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QTL mapping of fire blight resistance in apple

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Abstract

Fire blight caused by the bacterium *Erwinia amylovora* is a severe threat to apple and pear orchards worldwide. Apple varieties exhibit a wide range of relative susceptibility/tolerance to fire blight. Although, no monogenic resistance against fire blight has been identified yet, recent evidence indicates the existence of quantitative resistance. Potential sources of fire blight resistance include several wild *Malus* species and some apple cultivars. F1 progenies of 'Fiesta'×'Discovery' were inoculated with the Swiss strain Ea 610 and studied under controlled conditions to identify quantitative trait loci (QTLs) for fire blight resistance. Disease was evaluated at four time points after inoculation. Shoot lesion length and the area under disease progress curve (AUDPC) values were used for QTL analysis. One significant (LOD score of 7.5–8.1, $p < 0.001$) QTL was identified on the linkage group 7 of 'Fiesta' (F7). The F7 QTL explained about 37.5–38.6% of the phenotypic variation.

Abbreviations: AUDPC – Area under disease progress curve; DAI – Days after inoculation; F7 – Linkage group 7 of Fiesta; LOD – Logarithm of odds; MQM – Multiple QTL Model; PLL – Percent lesion length; QTL – Quantitative trait loci

Introduction

Fire blight, caused by the Gram-negative enterobacterium *Erwinia amylovora*, infects many members of the Rosaceae family and is a major economic threat to apple, pear and quince production worldwide. Since first being described in the Eastern USA in 1780, fire blight has spread throughout North America, and to, New Zealand, Western and Central Europe and the Middle East (Bonn and Van der Zwet 2000). Fire blight attacks flowers, leaves, branches, roots and fruits and in severe cases entire trees and orchards can be devastated within a season. The pathogen invades through natural openings and wounds. The occurrence and severity of fire blight is determined by the interaction between the pathogen, favorable weather conditions and host plant susceptibility/tolerance (Thomson 2000). Control options are largely limited to exclusion (quarantine), eradication, and antibiotics (banned in many countries) (McManus et al. 2002; Norelli et al. 2003). Some success has been achieved using growth regulators (Rademacher and Kober 2003) and biocontrol (Johnson and Stockwell 1998).

No fire blight resistant commercial cultivars are available although there are reports of a large variability among apple genotypes regarding fire blight susceptibility/tolerance. Little is known about the genetic basis of this tolerance (Norelli et al. 1987). Several wild *Malus* species have been identified as potential sources for fire blight resistance, including *M. robusta*, *M. sublobata*, *M. atrosanguinea*, *M. prunifolia* and *M. fusca* (Aldwinckle and Beer 1979). A promising level of resistance has also been observed in some *Malus*×*domestica* cultivars, such as 'Nova Easygro' and 'Florina' (Aldwinckle and van der Zwet 1979; Keck et al. 1997; Fischer and Fischer 1999). Monogenic resistance to fire blight has not been identified in apple and it is thought that disease resistance is a quantitative trait (Korban et al. 1988; Brisset et al. 2002; Dondini et al. 2004).

Quantitative Trait Loci (QTL) mapping can identify chromosomal regions controlling quantitative traits. This approach has been successfully used to study many agronomic traits including disease resistance in many plant species including tomato (Goldman et al. 1995; Mangin et al. 1999), maize (Berke and Rocheford 1995; Krakowsky et al. 2004), rice (Ishimaru et al. 2001; Zenbayashi et al. 2002) and wheat (Anderson et al. 1993; Otto et al. 2002). Genetic linkage maps, which are an essential foundation for QTL analysis, are now available for apple (Conner et al. 1998; Maliepaard et al. 1998; King et al. 2001; Liebhard et al. 2003b; Kenis and Keulemans 2005). Conner et al. (1998) identified QTLs for growth and development characteristics in juvenile apple trees. King et al. (2000, 2001) reported the mapping of QTLs for fruit flesh firmness and fruit texture. Liebhard et al. (2003a) mapped several quantitative physiological traits, such as stem diameter, height, leaf size, number of flowers, sugar content of fruit and fruit acidity. QTLs have also been found for apple scab resistance (Durel et al. 2003; Liebhard et al. 2003c). Recently the QTL mapping approach has been used to study fire blight resistance in pear (Dondini et al. 2004) and apple (Calenge et al. 2004a, 2005). In this study, we applied the available linkage map data for the apple cross 'Fiesta'× 'Discovery' to identify QTLs for fire blight resistance (Liebhard et al. 2003b).

