Pyramiding Quantitative Resistance with a Major Resistance Gene in Apple: from Ephemeral to Enduring Effectiveness in Controlling Apple Scab

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Abstract

Genetic resistance is a useful strategy to control plant disease, but its effectiveness may be reduced over time due to the emergence of pathogens able to circumvent the defences of the plant. However, the pyramiding of different resistance factors in the same plant can improve the effectiveness and durability of the resistance. To investigate the potential for this approach in apple to control scab disease we surveyed scab incidence in two experimental orchards located more than 300 km apart planted with apple genotypes carrying quantitative resistance and major gene resistance alone or in combination. Our results showed that the effectiveness of pyramiding in controlling scab was dependent on the site and could not be completely explained by the effectiveness level of the resistances alone.

Key words: apple, *Malus* × *domestica*, scab, *Venturia inaequalis*, durability, sustainability, Quantitative Trait Locus, major resistance gene, pyramiding
The control of plant diseases by chemical products presents risks to human health and the ecological environment. Furthermore, these treatments can be ineffective if they are not used at the appropriate time or if the pathogens adapt and become resistant. Plant genetic resistance constitutes an environmentally friendly way to control disease and to reduce chemical inputs. Plant resistance can be described by two traits: (1) its phenotype with either a complete or partial level of resistance, and (2) its mode of heredity with either a continuous or a bimodal distribution between resistant and susceptible phenotypes in a progeny (Niks et al. 2015). Plant resistance is generally classified in two extreme categories: qualitative resistance conditioned by a single gene (i.e. major gene) and quantitative resistance conditioned by multiple genes of partial effect (i.e. Quantitative Trait Locus = QTL), with possibilities of intermediate types of resistances between these two categories (Poland et al. 2009; Stuthman et al. 2007). Genetically homogeneous crops with qualitative resistance induce strong directional selection on pathogen populations when they are deployed at a large scale and can result in resistance breakdown (Bus et al. 2011; Peressotti et al. 2010; Rouxel et al. 2003). Quantitative resistance has been frequently considered to be more durable than qualitative resistance (Lindhout 2002; Parlevliet 2002; Stuthman et al. 2007). However, it was postulated that quantitative resistance might also exert selection on pathogen populations (Gandon and Michalakis 2000; Parlevliet and Zadoks 1977). Indeed, it was shown experimentally in several pathosystems that quantitative resistance can also be eroded, for instance for *Mycosphaerella graminicola* on wheat (Cowger and Mundt 2002), lettuce mosaic virus on lettuce (Pink et al. 1992), *Phytophthora infestans* on potato (Andrivon et al. 2007; Montarry et al. 2008), *Plasmopara viticola* on grape (Delmas et al. 2016; Delmotte et al. 2014) and *Venturia inaequalis* on apple (Caffier et al. 2014; Caffier et al. 2016). As a consequence, both types of resistance may be eroded or overcome more or less rapidly according to the evolutionary potential of the pathogen, the host characteristics and the agronomic practices. It is therefore necessary to develop strategies of resistance gene management that can improve the durability of resistance (Fabre et al. 2012; Mundt 2014; Sapoukhina et al. 2009; Sapoukhina et al. 2013).

Pyramiding genes of resistance in one cultivar is one of these strategies and is now feasible by marker-assisted breeding (Pilet-Nayel et al. 2017; Pink 2002). Several studies have demonstrated that combining resistance factors increases resistance effectiveness, either by cumulating major genes (Hittalmani et al. 2000; Tan et al. 2010; Koide et al. 2010), QTL (Castro et al. 2003; Hamon et al. 2010; Laloi et al. 2017; Richardson et al. 2006) or major genes and QTL (Eibach et al. 2007; Tabien et al. 2002). It has been experimentally observed that combinations of major genes and QTL can increase both resistance effectiveness and durability, for instance for *Leptosphaeria maculans* on rapeseed (Delourme et al. 2014), *Microcylus ulei* on rubber (Le Guen et al. 2011), *Globodera pallida* on potato (Fournet et al. 2013), *Meloidogyne* species on pepper (Barbary et al. 2014) and Potato
virus Y on pepper (Palloix et al. 2009; Quenouille et al. 2014). Indeed, major genes are totally
effective to control the avirulent fraction of the pathogen population, whereas QTL reduce the
pathogen population size, which limits the selection for virulent isolates and maintains a partial
effectiveness of the resistance towards the virulent isolates (Delourme et al. 2014). Theoretical
studies indicate that durability of cultivars pyramiding resistances will depend on the way that the
resistance genes are employed (Bourget et al. 2013). If each resistance gene is used separately,
concomitantly or before the use of cultivars pyramiding resistances, then the pathogen will be able
to overcome the resistance by incrementally stacking virulences (Ayliffe et al. 2008; Pink 2002;
Stuthman 2002) either by independent successive gain of virulence in the same strain or by sexual
recombination between mono-virulent strains. It should be noted that the durability of resistance
pyramiding may depend on the genetic structure of the pathogen populations and ability of
pathogen populations carrying single mutations to recombine, but this point has not yet been
explored experimentally.

