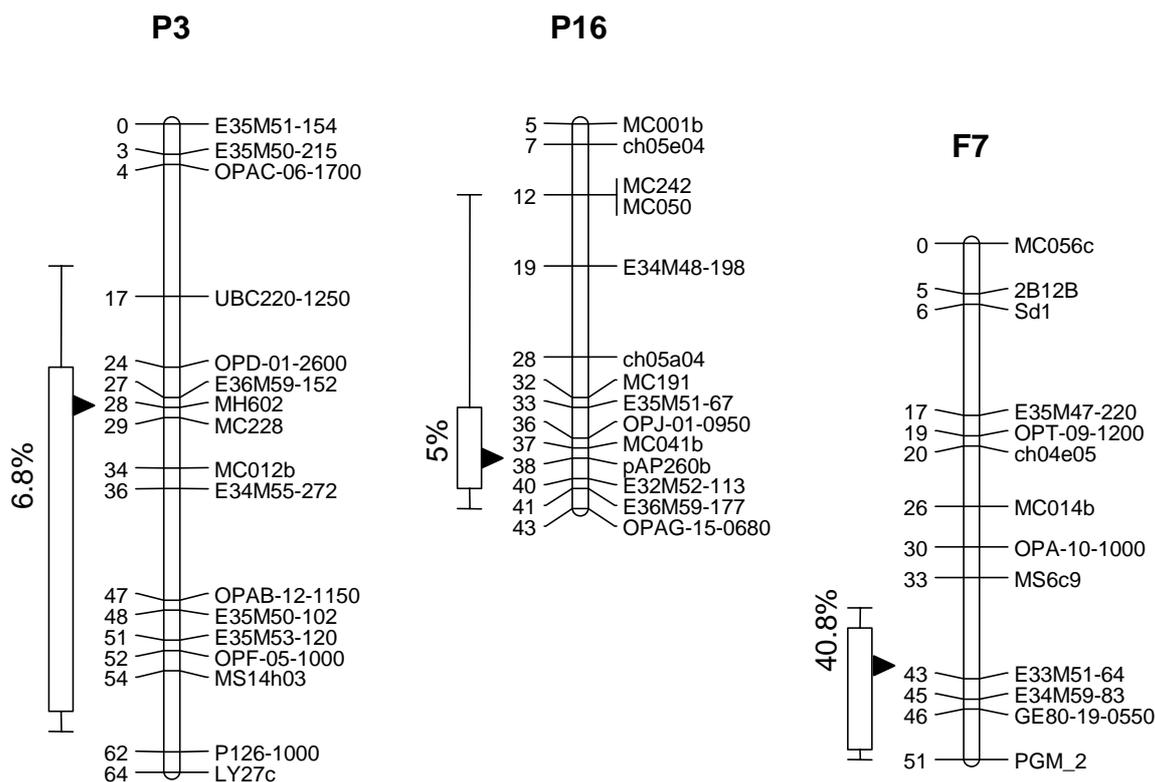


Fig. 2. Positions and percentages of phenotypic variation (R^2) explained by the QTL for resistance to fire blight identified in the 'Prima' x 'Fiesta' progeny using the mean length of necrosis measured 14 days after inoculation. Only linkage groups carrying QTL are shown. Black triangles indicate the position of the maximum LOD score. White rectangles indicate the LOD-1 confidence interval, and are prolonged on both sides by the LOD-2 confidence interval.

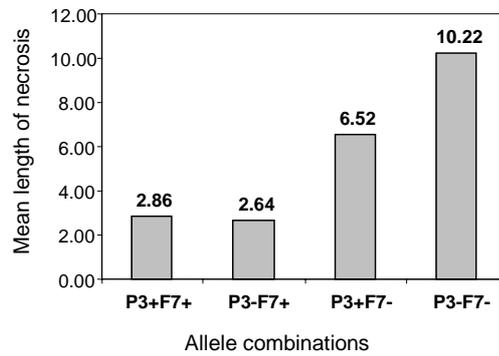


Fig. 3. Mean lengths of necrosis of the four groups of individuals of the 'Prima' x 'Fiesta' progeny displaying distinct allele combinations at the QTL detected on LG P3 and F7. QTL effects are estimated by the effects associated with the molecular markers closest to each QTL peak. + : favourable allele; - : unfavourable allele.