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Camilla Langlands-Perry

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Could the issue of "quantitative vs. qualitative" in plant-pathogen interactions be a false dilemma ? The wheat-*Zymoseptoria tritici* case study

Quantitatif versus qualitatif dans le cadre des interactions plante-pathogène, est-ce un faux dilemme? Le cas de l'interaction blé-Zymoseptoria tritici

Thèse de doctorat de l'université Paris-Saclay

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Titre : Quantitatif versus qualitatif dans le cadre des interactions plante-pathogène, est-ce un faux dilemme ? Le cas de l'interaction blé-*Zymoseptoria tritici*.

Mots clés : résistance partielle, pouvoir pathogène, quantitative trait locus (QTL), interaction gène-pour-gène

Résumé : Qualitatif et quantitatif sont deux notions qui s'opposent en phytopathologie depuis plus de 60 ans. La résistance qualitative se rapporte à une distribution discrète des données phénotypiques en classes distinctes, résistant ou sensible. Cette distribution s'explique par les interactions gène-pour-gène entre les gènes de résistance *R* côté hôte et les gènes d'avirulence *Avr* côté agent pathogène. La résistance quantitative, en revanche, parfois appelée résistance basale ou horizontale, est caractérisée par une distribution continue des phénotypes expliquée par une combinaison de loci à effet quantitatif (QTL) à effet plus ou moins fort et dont les gènes et mécanismes sous-jacents sont généralement inconnus. Depuis une vingtaine d'années, de plus en plus d'exemples semblent contredire la théorie qui prône une stricte dichotomie entre ces deux formes de résistance. L'objectif de cette thèse a été d'étudier les mécanismes sous-jacents aux interactions quantitatives avec comme finalité potentielle de mettre en évidence un besoin d'adapter les définitions conventionnelles aux dernières connaissances acquises. Nous avons choisi de travailler avec le pathosystème modèle *Zymoseptoria tritici*-blé tendre car les interactions entre l'hôte et l'agent pathogène sont considérées comme étant principalement quantitatives.

Le chapitre 2 concerne la caractérisation de l'architecture génétique de la résistance du cultivar de blé 'Renan' envers deux souches fongiques I05 et I07, démontrant qu'elle est effectivement quantitative, basée sur l'action combinée de trois QTL de résistance. Le chapitre 3 se focalise sur l'identification des sources de pathogénicité envers 'Renan' dans les souches fongiques I05 et I07. Trois QTL de pathogénicité ont été détectés dans ces souches. Pour deux de ces QTL, un gène candidat a été identifié puis cloné. L'un des clonages a démontré l'implication d'un gène codant pour une petite protéine sécrétée, typique des effecteurs, dans la pathogénie. Enfin, le chapitre 4 vise quant à lui à identifier des interactions QTL-pour-QTL expliquant des phénotypes quantitatifs. Nous avons été en mesure d'identifier des interactions QTL-pour-QTL et également une interaction gène-pour-gène dans ce pathosystème, démontrant que, malgré les définitions usuelles, quantitatif n'est pas nécessairement synonyme de large spectre et aspécifique, mais peut également englober des interactions gène-pour-gène spécifiques impliquant des effecteurs. Ces résultats mettent en lumière la nécessité de travailler les définitions actuelles de ces notions et de discuter la durabilité présumée de la résistance quantitative.

Title : Could the issue of "quantitative vs. qualitative" in plant-pathogen interactions be a false dilemma ? The wheat-*Zymoseptoria tritici* case study.