Here we investigated the effectiveness and durability of combining one major gene with two
QTL in the apple scab pathosystem. Apple scab, caused by the fungus Venturia inaequalis, is one of
the most important diseases in commercial orchards in all temperate countries, because it strongly
reduces fruit quality which is vital for fresh market sales. Disease control today generally depends on
the use of fungicides, spraying up to 20 treatments per year (Sauphanor et al. 2009). The perennial
trait of apple necessitates genotypes that can maintain resistance over many years. Major resistance
genes have been identified in wild or domesticated apple species (Bus et al. 2011; Gessler et al. 2006;
Soufflet-Freslon et al. 2008). The Rvi6 (= Vf) major resistance gene has been widely used in apple
breeding programs during the last 50-60 years (Laurens 1999), but this monogenic resistance has
been overcome by the pathogen (Bus et al. 2011; Lemaire et al. 2016). Many QTL, including specific
and broad-spectrum QTL, have also been identified (Calenge et al. 2004; Durel et al. 2003; Lê Van et
al. 2013; Liebhard et al. 2003a; Soufflet-Freslon et al. 2008). The combination of two broad-spectrum
resistance QTL, F11 and F17, has already been shown to be eroded in experimental trials (Caffier et
al. 2014). Thus, our aim was to evaluate the utility of pyramiding Rvi6 with QTL F11 and F17, in two
unsprayed orchards where resistance effectiveness of these QTL and this major gene has already
been challenged.

Materials and Methods

Plant material. A cross between cv. ‘Prima’ and cv. ‘Fiesta’ was carried out by the former
Cupro-DLO, which is now part of Wageningen-UR Plant Breeding, The Netherlands (Maliepaard et al.
1998). These progeny (referred to as the “J progeny”) segregate for two major genes, Rvi1 (= Vg) and
Rvi6 (= Vf), and two broad-spectrum QTL, since cv. ‘Prima’ carries Rvi1 and Rvi6 (Durel et al. 2000; Maliepaard et al. 1998) and cv. ‘Fiesta’ carries two QTL on linkage groups 11 and 17 (referred to as F11 and F17 in the present study) (Durel et al. 2004; Durel et al. 2003; Liebhard et al. 2003b). Using available molecular markers for the initial and curated marker positions published in Maliepaard et al. (1998) and Schouten et al. (2012) respectively, we selected 22 apple genotypes that carried either no favourable allele for QTL or for Rvi6 (Class-0), two favorable alleles for both QTL F11 and F17 only (Class-QTL), favourable allele for Rvi6 only (Class-R), and favorable alleles for F11, F17 and Rvi6 (Class-RQTL), from the 161 available progenies. As Rvi1 is largely overcome in V. inaequalis populations, we considered that this major gene had no effect, and that the classes with Rvi1 and Rvi6 represented the effect of Rvi6. The number of genotypes per combination varied from five to seven (Table 1).

**Orchard network.** During winter 2003-2004, each genotype was planted in two experimental orchards located more than 300 km apart, representing two different environments and climates (Angers in north-western France, and Villeneuve d’Ascq in the north of France, later referred to as “Villeneuve”). In each orchard, three replicates per genotype were planted in a randomised three-block design after grafting onto the apple rootstock, ‘Pajam 2’. The distance between the trees on a row was 1.5 m in Villeneuve and 1 m in Angers; the distance between the rows was 4 m. Pesticides were applied when necessary, but no fungicide treatment was applied against scab.