Materials and methods

Plant material

Eighty-six F1 progeny plants from the 'Fiesta'×'Discovery' cross previously used by Liebhard et al. (2003a) were chosen at random. Six replications for each of theses 86 progenies along with 12 replications of each parent were whip-grafted on 'virus-free M.9 T337' rootstocks. Plants were grown in the quarantine greenhouse facility at Agroscope FAW Wädenswil (Swiss Federal Research Station for Horticulture). Space limitation necessitated splitting the replications into two adjacent greenhouse cabins (three replications/greenhouse cabin). Temperature and humidity were controlled throughout the experiment (relative humidity was 70% and temperature was maintained at 21–25 °C).

Inoculation and evaluation of disease resistance

Inoculation was performed using the Swiss *E. amylovora* strain Ea 610. Inoculum was prepared by growing Ea 610 on plates of King's medium B (KB) for 24–36 h at 27 °C, scraping the bacterial lawn into tubes with phosphate-buffered saline (PBS, pH 7.2), and adjusting the concentration to approximately 1×10^9 cfu/ml based on optical absorbance at 600 nm. After 4–5 weeks, plants with minimum shoot length of 13.5 cm were inoculated as described by Momol et al. (1998). Inoculum was introduced to the shoot tip by inserting a syringe of 1.3-mm diameter (18-gauge) needle through the stem just above the youngest unfolded leaf. Plants in the two greenhouse cabins were inoculated two days apart. The first necrotic symptoms were visible at 3 days after inoculation. Lesion length (cm) was measured at four time points after inoculation (i.e., 6, 13, 20 and 27 days after inoculation (DAI)). After 27 days, disease progress completely ceased.

Statistical analysis

Statistical analysis was performed using SYSTAT software (SPSS 2000) (version 10; SPSS Corp., Chicago, IL). Prior to analysis, percent lesion

length (PLL) was calculated by dividing the lesion length (cm) by the shoot length (cm; measured at 6 DAI) (Norelli et al. 1984). An area under disease progress curve (AUDPC) value was calculated for each progeny (Campbell and Madden 1990). PLL measurements at 6, 13, 20 and 27 DAI were integrated using the formula

$$\text{AUDPC} = \sum_{i=1}^{n-1}[(t_{i+1} - t_i)(y_i + y_{i+1})/2]$$

where t is time in days of each measurement, y is the PLL at each measurement and n is the number of measurements.

Data were checked for outliers, normal distribution and progeny×greenhouse cabin interactions. Outlying data from two progeny were detected and removed. Differences between greenhouse cabins were significant for all measurements (6, 13, 20 and 27 DAI) and AUDPC. Plants in greenhouse cabin 1 consistently had longer PLL than plants in greenhouse cabin 2, however no progeny×greenhouse cabin interaction was found. Finally, data for both greenhouse cabins were pooled together. PLL for each progeny was averaged and used to calculate mean, range, standard deviation, and 95% confidence interval (CI). Data were not normally distributed and shoot length (cm), absolute lesion length (cm), PLL and AUDPC for each progeny was log transformed. Log transformed and non-transformed PLL and AUDPC were used to perform analysis of variance and to estimate broad sense heritability. Broad sense heritability was estimated by the formula,

$$h2 = \sigma_{2g}/\sigma_2 p \text{ and } \sigma_{2p} = (\sigma_{2g} + \sigma_2 e/n)$$

where, σ_{2g} is genetic variance, σ_{2p} is phenotypic variance; σ_{2e} is environmental variance and n is the mean number of replicates per genotype (Calenge et al. 2004b).

