Fire blight resistance breeding

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Abstract

Fire blight, caused by the Gram-negative bacterium *Erwinia amylovora*, is a disastrous disease to pome fruit. Although fire blight is now a regulated non-quarantine pest (RNQP) in the European Union, for a long period it was regarded as a quarantine disease in Europe. Highly effective control is only possible with the application of antibiotics, but most European countries have banned their use. A sustainable approach would be the growing of fire blight resistant cultivars. Since most apple cultivars grown worldwide are susceptible to the disease, breeding of new apple cultivars resistant to fire blight would be an ecological and sustainable approach to overcome the menace of the disease. Major fire blight resistance QTLs have been mainly detected in wild *Malus* species, but the conventional introduction of these QTLs into cultivated apple takes decades. Methods using biotechnological approaches can reduce the time needed for the development of fire blight resistant cultivars. We will highlight the possibilities and difficulties in breeding for resistance to fire blight in apple (*Malus*).

Keywords: *Erwinia amylovora*, *Malus*, genetic resources, resistance QTLs

INTRODUCTION

The economic impact of fire blight, caused by the bacterium, *Erwinia amylovora* (Burrill) Winslow et al. (Winslow et al., 1920), of apples (*Malus domestica*) ranges from inexpensive pruning as a sanitary measure, to millions of dollars for the loss of whole trees and in fact entire orchards (van der Zwet et al., 2012). With limited options for containing the disease, particularly since many European countries have banned the application of antibiotics. Breeding for resistance remains as the most sustainable means of controlling fire blight, the most destructive bacterial disease of apples. Knowledge of the causative bacteria and its interaction with *Malus* hosts is essential in developing breeding strategies. The bacteria enter hosts through flowers or wounds and uses the type III secretion system (T3SS) to translocate effector proteins (Oh and Beer, 2005), e.g. avrRpt2$_{EA}$, DspA/E, Eop1, Eop3, HopPtoC$_{EA}$ (Zhao et al., 2006) into host cells, to cause disease. Susceptible hosts, as many of the apple cultivars that dominate the global market industry are, show typical symptoms such as progressive necrosis, the so-called shepherd's crook, and bacterial ooze (Peil et al., 2009). However, there is a variability of resistance responses in the *Malus* genus triggered by the ability to recognize bacterial effectors either directly or indirectly and subsequently inducing specific immune responses.

Malus genetic resources are reservoirs of fire blight resistance genes. For breeding for resistance, it is crucial to first identify fire blight resistance donors from germplasm by artificially phenotyping with the bacteria. Mapping of fire blight resistance in segregating populations led to the identification of several major and minor QTLs for resistance to fire blight in apple cultivars as well as in wild *Malus* accessions (Emeriewen et al., 2019). Fine mapping approaches combined with increased population size led to the identification of candidate resistance genes in the ornamental apple cultivar 'Evereste' (Parravicini et al., 2011), *M. fusca* accession MAL0045 (Emeriewen et al., 2018) and *M.* × *robusta* 5 (Mr5)

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(Fahrentrapp et al., 2013). Broggini et al. (2014) showed that the candidate gene for Mr5 *FB_MR5* confers resistance to susceptible 'Gala' using a transgenic approach. Functional validation of the other candidate genes is still in progress.

An important consideration in breeding for resistance is the pathogen strain-specificity of *Malus* resistance (Norelli and Aldwinckle, 1986). Some resistance genes are overcome by highly virulent/mutant strains (reviewed by Emeriewen et al. (2019)), most notably the breakdown of Mr5 fire blight resistance (Peil et al., 2011; Vogt et al., 2013; Wöhner et al., 2014). Therefore, the ultimate goal of breeding for resistance is to develop elite cultivars with durable resistance by pyramiding differently acting genes. This review highlights the possibilities and difficulties in breeding for resistance to fire blight in apple.