**Disease assessment.** Scab incidence was scored on each tree in June after the period of primary infection, in 2006-2008 at both sites. In Villeneuve, data from 2007 could not be interpreted because of a very low level of disease on the susceptible trees. In Angers, where the orchard was maintained after the end of the project, an additional assessment was carried out in 2012. On each tree, disease incidence was scored using an ordinal scale adapted from Lateur and Populer (1994). This scale consists of nine levels, described as intervals around the following values: 0%, 1%, 3%, 12.5%, 25%, 37.5%, 50%, 75% and 90% of scabbed leaves.

**Statistical analysis.** All statistical analyses were performed on the variable “disease incidence”. Within each site and each year, the four classes of genotypes were compared for their disease incidence using a linear mixed effect model LME (Pinheiro and Bates 2000). The ‘genotype’ was treated as a random factor nested in the class of genotypes, and ‘block’ as a fixed factor. The model was fitted by maximizing the log-likelihood and the best model was selected using the Bayesian Information Criterion after including each factor (‘class of genotypes’ and ‘block’) through an ascendant selection (Pinheiro and Bates 2000). The data were transformed into arcsine square root to normalise and homogenise the residuals. The resistance of Class-R, Class-QTL or Class-RQTL was considered as effective if “disease incidence” was significantly different from “disease incidence”
of Class-0. All statistical analyses were performed with R software, version 3.1.2 (R Core Team 2016),
using the ‘nlme’ package (Pinheiro et al. 2016).

Results

In both sites, the level of disease on the control (Class-0) was high with fluctuations from year
to year ranging from 51% to 83% scabbed leaves. In Angers, the resistance based on \( Rvi6 \) (Class-R)
was completely effective in 2006 with no scabbed leaves (fig. 1a). This resistance was still effective in
2007 but a higher level of disease was observed (22% scabbed leaves). This resistance became
ineffective by 2008 (67% scabbed leaves). The QTL combination F11F17 (Class-QTL) significantly
reduced the disease each year compared to Class-0, even if the level of control decreased slowly over
time (not shown in the present study, see Caffier et al. 2014). In 2006, the pyramiding of \( Rvi6 \) and
F11F17 (Class-RQTL) was completely effective with no scabbed leaves, due to the effectiveness of
\( Rvi6 \). In 2007, this pyramiding was significantly more effective (6% scabbed leaves) than the
resistance factors \( Rvi6 \) (Class-R with 22% scabbed leaves) or F11F17 (Class-QTL with 55% scabbed
leaves) alone. In 2008 and 2012, the resistance of Class-RQTL was still effective with 20% and 64%
scabbed leaves respectively, compared to 75 and 83% for Class-0. However, the added value of \( Rvi6 \)
was lost from 2008, while the effect of F11F17 remained with no significant difference between
Class-RQTL and Class-QTL in 2008 and in 2012. By contrast, in Villeneuve, both the major resistance
\( Rvi6 \) (Class-R) and the QTL combination F11F77 (Class-QTL) were ineffective from the beginning of
the experiment with more than 40% scabbed leaves. However, the apple genotypes carrying the
pyramiding of \( Rvi6 \) and F11F17 (Class-RQTL) were significantly less diseased than Class-0 with less
than 20% scabbed leaves for the two observed years 2006 and 2008 (fig. 1b).

Discussion

We evaluated the effectiveness of pyramiding quantitative resistance with a major resistance
gene in two contrasting situations: one site (Angers) where the resistance factors alone were
progressively overcome during the experiment trial and one site (Villeneuve) where the resistance
factors alone were already overcome when the experiment started (Caffier et al. 2014). Our results
showed the effectiveness of pyramiding resistances for controlling scab in both sites, but to a
different extent depending on the site. In Angers, the added value of pyramiding quantitative
resistance with a major gene to control scab was ephemeral and disappeared when the level of
disease control based on resistance factors alone decreased, whereas in Villeneuve, the pyramiding
(Class-RQTL) remained effective both in 2006 and 2008, even if the resistance factors alone where
not effective compared to Class-0.
These results agree with other studies suggesting pyramiding QTL with major resistance genes improves effectiveness and increases durability of resistance (Delourme et al. 2014; Le Guen et al. 2011; Quenouille et al. 2014). The effectiveness of pyramiding may be due to the low probability of a pathogen strain to accumulate independent mutations necessary to overcome all resistance genes in the pyramid (Mundt 2014), the reduction in the pathogen size population or the diversification in the selection pressures exerted on the pathogen population (Pilet-Nayel et al. 2017). It is surprising however that the added value of pyramiding was maintained in Villeneuve whereas it was lost in Angers. Indeed, at the Villeneuve site, the effectiveness of pyramiding the Rvi6 gene with QTL was observed although apple genotypes with single non-effective resistance factors (Class-R or Class-QTL) were planted in the same orchard, which putatively facilitates stacking pathogenicity loci by mating. In such a situation, we could have expected disease control associated with pyramiding to decrease or be completely lost over time (Ayliffe et al. 2008; Pink 2002; Stuthman 2002), which was not observed. We assume that the effectiveness of resistance observed at this site was probably due to a lack of recombination between pathogen populations growing on class-R and populations growing on class-QTL. Indeed, a strong genetic differentiation was observed in the northwest of France between populations virulent towards Rvi6 and populations avirulent towards Rvi6 (Gladieux et al. 2011). At the Angers site, the improved resistance associated with pyramiding instead of single resistance factors was also observed in 2007 at the beginning of Rvi6 breakdown, but was lost from 2008, with the level of disease control associated with pyramiding QTL and the major gene (Class-RQTL) becoming similar to the level of disease control observed with the QTL (Class-QTL) alone. At this site, Leroy et al. (2016) showed a low genetic differentiation between populations virulent towards Rvi6 and populations avirulent towards Rvi6 due to recombination between the two populations. Such a recombination may have resulted in the development of strains that were well adapted to all resistance factors incorporated in the pyramiding. Our results suggest that the effectiveness of pyramiding resistance genes not only depends on the way the resistance genes are used (Ayliffe et al. 2008; Bourget et al. 2013; Pink 2002; Stuthman 2002) but also on the capacity of pathogen populations carrying virulence and aggressiveness traits to recombine. We assume that the more gene flow that occurs between populations able to overcome different resistances the more quickly the resistance of the pyramiding will be eroded.

Even in case of ephemeral effectiveness, these results are promising for the development of environmentally friendly strategies to control apple scab and to reduce chemical treatments. Indeed, in a previous study, Didelot et al. (2016) showed that the breakdown of the major resistance Rvi6 by virulent isolates could be delayed by the removal of leaf litter in autumn associated with spraying of fungicides in case of moderate or high risks of scab infection. We can expect that the association of all these control methods, i.e. pyramiding of quantitative resistance with major resistance genes,

Valérie Caffier
Plant Disease
sanitation and chemical treatment applied only in case of great risk of scab infection, will provide a sustainable management of disease in a low-fungicide input system, which is crucial for a perennial plant.

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Valérie Caffier


Valérie Caffier

*Plant Disease*


Figure Legend

Figure 1. Bar plots of apple scab incidence in two French orchards situated in: 1) Angers and 2) Villeneuve d'Ascq, for four classes of apple genotypes of the cv. ‘Prima’ x cv. ‘Fiesta’ progeny: Class-0 with no resistance factor, Class-R carrying the major resistance gene Rvi6, Class-QTL carrying the resistance QTL F11 and F17 and Class-RQTL carrying the pyramiding of Rvi6 with F11 and F17. Different letters indicate significant differences between disease incidence of the four classes for each year and each site with linear mixed-effect modelling (p<0.05).
Table 1. Description of the apple genotypes of cv. ‘Prima’ x cv. ‘Fiesta’ progeny used in the present study.

<table>
<thead>
<tr>
<th>Resistance factors</th>
<th>Class-0</th>
<th>Class-R</th>
<th>Class-QTL</th>
<th>Class-RQTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rvi6</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>F11</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>F17</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>J25</td>
<td>J3</td>
<td>J28</td>
<td>J46</td>
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<td>J32</td>
<td>J43</td>
<td>J63</td>
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<td>J51</td>
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<td>J80</td>
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<td>J150</td>
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</tr>
</tbody>
</table>

\( a \) Absence of the resistance factor

\( b \) Presence of the resistance factor

\( c \) Apple progenies of the cross between cv. ‘Prima’ and cv. ‘Fiesta’