Keywords : partial resistance, pathogenicity, quantitative trait locus (QTL), gene-for-gene interaction

Abstract : Qualitative and quantitative notions of disease resistance have been opposed for the last 60 years. Qualitative resistance relates to a discrete distribution of phenotypic data into distinct resistant or non-resistant classes, distribution explained by gene-for-gene interactions between resistance *R* genes host-side and avirulence *Avr* genes pathogen-side. Quantitative resistance, on the other hand, sometimes referred to as basal, horizontal or even field resistance, relates to a continuous distribution of phenotypes explained by a combination of quantitative trait loci (QTL) with varying effects and with generally unknown underlying genes and mechanisms. Over the last 20 years or so, more and more examples have cropped up which seem to contradict the strict theoretical dichotomy. The objective of this thesis was to investigate the mechanisms underlying a quantitative system with the potential of highlighting a need to adapt conventional definitions to new findings. We worked with the model pathosystem *Zymoseptoria tritici*-wheat as interactions between the host and pathogen are thought to be mostly quantitative.

Chapter 2 focuses on characterizing the genetic architecture of resistance in the wheat cultivar 'Renan' towards two fungal strains I05 and I07, demonstrating that it is indeed quantitative, based on the combined action of three resistance QTL. Chapter 3 focuses on identifying the sources of pathogenicity towards 'Renan' in the fungal strains I05 and I07, which were attributed to three pathogenicity QTL. For two of these QTL, candidate genes were cloned, and one of these was shown to be involved in pathogenicity. This validated candidate encodes a small secreted protein typical of effectors. Finally, in Chapter 4 we evidenced that QTL-for-QTL interactions, and gene-for-gene interactions, can underlie quantitative phenotypes in this pathosystem. This demonstrates that despite previous definitions, quantitative does not necessarily equate broad-spectrum and non-specific, it can also englobe specific gene-for-gene interactions involving effectors. We are able to confirm a need for an update in definitions and can also question the purported durability of quantitative resistance.

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LIST OF ABBREVIATIONS

APR	Adult plant resistance
ATMT	<i>Agrobacterium tumefaciens</i> mediated transformation
AUDPC	Area under the disease progress curve
<i>Avr</i>	Avirulence gene
DAMP	Damage associated molecular pattern
dpi	Days post inoculation
ET	Ethylene
ETD	Effector-triggered defence
ETI	Effector-triggered immunity
GWAS	Genome-wide association study
HR	Hypersensitive response
JA	Jasmonic acid
MAMP	Microbe-associated molecular pattern
NBS	Number of spores per pycnidium
NLR	Nucleotide-binding site leucine-rich repeat immunoreceptors
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
PRR	Pattern recognition receptor
PTI	PAMP-triggered immunity
PYC	Pycnidia density
qPCR	Quantitative PCR
QTL	Quantitative trait locus
<i>R</i>	Major resistance gene
RIL	Recombinant inbred line
RLK	Receptor-like kinase
RLP	Receptor-like protein
ROS	Reactive oxygen species
S20	Sporulating area at 20 dpi (%)
S26	Sporulating area at 26 dpi (%)
SA	Salicylic acid
SNP	Single nucleotide polymorphism
SSP	Small secreted protein
STB	Septoria leaf blotch or <i>Septoria tritici</i> blotch
TE	Transposable element
UTR	Untranslated region

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CHAPTER 1: GENERAL INTRODUCTION

I. WHY ARE WE INTERESTED IN PLANT PATHOGENIC FUNGI?

Crops are the target of a multitude of phytopathogenic organisms including fungi, bacteria, viruses, protists, and more. These organisms live off the damage they cause to plants and thus affect human activity, in particular when it comes to agriculture. Fungi and oomycetes are the leaders in terms of potential threat to global food security (Fisher et al., 2012), being responsible for 70 to 80% of all plant diseases (Zeilinger et al., 2016). Fungi cause yield losses in calorie crops such as rice, wheat, maize, cassava, potato and soybean, but also in commodity crops such as banana, coffee, tomatoes and barley (Fones et al., 2020; Steinberg and Gurr, 2020). With 7.9 billion people to feed, it is necessary to attempt to negate or at least control the effects of fungal diseases in crops and a better understanding of fungal biology and plant pathogenicity would highly contribute to these efforts (Almeida et al., 2019).