Phenotyping

Reliable phenotyping is a prerequisite for the estimation of resistance/susceptibility of a specific genotype to fire blight. Although primary infection occurs on flowers, artificial inoculation for phenotyping of apple cultivars, wild *Malus* species accessions or whole populations for mapping purposes are mainly done by artificial inoculations of young, actively growing shoots. Mainly two methods of shoot inoculation are performed. One is inoculation by hypodermic needle, where the needle is inserted through the stem just above the youngest unfolded leaf and inoculum is introduced to fill the wound and leave visible drops on both sides of the wound (Norelli et al., 1984; Paulin and Lespinasse, 1989; van der Zwet and Bell, 1995). Another method, currently the most common one, is inoculation by bisecting the two youngest leaves of one shoot of each plant with scissors dipped in bacterial suspension (Horner et al., 2015; Richter and Fischer, 2002; Rothleutner et al., 2014), or by cutting-off their tips just below the first undeveloped leaf (Sobiczewski et al., 2015). Disease progression for each inoculated shoot is expressed as the percentage quotient of the length of the necrotic lesion and the total shoot length, i.e. percentage lesion length (PLL). Whereas Thibault and Le Lezec (1990) found only a weak correlation between susceptibility in shoots and flowers after artificial inoculation of shoots and/or blossoms with *E. amylovora*, Peil et al. (2019) showed that at least for Mr5 that the same locus on linkage group 3 (LG3) conditioned resistance in both shoots and flowers.

Standardization of inoculum source is important to obtain reproducible and reliable results (Quamme and Bonn, 1981) as well as using a sufficient number of replicates as there can be considerable variation in the amount of disease in the replicates of a genotype. The inoculum concentration used in phenotyping differs between 10^7 cfu mL^{-1} (Rothleutner et al., 2014; Sobiczewski et al., 2015) and 10^{10} cfu mL^{-1} (Aldwinckle and Preczewski, 1979). The use of water for suspending the bacterial inoculum or the use of phosphate buffered solution (PBS) did not result in significant differences in the incidence of fire blight infection (Horner et al., 2015). The choice of a specific bacterial strain for phenotyping is of great importance since Norelli et al. (1984) found differential host pathogen interactions among cultivars of apple and strains of *E. amylovora*. For that reason, Richter and Fischer (2002) used a mixture of three highly virulent strains, selected in a previous virulence test, for their annual fire blight screenings. This procedure was applied for phenotyping of the genetic resources described below. Phenotyping of populations for the mapping of resistance loci are generally performed with single strains.

Environmental and host conditions have a large influence on fire blight infections. Artificial inoculations of shoots in the field may be more or less successful depending on weather conditions, thus underestimating susceptibility if sub-optimal. Phenotyping fire blight in a greenhouse under controlled conditions increases the chance of infection and enables the efficient identification of resistant genotypes (Peil et al., 2009), but may overestimate susceptibility (Peil et al., 2019).

Genetic resources

Many genetic resources *M. domestica* cultivars and *Malus* wild species accessions are available for apple breeding. Both the apple cultivar and the wild *Malus* species collections of the fruit gene bank of the Institute for Breeding Research on Fruit Crops (ZO) have been

phenotyped for fire blight by artificial shoot inoculation in the green house. Only genotypes with a minimum of five inoculated replicates were considered, i.e. a total of 299 wild species accessions and 690 cultivars contributed. Whereas a large proportion of wild species accessions (25.1%) showed necrosis length lower than 25% (Figure 1a), only five accessions (0.7%) showed such low necrosis rates in the *Malus* cultivars (Figure 1b). Those with the lowest PLL were 'Remo' (8.3), 'Joachim Gauck' (12.3), 'Enterprise' (16.0), 'Crimson Beauty' (20.3), 'Altländer Pfannkuchenapfel' (22.7), 'Rewena' (25.2), and 'Rea Agata' (26.0). 'Altländer Pfannkuchenapfel', (~1840 in Germany) and 'Crimson Beauty' (~1880 in Canada) are original cultivars. The other four cultivars are descendants of *M. floribunda* 821, which carries a major QTL for fire blight resistance (Figure 2). 'Enterprise' was developed by the PRI, USA, breeding program, and 'Remo', 'Joachim Gauck', 'Rewena' and 'Rea Agata' by the Dresden-Pillnitz, Germany, breeding program. Kostick et al. (2019) used cluster analysis to classify resistance/susceptibility groups of 94 apple cultivars and found 16 cultivars with moderately to highly resistance responses.

