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Research paper

Slow erosion of a quantitative apple resistance to *Venturia inaequalis* based on an isolate-specific Quantitative Trait Locus

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ABSTRACT

Quantitative plant resistance affects the aggressiveness of pathogens and is usually considered more durable than qualitative resistance. However, the efficiency of a quantitative resistance based on an isolate-specific Quantitative Trait Locus (QTL) is expected to decrease over time due to the selection of isolates with a high level of aggressiveness on resistant plants. To test this hypothesis, we surveyed scab incidence over an eight-year period in an orchard planted with susceptible and quantitatively resistant apple genotypes. We sampled 79 *Venturia inaequalis* isolates from this orchard at three dates and we tested their level of aggressiveness under controlled conditions. Isolates sampled on resistant genotypes triggered higher lesion density and exhibited a higher sporulation rate on apple carrying the resistance allele of the QTL T1 compared to isolates sampled on susceptible genotypes. Due to this ability to select aggressive isolates, we expected the QTL T1 to be non-durable. However, our results showed that the quantitative resistance based on the QTL T1 remained efficient in orchard over an eight-year period, with only a slow decrease in efficiency and no detectable increase of the aggressiveness of fungal isolates over time. We conclude that knowledge on the specificity of a QTL is not sufficient to evaluate its durability. Deciphering molecular mechanisms associated with resistance QTLs, genetic determinants of aggressiveness and putative trade-offs within pathogen populations is needed to help in understanding the erosion processes.

1. Introduction

Plant genetic resistance to pathogens offers an interesting alternative to methods of disease control based on the use of pesticides. However, it needs to gain in durability since plant resistance is confronted with the evolution of pathogen populations. Pathogen populations are able to adapt to their host, which can result in the breakdown of the resistance.

Two extreme categories of resistance are generally recognized: qualitative resistance, which affects the qualitative component of pathogenicity (i.e. the ability of a pathogen to infect a plant), and quantitative resistance, which affects the quantitative traits of pathogenicity (for instance: rate of infection, latent period and rate of sporulation). In the present paper, we will use the term virulence for the ability of a pathogen to infect a plant and the term aggressiveness for the quantitative traits of pathogenicity (Lannou, 2012). Quantitative resistance induces a partial reduction of pathogen development, and is frequently considered to be more durable than qualitative resistance (Parlevliet, 2002), but few experimental data are available (St Clair, 2010). The expected durability of quantitative resistance can be explained by different and not exclusive hypotheses. First, the quantitative resistance may exert a low selection pressure on the pathogen populations. The isolates that are selected by the resistance have a low advantage compared to the other isolates and increase slowly in frequency (Lindhout, 2002; Poland et al., 2009). Second, the durability of quantitative resistance may be related to the number of quantitative trait loci (QTLs) involved (Parlevliet, 2002) and to the mechanisms that underlie quantitative resistance, like basal defence, chemical warfare, defence signal transduction or weaker forms of major resistance genes (Poland et al., 2009). An increase in aggressiveness may be rendered difficult if the different loci involved in a quantitative resistance act on different components of aggressiveness (Azzimonti et al., 2014). The selection of more fit isolates may be progressive with a step-by-step increase in aggressiveness toward each specific locus (Geffroy et al., 2000), as modelled by Bourget et al. (2015). Third, the durability could be based on the broad spectrum

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of the resistance, i.e. efficiency towards all the isolates of a pathogen species (Parlevliet, 2002). However, quantitative resistance can involve either broad-spectrum loci or isolate specific loci, as shown for instance in the resistance of *Malus* × *domestica* to *Venturia inaequalis* (Calenge et al., 2004), and in the resistance of *Medicago truncatula* to *Aphanomyces euteiches* (Hamon et al., 2010). A quantitative resistance can also be broad-spectrum because of many isolate specific loci (Talukder et al., 2004; Lapin et al., 2012). Specific interactions may render quantitative resistances non-durable in a same manner than major genes following gene-for-gene relationships (Flor, 1971). In this case, quantitative resistances are likewise called minor-gene for minor-gene relationships (Parlevliet and Zadoks, 1977). However, the consequences of specific interactions on the durability of quantitative resistance are still not well documented (Azzimonti et al., 2013; Mundt, 2014).

In agricultural systems, erosion of quantitative resistance can occur, as observed for wheat leaf blotch (Mundt et al., 2002). Quantitative resistant cultivars can select for more aggressive isolates, as observed for *Mycosphaerella graminicola* on wheat (Cowger and Mundt, 2002), *Phytophthora infestans* on potato (Flier et al., 2003; Andrivon et al., 2007; Montarry et al., 2008) and grapevine downy mildew (Delmotte et al., 2014; Delmas et al., 2016). However, the relationship between selection of aggressive isolates and erosion has rarely been demonstrated (Bjor and Mulelid, 1991; Caffier et al., 2014) since the strong effect of environmental conditions on both the levels of quantitative resistance and levels of pathogen aggressiveness makes the detection of a gradual erosion of resistance extremely difficult (Young, 1996; McDonald and Linde, 2002; Pariaud et al., 2009; Lannou, 2012).