II. FUNGAL DISEASE CONTROL IN CROPS

A wide array of factors can contribute to disease outbreaks including climate change, pathogen variation or maladapted farming methods (Lv et al., 2020). In order to compensate, an equally wide array of possible solutions have been implemented over time. A non-exhaustive list of these solutions include quarantine regulations and seed testing for pathogen strains (McGee, 1995), early planting (Finckh et al., 2006; Reynolds, 2010), roguing, which is the practice of simply removing infected plants (Sisterson and Stenger, 2013), burning crop residues (Malhi and Kutcher, 2007) or reducing plant density (Farias et al., 2019; Finckh et al., 2006; Legard et al., 2000). Crop rotation (Bailey and Duczek, 1996; Fry, 2012; Gulley et al., 1884; Peters et al., 2003), fallowing (Curl, 1963) and intercropping (Reynolds, 2010) have also been shown to reduce disease impact, though not in all cases (Kincaid, 1960; Umaerus et al., 1989), and to improve soil quality (Li et al., 2021; Venter et al., 2016; Y., Wang et al., 2010). Soil quality overall contributes to reducing disease incidence (Larkin, 2015). Crop diversification, switching cultivars and cultivar mixtures are also means of reducing disease and disease spread (Borg et al., 2018; Gigot et al., 2013; Orellana-Torrejon et al., 2021; Reynolds, 2010; de Vallavieille-Pope, 2004; Vidal et al., 2017). Finally, nitrogen inputs can modulate plant defence responses, though the impact on the pathogen is highly variable (Sun et al., 2020). Nowadays however, in combination with agronomic practices just listed, fungicides are widely used to maximise yield despite the presence of pathogens (Oerke, 2006). These phytosanitary products have an array of modes of action and can target membrane components, amino acid and protein synthesis, signal transduction, respiration, cell division, nucleic acids synthesis or have multisite activity (Yang et al., 2011). Regardless of the mode of action, they all come with downsides. Some can have

adverse effects on non-target organisms such as microbial communities in soils (Komárek et al., 2010) or cause accumulation of heavy metals in soils and plants (Semu and Singh, 1995). Some create pollution with consequences on health (Fushiwaki et al., 1990) or hinder metabolic pathways in plants they are meant to shield (Petit et al., 2012). Pathogens have however shown ability to adapt to fungicides (Lucas et al., 2015), making this chemical warfare less effective and unsustainable. Moreover, fungicides represent a vast cost for farming. The annual global chemical-pesticide market, including a majority of fungicides, is associated with USD 40 billion (about 35 billion euros) in expenditures (Popp et al., 2013). Biocontrol of fungal diseases has gained interest in an increasingly green-minded world, but if solutions have been discovered for some pathosystems, for now these methods remain a minority (Carmona-Hernandez et al., 2019; Karise et al., 2016; Vos et al., 2014). Moreover, the introduction of a non-native antagonistic organism for biocontrol purposes presents with its own set of risks (Schulz et al., 2019). Crop protection focused on plant immunity then seems a promising alternative solution, wherein cultivars carrying genetic resistance to diseases are grown. Indeed, a cropping strategy involving a combination of the implementation of genetically resistant cultivars and other plant protection methods could greatly reduce the need for systematic fungicide or pesticide application. Genetic resistances can however be overcome by pathogens, particularly if they are not well deployed such as in many reported cases of overuse of a single strong effect resistance gene over a large cultivated area during a long period of time. The main stake for crop protection by use of genetically resistant cultivars is therefore durability, attainable by reducing selection pressure on pathogen populations.