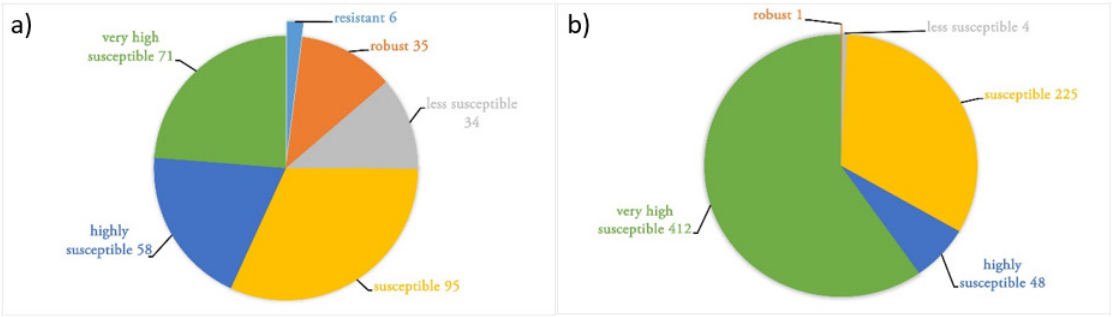

Figure 1. Number of a) *Malus* wild species accessions and b) *M. domestica* cultivars present in the classes according to their percentage lesion length (PLL) after artificial inoculation with *Erwinia amylovora*: 0 - resistant, 0< - <10 moderately resistant, 10 - <25 lowly susceptible, 25 - <50 susceptible, 50 - <75 highly susceptible, ≥75 extremely susceptible.

Figure 2 shows the position of major and minor fire blight resistance QTLs on the respective linkage groups of apple. Major QTLs explaining more than 40% of the phenotypic variation were identified on LG3 of *M.* × *robusta* 5 (Peil et al., 2007), on LG7 of 'Fiesta' (Khan et al., 2006), on LG10 of *M. fusca* acc. MAL0045 (Emeriewen et al., 2014), and on LG12 of *M.* × *arnoldiana* acc. MAL0004 (Emeriewen et al., 2017), *M. floribunda* 821 (Mf821) and the ornamental cultivar 'Evereste' (Durel et al., 2009). Several minor QTLs were found in the main progenitor of the domesticated apple *M. sieversii* acc. GMAL4593 (Desnoues et al., 2018) and in a number of apple cultivars (Calenge et al., 2005; Khan et al., 2013; Le Roux et al., 2010; van de Weg et al., 2018). Additional minor QTLs in *M.* × *robusta* 5 were detected after inoculation with different *E. amylovora* strains (Gardiner et al., 2012; Wöhner et al., 2014) and in 'Evereste' on LG15 (Durel et al., 2009).

E. amylovora strain specificity

Resolving the crucial understanding of fire blight host-pathogen interactions would greatly facilitate breeding, hence the analysis of *E. amylovora* strains with differential host ranges have become a starting point to identify resistance mechanisms. Asselin et al. (2011) showed that the eop1 effector gene of *Rubus*-pathogenic *E. amylovora* isolates limits virulence on apple and pear. Its deletion did not alter virulence on *Rubus*, while the deletion indeed resulted in gain of virulence for *Rubus* strains on immature pear fruits. An *eop1* deletion in Spiraeoideae-infecting wild type strains did not alter virulence on common apple and pear cultivars, whereas Wöhner et al. (2018) showed virulence on the ornamental cultivar 'Evereste' and the crab apple Mf821, but not on *M.* × *arnoldiana*. While QTL mapping with all three genotypes points to a similar region on LG12 (Figure 2), the different reactions to the

eop1 mutant prove the presence of independent resistance mechanisms. Naturally occurring mutants in the effector gene of *E. amylovora* resulted in the first demonstration of a gene-for-gene interaction for this pathogen (Vogt et al., 2013). A single nucleotide exchange resulted in the exchange of a cysteine amino acid to serine at position 156 of the cysteine protease. While Zhao et al. (2006) showed a reduced virulence for an *avrRpt2*-deletion mutant on commonly susceptible apple and pear accessions, inoculation with the *avrRpt2*-deletion mutant led to the breakdown of fire blight resistance in Mr5, but not of *M. fusca* MAL0045 and *M. arnoldiana* MAL0004 resistance. The fire blight resistance gene *FB_MR5* (Broggini et al., 2014; Fahrentrapp et al., 2013) was recently shown to be activated by ACP3 released upon cleavage of MdRIN4-1 by AvrRpt2 (Prokchorchik et al., 2020). While the stepwise identification of resistance genes will facilitate more targeted breeding approaches, the quick distinction between different resistance pathways early on by inoculation with different *E. amylovora* strains and mutants will help in economic selection of resistance donors. Further analysis of effector mutants in different genomic backgrounds of *E. amylovora* could improve this significantly. The constant progress in techniques for bacterial genome editing, such as recombineering or CRISPR/Cas (Choudhury et al., 2020), will enable the construction of a broad "strain library" for resistance screening.