In apple, many resistance QTLs to *Venturia inaequalis* (fungal causal agent of scab) have been described, either broad-spectrum or isolate-specific (Durel et al., 2003; Liebhard et al., 2003; Calenge et al., 2004; Soufflet-Freslon et al., 2008). *V. inaequalis* has a great evolutionary potential (Gladieux et al., 2008; Bus et al., 2011; Lê Van et al., 2012; Leroy et al., 2016). In a previous study, we showed that a quantitative resistance based on the combination of two broad-spectrum QTLs (QTLs F11 and F17) decreased over time through the selection of isolates that were aggressive on both resistant and susceptible apple genotypes (Caffier et al., 2014). In the present study, our aim was to test the hypothesis that a quantitative resistance based on a specific QTL may be eroded through the selection of isolates that are aggressive on the resistant apple genotypes carrying this QTL. For this purpose, we investigated the efficiency over time of the specific quantitative resistance QTL T1 to scab. This QTL is isolate specific since it was detected in an apple progeny after inoculation with five out of eight tested isolates of *V. inaequalis* (Calenge et al., 2004) and induced a modification in the frequency of the isolates able to sporulate on infected leaves 28 days after inoculation of the isolates together in mixture (Lê Van et al., 2013). On the apple genetic map, the QTL T1 co-localizes with the major resistance gene *Rvi6* (Calenge et al., 2004), which is overcome by virulent isolates (Lemaire et al., 2016). The fact that this QTL had weak or no effect on three isolates able to overcome the major resistance gene *Rvi6* (Calenge et al., 2004) raises the question of a relationship between virulence towards *Rvi6* and aggressiveness towards the QTL T1. In the present study, we defined four questions: (1) Does the efficiency of the resistance QTL T1 evolve over time in orchard?, (2) Does the QTL T1 select for aggressive isolates of *V. inaequalis* and is there a link between virulence towards *Rvi6* and aggressiveness on the QTL T1? (3) Which components of aggressiveness of *V. inaequalis* does the QTL T1 act on? (4) Does the level of aggressiveness or frequency of aggressive isolates evolve over time? We surveyed scab incidence over an 8-year period in an orchard planted with apple genotypes carrying or not the QTL T1. We sampled *V. inaequalis* isolates on these different apple genotypes at three dates and we tested their level of aggressiveness on susceptible and resistant apple genotypes under controlled conditions by scoring five components of aggressiveness: disease severity, density of lesions, level of sporulation, size of lesions and latent period.

2. Materials and methods

2.1. Orchard

We used progenies of the cross between the apple cvs. TN10-8 and Prima (referred to as the "E progeny"). This progeny segregates for the two major genes, *Rvi1* (= *Vg*) and *Rvi6* (= *Vf*), present in cv. Prima at the heterozygous state (Maliepaard et al., 1998; Durel et al., 2000) and for one specific QTL on linkage group 1 (referred to as T1 in the present study) detected in TN10–8 (Calenge et al., 2004; Lê Van et al., 2013). Using available molecular markers, we selected 57 apple genotypes that did not carry the resistance allele for QTL T1 (Class0) and 50 apple genotypes that carried the resistance allele for QTL T1 (ClassT1). None of the selected genotypes carried the major resistance gene *Rvi6*. Some of the selected genotypes carried the major resistance genes *Rvi1*, but this had no impact on the present study since all isolates of *V. inaequalis* were virulent to *Rvi1* (see below). For the sake of simplicity, "presence/absence of the resistance allele of a given QTL" will later be indicated by "presence/absence of the QTL".

Each genotype (one tree per genotype) was planted "on its own roots" in an orchard in Angers in north-western France (with 4 m between the rows and a tree-to-tree distance of 1 m). No fungicide treatment was applied to control scab in order to allow the presence of the natural inoculum of *V. inaequalis*.

Scab incidence was scored on leaves on each tree in June or July at the end of the primary infection period for six years between 2007 and 2015, except in 2007 where the scoring was done in September. The scoring scale was an ordinal scale, with nine levels of disease incidence, described as intervals around the following values of percentage of scabbed leaves: 0%, 1%, 3%, 12.5%, 25%, 37.5%, 50%, 75% and 90% (Caffier et al., 2014). Two annotators, one standing on each side of the tree, agreed on a common score for each tree.

2.2. Pathogenicity tests

In 2008, 2009 and 2012, scabbed leaves were sampled in June on 9 to 20 different apple genotypes of ClassT1 and on 10 to 19 different apple genotypes of Class0 (Table 1). Monoconidial isolates were obtained from scabbed leaves and multiplied on cellophane sheets overlaid on malt agar in Petri dishes during 7–10 days of incubation at 17 °C with 16 h of light (Caffier et al., 2010). The cellophane sheets were dried and frozen until use. For each isolate, conidial suspensions were obtained from the cellophane sheets, and were inoculated with manual sprayers until run-off on apple trees grafted on MM106. The inoculated plants (one shoot per tree) were incubated in controlled conditions as described in Caffier et al. (2010).

2.2.1. Tests for virulence

To test the relationship between the virulence towards *Rvi6* and the aggressiveness on T1, we first characterized the isolates for their virulence/avirulence towards *Rvi6* on cv. Prima (*Rvi1* + *Rvi6*) with cv. Gala (scab susceptible) and cv. Golden Delicious (*Rvi1*) as control cultivars. Three plants of each cultivar were inoculated by depositing 2 to 4 droplets of 10 µl of conidial suspension (1.5×10^5 spores ml^{-1}) on each of the two youngest unfurled leaves (named L0 and L1) at the tip of the shoots. The resistance symptoms were characterized 14 and 21 days after inoculation as pinpoints, chlorosis or necrosis. An isolate was considered to be avirulent if it induced resistance symptoms (incompatible interaction) and virulent if it induced sporulation (compatible interaction). All isolates were virulent on cv. Golden Delicious (*Rvi1*). One of the tested isolates could not be classified as virulent or avirulent towards *Rvi6*, because it induced both sporulation and resistance symptoms on cv. Prima.