III. MECHANISMS UNDERLYING DISEASES DUE TO FILAMENTOUS ORGANISMS IN CROPS

A. AN OVERVIEW OF PLANT PATHOGENIC FUNGI AND OOMYCETES

i. Diversity of plant pathogenic fungi and oomycetes

Fungi and oomycetes are filamentous organisms, relating to the filamentous structures or hyphae they are able to produce. They are uni- or multicellular eukaryotic heterotrophic organisms (de Wit, 2015). Fungi and oomycetes used to be considered very similar, oomycetes thought to be true fungi. However, through a multitude of genomic analyses, they now find themselves on opposite sides of the eukaryote phylogenetic tree. Their lineages separate even before the split between fungi, plants and animals (Keeling and Burki, 2019) (Figure 1). Fungi are opisthokonts and oomycetes are stramenopiles. Though they are phylogenetically distant, plant pathogenic fungi

and oomycetes have many common characteristics including filamentous growth and modes of plant infection (de Wit, 2015), one major difference is in cell wall composition, where fungal cell walls contain mostly chitin and glucan, oomycete cell walls contain cellulose rather than chitin (Raffaele and Kamoun, 2012; de Wit, 2015). The two major phyla of the fungal kingdom for plant pathogenic fungi are ascomycetes and basidiomycetes (Möller and Stukenbrock, 2017) with Ascomycota representing two thirds of all described fungal species (Naranjo-Ortiz and Gabaldón, 2019). The third largest group of plant pathogens corresponds to the oomycetes (de Wit, 2015). Of all the known oomycetes, 60% are pathogenic to plants (Raffaele and Kamoun, 2012). Estimates of the number of fungal and oomycete species able to cause disease on plants vary, some estimating it at around 8,000 (Hariharan and Prasannath, 2021), others a little higher at 10,000 (Zeilinger et al., 2016) and others at twice that at 20,000 (Ray et al., 2017). Some even suggest that these numbers could be grossly underestimated, due to an overwhelming fungal diversity as of yet undiscovered (Blackwell, 2011; Hawksworth and Lücking, 2017).