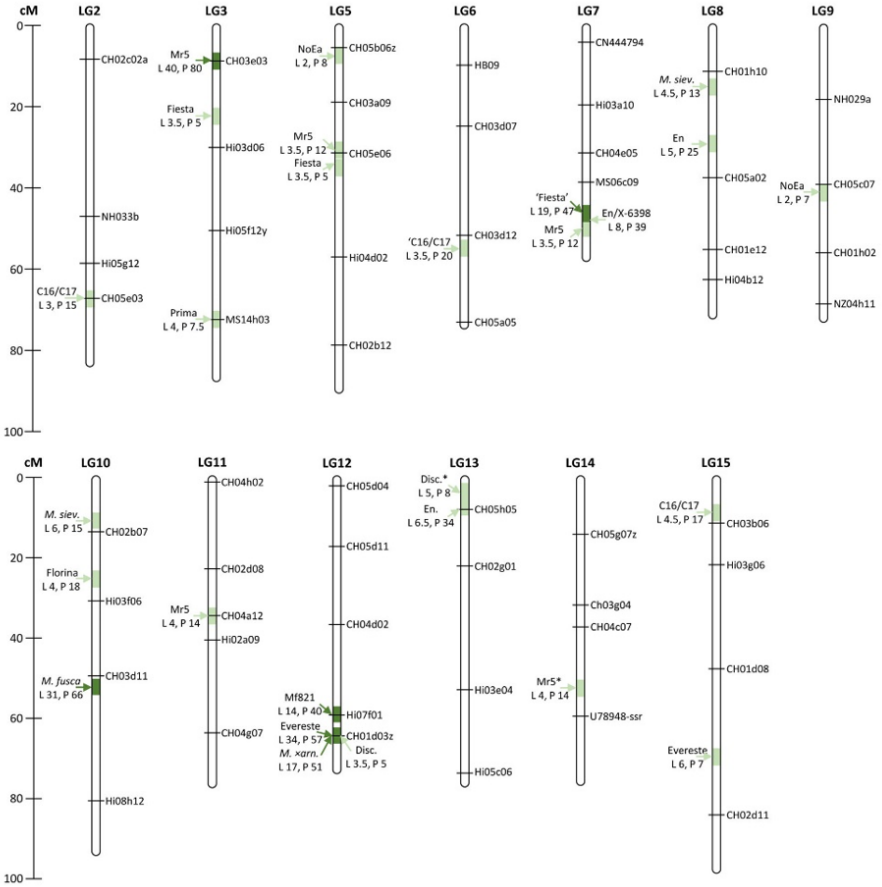

Figure 2. Estimated central position of minor (light green) and major (dark green) quantitative trait loci (QTLs) for fire blight resistance on linkage groups (LGs) of apple in relation to SSRs found in different genotypes/populations. SSRs on LGs reflect their position on the apple integrated map (users.unimi.it/hidras). C16/C17 – Co-op16/Co-op17; Mr5 – *M.* × *robusta* 5; NoEa – 'Nova Easygro'; *M. siev*. – *M. sieversii* GMAL 4593; En – Enterprise; *M. fusca* – *M. fusca* MAL0045; Disc. – 'Discovery '; *M.* × *arn*. – *M.* × *arnoldiana* MAL0004; Mf821 – *M. floribunda* 821; L: logarithm of the odds; P: percentage variation explained.

Breeding

Breeding in apple in general is aimed at the development of cultivars that are competitive in the market. Breeding of resistant apple cultivars has an additional aim: durable resistance. At the beginning of the breeding process, the breeder has to decide what he wants to achieve, a cultivar with reduced susceptibility or a cultivar with a stable and strong resistance. Dependent on this decision is the choice of the parents. Donors inheriting strong QTL effects have been found mainly in different wild species accessions with unpalatable fruits. Due to the long juvenile phase of apples (Hanke et al., 2007), the introgression of resistance genes from wild relatives needs a certain number of backcrosses and lasts decades to eliminate genetic drag. Furthermore, since resistance to fire blight is strain-dependent, pyramiding of differently acting resistance genes is necessary to achieve durable resistance. Differently acting fire blight resistance QTLs have been found in e.g. Mr5, *M. fusca* MAL0045 and the ornamental cultivar 'Evereste' (Wöhner et al., 2018). Whether pyramiding of differently acting resistance QTLs really results in resistance to highly virulent strains like Ea3049 still has to be proven. Although MAL0045 and MAL0004 are resistant to Ea3049, progenies inoculated with this strain showed an increase in average susceptibility from about 23 to 62%, and from around 33 to 66% PLL, respectively, compared with Ea222 (Emeriewen et al., 2017, 2015). More knowledge on host pathogen interaction is required. Three major QTLs and a minor one are located in the same region on LG12 (Figure 2). Whether the underlying genes are allelic or at separate loci is still unknown, but this fact is important for developing a breeding strategy for pyramiding. If genes are at different loci, they can be combined by crossing and inherited together in the breeding process. If genes are allelic, pyramiding is only possible in a final crossing step as they cannot be inherited together.

Using resistances from elite cultivars could enhance the development of new, competitive fire blight resistant cultivars. The moderate fire blight resistance QTL identified in *M. domestica* cultivar 'Fiesta' (Calenge et al., 2005; Khan et al., 2006) is promising for fire blight resistance breeding since it is from an elite background, but the locus is not sufficient to provide good resistance. Until now, several minor QTLs have been described in apple cultivars. With the application of molecular tools, it should be possible to pyramid several minor QTLs by crossing, which could lead to good resistance. Whether these minor QTLs have an epistatic and/or additive effect has to be analyzed. Furthermore, the combination of several minor QTL requires a large population size.

Of interest to breeders is the identification of moderate resistance in some accessions of the primary progenitor of apple – *M. sieversii* (Harshman et al., 2017), as it has the closest resemblance of cultivated apples. However, more studies are required to characterize and fully understand the fire blight resistance of these *M. sieversii* QTLs (Desnoues et al., 2018).

Biotechnological tools can improve the development of fire blight resistance cultivars either by reducing the generation cycle of apple or by introducing fire blight resistance genes using genetic engineering.

CONCLUSIONS

Breeding of fire blight resistant cultivars is a long-term process, which requires knowledge on resistances and their genetics, and on the pathogen as well as on host-pathogen interactions. More research is needed to fulfill the aim of competitive apple cultivars with durable resistance to fire blight caused by various strains of *E. amylovora*.

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