QTL mapping

Only log transformed data were used for further analysis. Least Square mean values were calculated for PLL at four different intervals and AUDPC for each progeny using the GLM procedure of SYSTAT and then used for QTL analysis (Krakowsky et al. 2004). Least Square mean values for shoot length and absolute lesion length were also estimated and used in QTL mapping. The maps used in QTL analysis were those already used by Liebhard et al. (2003b) for both 'Fiesta' and 'Discovery'. Maps consisted of a total of 734 markers, whereas 'Fiesta' had 345 markers including 137 AFLP, 108 SSR, and 100 RAPD markers, and 'Discovery' had 389 markers including 160 AFLP, 103 SSR, 1 SCAR and 125 RAPD markers with 91 SSRs in common on both maps (Liebhard et al. 2003b). The maps were calculated with 251 individuals of each parent. The average linkage group length was 66.96 cM for 'Fiesta' and 84.36 cM for 'Discovery'. Logarithm of odds (LOD) threshold value was calculated following Van Ooijen (1999). Significant (LOD >4.5) threshold was set to declare a QTL significant at the 95% confidence level (King et al. 2000).

MapQTL version 4 (Van Ooijen et al. 2000) was used for QTL mapping. Preliminary QTL analysis was done by interval mapping and Kruskal–Wallis test. Multiple QTL mapping (MQM) was performed only for QTL with LOD score exceeding the significant LOD threshold by interval mapping (Van Ooijan, personal communication). For MQM, marker with highest LOD value was taken as a co-factor (Hunt et al. 1998). The 2-LOD support interval was calculated to estimate the position of significant QTL with ~95% confidence (King et al. 2000; Durel et al. 2003). Interval mapping, Kruskal–Wallis test and MQM results were used to characterize the QTL (Atienza et al. 2004). Phenotypic variation explained by QTL was estimated by the multiple regression method (Lauter and Doebley 2002).

The 'Fiesta'×'Discovery' population was divided into sub-populations based upon the alleles of marker closest to the significant QTL at 6, 13, 20 and 27 DAI and AUDPC and then the average PLL was calculated for these sub-populations. Analysis of variance was performed to verify statistical differences between the two sub-populations at 6, 13, 20 and 27 DAI and AUDPC.

Results

Phenotypic evaluation of fire blight

Shoot length at the time of inoculation ranged from 13.7 to 48.4 cm with the average length of 25.7, 26.2 and 29.0 cm for 'Fiesta', 'Discovery' and

their progenies ('Fiesta'×'Discovery'), respectively (Table 1). At 6 DAI, most of the plants showed disease symptoms and the average PLL was almost double at 13 DAI while it remained constant after 20 days (Table 1). The mean PLL of the progenies ranged from 2.6 to 26.4% at 6 DAI, from 4.2 to 49.4% at 13 DAI and from 4 to 51.9% for both 20 and 27 DAI. The 95% CI for mean PLL at 6 DAI did not overlap the 95% CI for average PLL at 13, 20 and 27 DAI, which means that PLL at 6 DAI was significantly different from PLL at 13, 20 and 27 DAI. The data was positively skewed and transformation of the data normalized the distribution (Table 2). Genetic variation among the progenies in our population were significant ($p < 0.001$) for all measurements (6, 13, 20 and 27 DAI) and AUDPC. Variance was less for 6 DAI than 13, 20 and 27 DAI; however these latter three had almost the same variance. Broad sense heritability ranged from 0.90 to 0.94 (Table 2).

Table 1. Basic statistics for the mean PLL of parental cultivars and progeny plant of 'Fiesta'×'Discovery'.

Trait	Basic statistics[a]	Fiesta	Discovery	Fiesta×Discovery
Shoot length	Mean	25.70	26.22	29.01
	Standard deviation	4.54	4.63	6.29
	Range	18–31	18–34.50	13.75–48.42
	95% CI	22.46–28.94	22.91–29.53	27.66–30.36
6 DAI	Mean	10.78	13.14	12.56
	Standard deviation	7.08	6.93	5.13
	Range	2.00–24.50	4.30–25.90	2.64–26.40
	95% CI	5.72–15.84	8.19–18.10	11.46–13.66
13 DAI	Mean	17.33	19.58	23.26
	Standard deviation	12.88	12.39	10.50
	Range	2.00–40.70	6.40–46.40	4.25–49.38
	95% CI	8.12–26.54	10.71–28.44	21.01–25.51
20 DAI	Mean	18.87	20.63	25.19
	Standard deviation	13.71	12.47	11.55
	Range	2.00–42.40	6.40–47.80	4.04–51.73
	95% CI	9.06–28.68	11.71–29.55	22.71–27.66
27 DAI	Mean	19.04	21.49	25.69
	Standard deviation	13.59	12.25	11.54
	Range	2.00–42.40	6.40–47.80	4.04–51.95
	95% CI	9.32–28.76	12.73–30.25	23.22–28.16
AUDPC	Mean	357.73	402.80	472.98
	Standard deviation	253.36	229.62	201.80
	Range	42.00–794.90	126.60–867.40	88.23–949.53
	95% CI	176.48–538.98	238.54–567.06	429.71–516.24

[a]Mean, range, standard deviation, and 95% CI were calculated for the mean shoot length (cm), mean PLL at 6, 13, 20, 27 DAI and mean AUDPC for parental cultivars and progeny plant of 'Fiesta'×'Discovery' separately.

Table 2. Comparison among PLL and log transformed PLL data for population of 'Fiesta'×'Discovery'.

Trait	6 DAI		13 DAI		20 DAI		27 DAI		AUDPC	
	PLL	Log trans.[a]	PLL	Log trans.	PLL	Log trans.	PLL	Log trans.	PLL	Log trans.
df	85									
Variance components	42.2	0.06	147.0	0.08	174.0	0.08	177.3	0.08	54737.5	0.07
Broad sense heritability	0.90	0.91	0.92	0.94	0.92	0.94	0.92	0.94	0.92	0.94

Variance components, *F*-test results of corresponding mean squares and broad sense heritability at 6, 13, 20, 27 DAI and AUDPC. *F*-test results in all cases were significant at $p < 0.001$.
[a]Log-transformed PLL data.

QTL mapping

A significant QTL ($p < 0.05$) was identified on linkage group 7 of 'Fiesta' (F7) (Figure 1). The F7 QTL was found in both preliminary interval mapping and MQM with the maximum likelihood position at 50.1 cM. It was associated with the AFLP marker E37M40-0400, for all time points (except at 6 DAI) and AUDPC. Kruskal–Wallis analysis showed highly significant ($p < 0.0001$) association of AFLP marker to fire blight resistance at 13, 20 and 27 DAI and AUDPC (Table 3). For MQM, the marker E37M40-0400 was selected as cofactor. The LOD score and phenotypic variation explained by F7 QTL for PLL at 13, 20 and 27 DAI and AUDPC were 7.5 (37.5%), 8.1 (38.2%), 7.8 (37.5%) and 7.7 (38.6%) (Table 3, Figure 1), respectively. The 2-LOD support interval for the F7 QTL based on MQM results ranged from map position 46.5–51.5 cM (Figure 1).

There was a significant difference ($p < 0.05$) between the mean PLL for the subpopulations divided based upon the presence/absence of AFLP marker E37M40-0400 on linkage group 7 of cultivar 'Fiesta' at 13, 20 and 27 DAI and AUDPC, whereas at 6 DAI it was not significant (Figure 2). The F7 QTL was also identified with log transformed absolute lesion length (Table 3). No QTL was found associated with shoot length (LOD > 2) (data not shown).

Discussion

The same QTL was identified with log transformed lesion length and PLL (percentage of diseased shoot length) data. However, the data corrected for the variability of the shoot length (PLL) resulted in an increased LOD score and a higher percentage of the phenotypic variation was explained by the identified QTL. No QTL was identified with shoot length data, which means that the fire blight QTL identified with PLL is not artifacts due to the combination of shoot and lesion length.

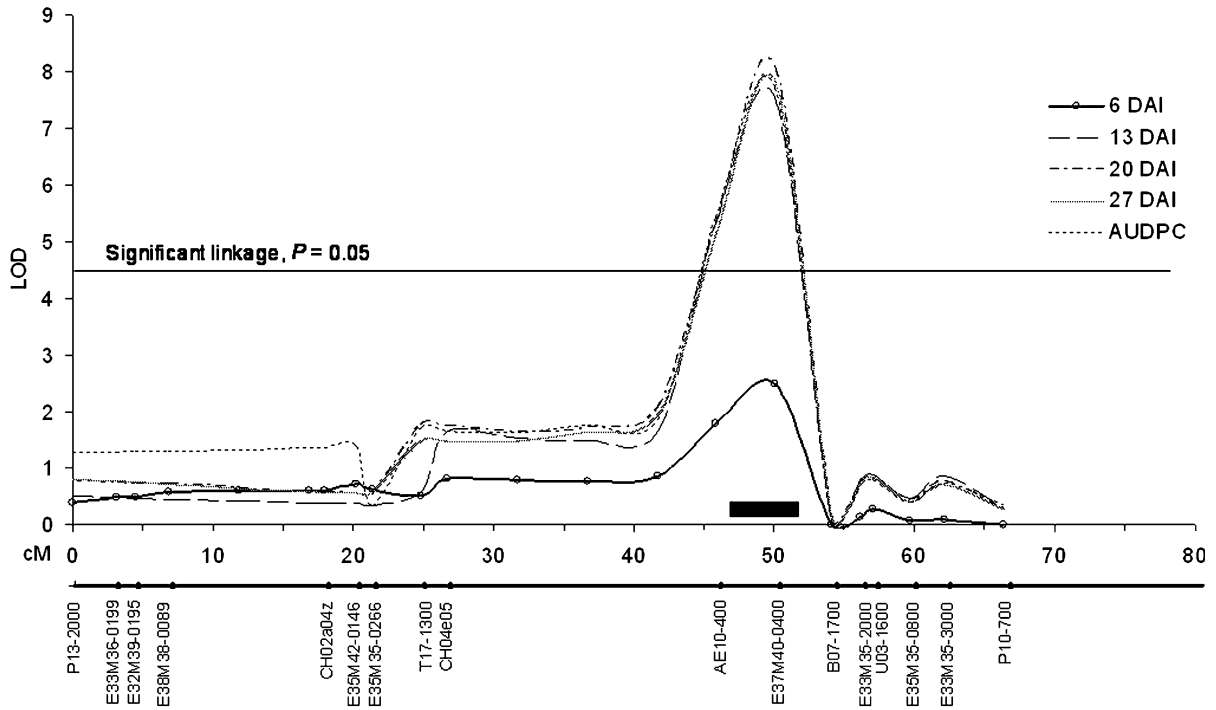

Figure 1. Multiple QTL mapping (MQM) results for the QTL identified on linkage group 7 of 'Fiesta' with log transformed PLL at 13, 20, 27 DAI and AUDPC. The x-axis indicate the linkage map of 'Fiesta' in cM; the y-axis show the LOD scores. The smooth horizontal line represents the significant ($p < 0.05$) threshold, and the solid black bar indicates 2-LOD support interval for the position of the QTL.

Table 3. QTL identified in the segregating population of 'Fiesta'×'Discovery' at 13, 20, 27 DAI for log-transformed lesion length, PLL and AUDPC showing chromosome, genetic locus, closest marker, DAI, LOD score, PVE, and Kruskal–Wallis results.

Chr	Locus	Closest marker	Parent/Method	Trait	Lesion length (cm)			PLL		
					LOD score[a]	PVE[b] (%)	Kruskal–Wallis	LOD score	PVE (%)	Kruskal–Wallis
7	50.1	E37M40-0400	Fiesta/MQM	13 DAI	6.22	34.0	*******	7.5	37.5	*******
				20 DAI	6.91	35.5	*******	8.1	38.2	*******
				27 DAI	6.66	34.7	*******	7.8	37.5	*******
				AUDPC	N/A			7.7	38.6	*******

[a]Significant linkage (LOD >4.5).
[b]PVE = phenotypic variance explained by QTL. N/A = Not available, ******* < 0.0001.

The QTL identified on linkage group 7 of 'Fiesta' (F7) explained approximately 38% of the phenotypic variation. Kruskal–Wallis test also showed highly significant association between the marker E37M40-0400 and this QTL. The F7 QTL was identified at 13, 20 and 27 DAI, and the same QTL could also be identified with AUDPC values which might be explained by the strong correlation between PLL measurements and AUDPC. In contrast to Calenge et al. (2005), we could not identify any QTL at one week after inoculation which could be due to different strains or may be due to different methods of inoculation used in both studies (cutting vs. injection). Lesion measurements and PLL at 6, 13, 20 and 27 DAI showed significant differences ($p < 0.05$) between progenies therefore, different intervals after inoculation (6, 13, 20 and 27 DAI) can be very informative about the disease development and could be used to calculate the AUDPC (Jorge and Verdier 2002). One possible explanation for the large amount of phenotypic variation attributed to the F7 QTL is the relatively small size of the progeny population. Beavis (1998) reported that when population size is small, QTLs with small effect could be overestimated and vice versa for QTLs with large effect. In this study, the reduced population size may have led to underestimation of a number of QTLs, overestimation of QTL effects, and a failure to quantify QTL interactions (Melchinger et al. 1998). More likely, however, F7 is a real QTL for fire blight resistance. This explanation is supported by the results of Calenge et al. (2004a, 2005), who identified a QTL in Fiesta in the same region of chromosome 7 using the bacterial strain CFBP 1430 in two different genetic backgrounds. The results reported here and the work of Calenge et al. (2004a, 2005), shared a common SSR (CH04e05) on linkage group 7 of the map of cultivar 'Fiesta'. The distance of this SSR marker and the 2-LOD support interval of the QTL identified in both studies overlap suggesting they may be the same. Interestingly, the F7 QTL was identified with two different strains.

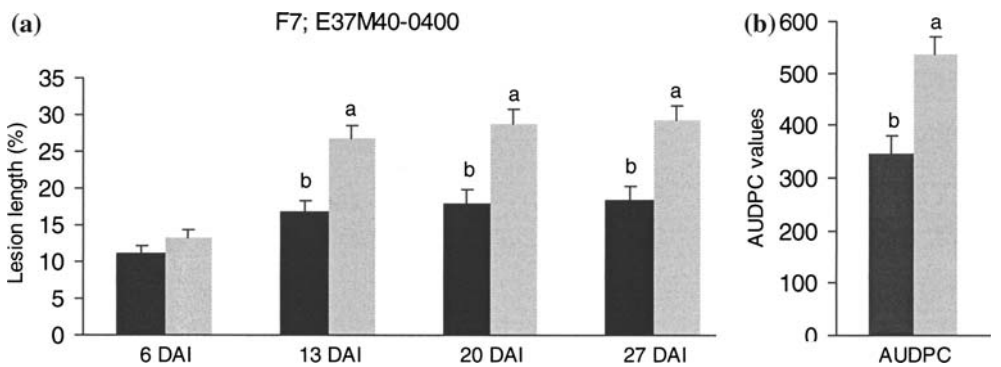

Figure 2. Mean PLL of the two subpopulations of progeny plants ('Fiesta'×'Discovery') divided based upon the presence/absence of AFLP marker 'E37M40-0400' at 6, 13, 20, 27 DAI (a) and AUDCP (b). Black and gray columns represent presence and absence of E37M40-0400 band, respectively. Letters indicate significant differences ($p < 0.05$) and bars represent the ±standard error.

Therefore, the F7 QTL can be considered to be a stable QTL since it is consistent in different genetic backgrounds and at least for two different *E. amylovora* strains.

Minor QTLs identified by Calenge et al. (2005) on linkage group 3 of cultivar 'Fiesta' and linkage groups 12 and 13 of cultivar 'Discovery' could not be identified in our experiments. The phenotypic variation explained by F7 QTL (37.5–38.6%) was less than the broad sense heritability, indicating that there are additional genetic elements contributing to fire blight resistance that were not identified in this study.

The data presented here together with those presented by Calenge et al. (2005) identify a QTL on chromosome 7 of 'Fiesta' associated with resistance to fire blight. However, F7 QTL should be further validated in other genetic background and with other pathogen strains. DNA markers for this QTL have genuine potential to be translated into an easy-to-use tool for rapid selection of genotypes conferring increased resistance to *E. amylovora* which could lead to the development of fire blight resistant cultivars.

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