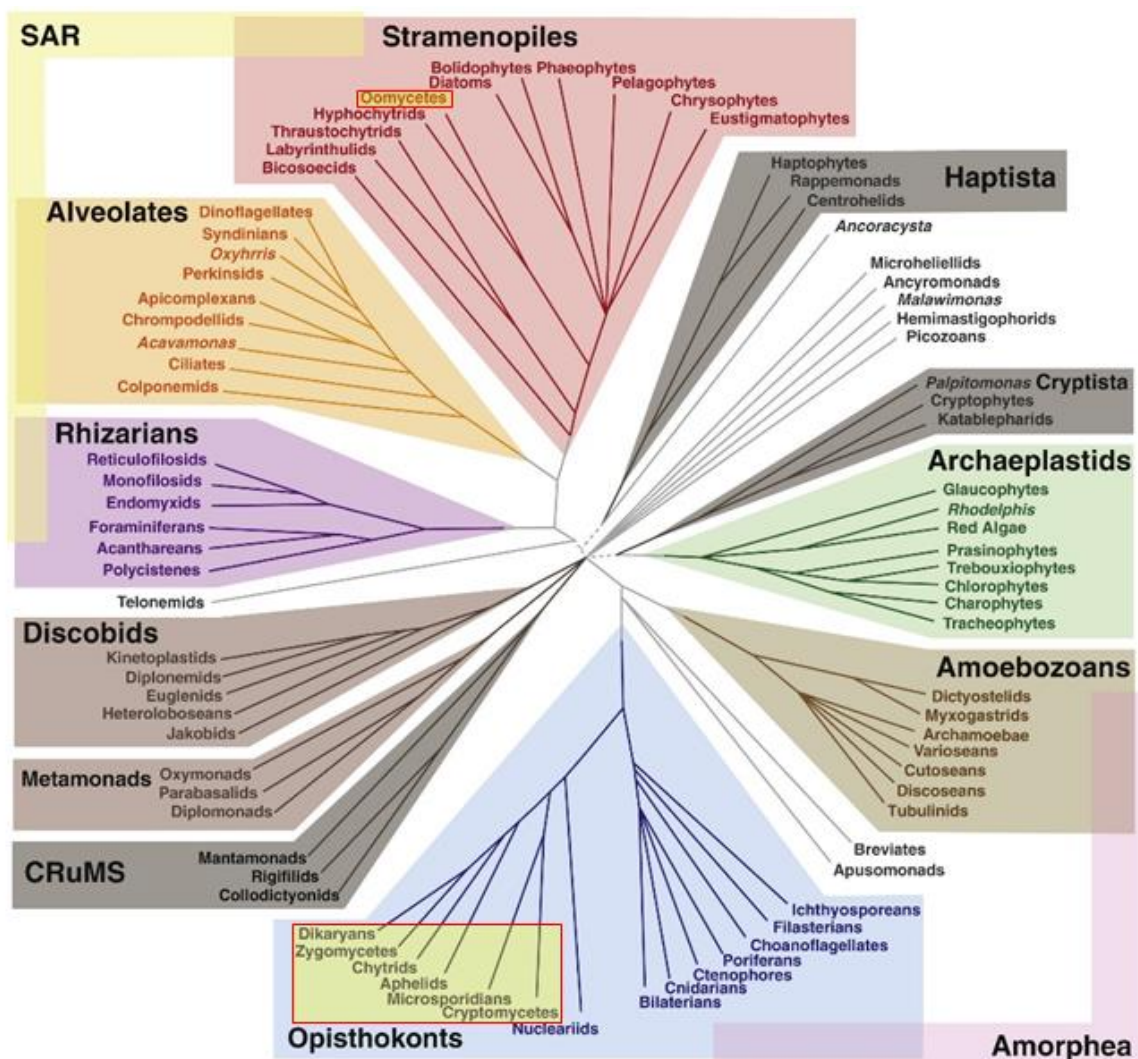


Figure 1: Phylogenetic tree of eukaryotes

Schematic tree of eukaryotes with undisputed supergroups represented in colour and disputed groups represented in grey. Boxed in red and highlighted in yellow are the oomycetes and all *Fungi* phyla. Adapted from Keeling and Burki (2019).

A 2012 survey-based study named the 'top ten' fungal pathogens with regards to scientific and economic importance (Dean et al., 2012). These are *Magnaporthe oryzae*, *Botrytis cinerea*, *Puccinia* spp., *Fusarium graminearum*, *Fusarium oxysporum*, *Blumeria graminis*, *Mycosphaerella graminicola* (now known as *Zymoseptoria tritici*), *Colletotrichum* spp., *Ustilago maydis* and *Melampsora lini*. Of these ten, seven target cereals while the three others have a very wide host range. *B. cinerea* can cause grey mould on more than 1400 known host species (Mercier et al., 2019). *Colletotrichum* spp. are known to target around 1350 different hosts (Talhinhas and Baroncelli, 2021). Finally, *F. oxysporum* is more modest, targeting more than 100 plant species (Husaini et al., 2018). For oomycetes, the most notorious members of the genus are the *Phytophthora* (de Wit, 2015), a name built from the Greek words φυτό (phyto), which means plant, and φθορά (phthora), which means destruction or perish, making *Phytophthora* aptly named plant killers. *Phytophthora infestans* was made famous for causing the Irish potato famine between 1845 and 1847 (Ristaino, 2002). Other destructive members of this genus are *P. palmivora* and *P. capsici* which have a broad host range including cacao, oil palms and vegetable crops (Bronkhorst et al., 2021). Filamentous organisms are therefore considered of high interest either when they target staple foods such as wheat, rice or potatoes, or when their highly diverse and large host range makes them a threat on many fronts.

ii. Life cycle of plant pathogenic filamentous organisms

For a pathogen to survive and spread, like any species, it must reproduce. The life cycle of any given plant pathogen therefore revolves around multiplication. This reproduction can be either sexual, leading to an increase in diversity through crossing-events during meiosis (Casselton and Zolan, 2002; Wittenberg et al., 2009) or asexual, leading to clonal populations, although mutations are possible. Life cycles therefore contain two parallel cycles (Figure 2). Sexual reproduction can occur on the host plant, examples are *U. maydis* (Wahl et al., 2010) and *P. infestans* (Yuen and Andersson, 2013), or on culture residues as for *Z. tritici* (Suffert et al., 2019) and *Leptosphaeria maculans* (Gout et al., 2006a) or on alternative hosts as for *P. triticina* (Bolton et al., 2008), and different spore types are involved. Not all pathogens include sexual reproduction on their life cycles, *F. oxysporum* has an asexual lifestyle for example (Wallen and Perlin, 2018). Additionally to the sexual and asexual modes of reproduction, a parasexual cycle

has been described in some organisms such as *Aspergillus nidulans* involving the fusion of haploid nuclei followed by mitosis and haploidization (Casselton and Zolan, 2002). Sexual and asexual spores alike can serve as primary inoculum for an epidemic.

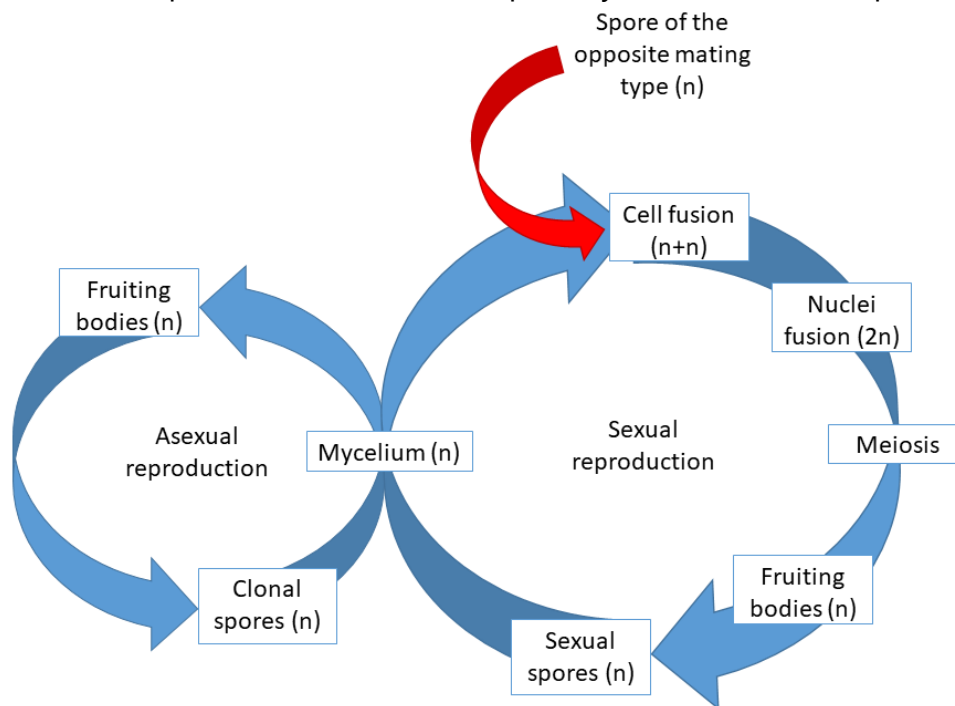


Figure 2: Simplified example of the life cycle of a heterothallic haploid filamentous fungal plant pathogen.

iii. Infectious process employed by plant pathogenic filamentous organisms

The types of spores involved in infection and reproduction for basidiomycetes, ascomycetes and oomycetes are not the same, however, for all, a very general and simplified infectious process can be drawn, divided into three main stages. The first stage corresponds to the germination of a spore on a host plant, and penetration of hyphae into the host tissues (Tucker and Talbot, 2001).

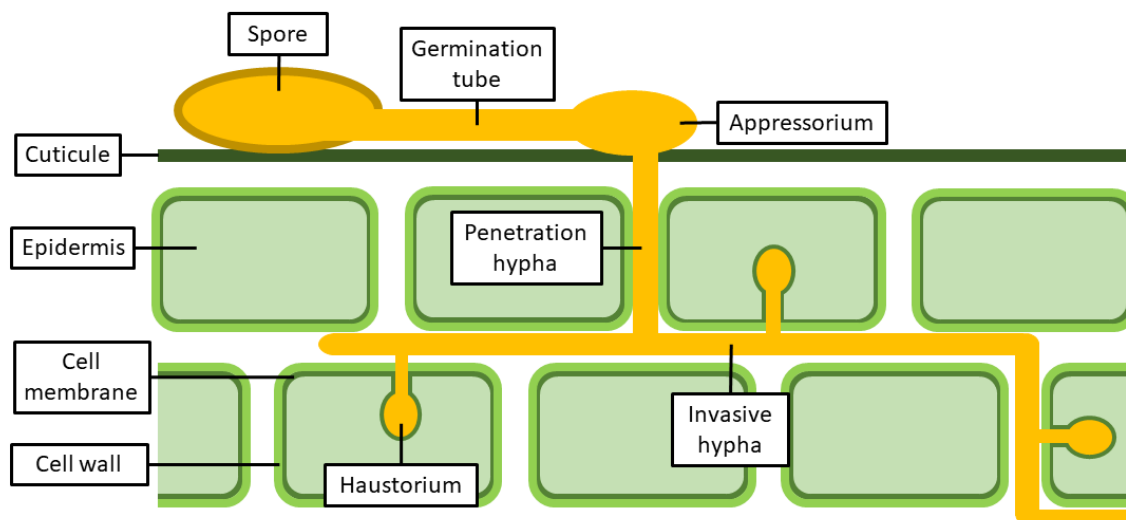


Figure 3: Examples of organs formed during the infection process in fungal and oomycete plant pathogens. Adapted from Meng et al. (2009) and Lo Presti et al. (2015).

Depending on the pathogen, additionally to hyphae, an appressorium will form, and if the pathogen penetrates beyond the host cell wall, haustoria will invaginate the host cell membrane (Lo Presti et al., 2015) (Figure 3). Different pathogens can target the host on different levels, while some are strictly foliar pathogens, such as *Z. tritici* (Steinberg, 2015) and *Ramularia collo-cygni* (Havis et al., 2015), others will attack the root system such as *F. oxysporum* (Husaini et al., 2018) or indeed other organs. *P. infestans* can initiate infection on the leaf surface, on fruits or on the root system indiscriminately (Boevink et al., 2020). Penetration can occur at natural openings in the leaf surface such as stomata, this is the case for *Z. tritici*, and *Puccinia triticina* for example (Fantozzi et al., 2021; Solanki et al., 2019a). It can also occur when the pathogen creates a wound through which to enter into the host tissue, this is the case for *P. infestans* (Bronkhorst et al., 2021), or by formation of a fortified appressorium from which a narrow penetration peg grows, forcing its way through the leaf surface into epidermal cells as is the case for *M. oryzae* (Nakao et al., 2011) and *Colletotrichum higginsianum* (Yan et al., 2018). The second stage corresponds to host colonization, where the pathogen invades the host tissues, generally resulting in visible disease symptoms highly variable between pathogens, for example necrosis and mycelium growth, as is the case for *B. cinerea* (Zhu et al., 2017), the formation of tumours as with *U. maydis* (Kahmann and Kämper, 2004), tuber blemish as with black dot caused by *Colletotrichum coccodes* (Lees and Hilton, 2003) or the formation of orange pustules surrounded by chlorotic halos as is the case with *P. triticina* (Panwar et al., 2018). This stage is generally associated with asexual reproduction, which can occur over several cycles. