Malus fusca fire blight resistance: Identification of a candidate gene on LG10 and a novel minor locus on LG16

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Fire blight resistance of the wild apple species *Malus fusca* (accession MAL0045) has been previously reported. This accession crossed with the domesticated apple cultivar, 'Idared', allowed for studies on the genetics of the resistance of this crabapple with the resultant F1 population. A major fire blight resistance locus *(Mfu10*) found on chromosome 10 explained up to 66% of the phenotypic variance amongst the *M. fusca* × 'Idared' progenies (Emeriewen *et al*. 2014). Although fire blight resistance is strain specific for some *Malus* accessions, leading to the breakdown of resistance in few resistance donors by highly aggressive strains of *Erwinia amylovora*; no strain able to breakdown the resistance of *M. fusca* itself or *Mfu10* has been found. This makes this wild apple an interesting model for resistance studies with different wild-type and mutant strains of *E. amylovora*. A candidate gene (*FB_Mfu10*) underlying the major locus was recently proposed (Emeriewen *et al*. 2018). Furthermore, with a dense genetic map of *M. fusca* and studies with a mutant of an aggressive strain of *E. amylovora*, a minor fire blight locus has been identified.

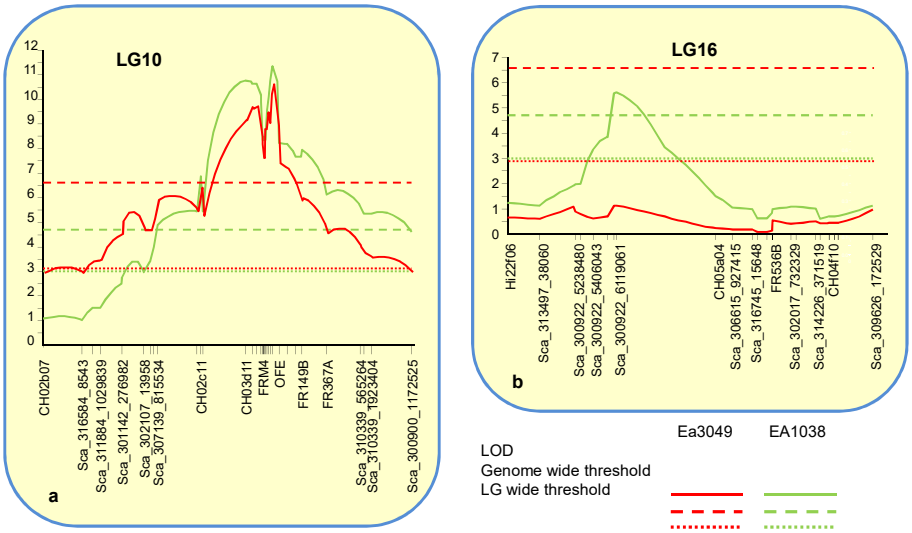

Figure 3: QTLs detected on LG10 (a) and LG16 (b) after inoculation with two *E. amylovora* strains. Some markers were deleted due to a better readability.

Identification of a candidate resistance gene (*FB_Mfu10*)

A genome walking approach was used to identify a BAC-clone (46H22) spanning the resistance locus on LG10 of *M. fusca*. Analyses of the sequences of 46H22 revealed one candidate resistance gene comprising 8 exons and 880 amino acids encoding B-lectin and serine/threonine kinase domains (Figure 1). Preliminary functional analyses showed that the open reading frame (ORF) together with its border sequences upstream of the start codon and downstream of the stop codon (~ 6000bp) is present only in resistant F1 genotypes, whereas 8bp distinguishes between susceptibility and resistance within the ORF.

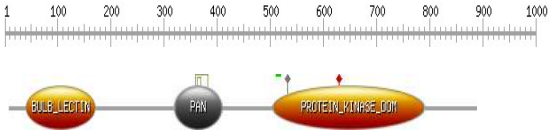

Figure 1: Predicted domains of the candidate gene for fire blight resistance

Establishment of a dense genetic map of *M. fusca*

A dense genetic map of *M. fusca* was established with SNP and SSR markers on 140 progenies of *M. fusca* × 'Idared'. SNP markers were developed by tGBS (tunable Genotyping by Sequencing – Data2Bio, Ames, IA). The map, calculated with JoinMap 4 (van Ooijen, 2006) consists of 17 LGs with a total length of 1079 cM comprising 613 markers, i.e. 0,6 marker per cM. Figure 2 shows the map of LG10.

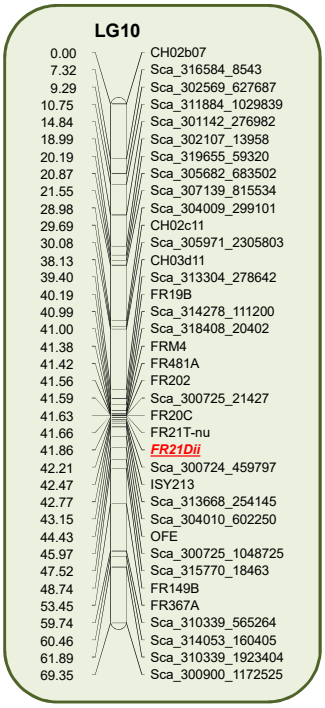

Figure 2: Dense genetic map of LG10. ***FR21Dii*** is the marker closest to FB_Mfu10

Phenotyping of *M. fusca* × 'Idared' progenies

Inoculation of progenies was done by cutting the two youngest leaves of actively growing shoots with scissors dipped in a bacterial suspension with a density of 10^9 cfu. Up to ten replicates per progeny were inoculated with *E. amylovora* strain Ea3049, which bears the S-allele of *avrRpt2*$_{EA}$, and strain Ea1038, i.e. Ea3049 ΔAvrRpt2$_{EA}$ complemented with the C-allele of *avrRpt2*$_{EA}$.

QTL-mapping

The developed genetic map of *M. fusca* established with SNP and SSR markers together with results from phenotyping with Ea3049 and Ea1038 were used for interval mapping (van Ooijen, 2004). Although both strains showed nearly identical results on LG10 (Figure 3a), only inoculation with strain Ea1038 resulted in the detection of a minor-QTL on LG16 with a LOD above the genome wide threshold (Figure 3b). This is further evidence of the contribution of the C-allele of the AvrRPT2$_{EA}$ effector on fire blight resistance of *M. fusca* accession MAL0045.

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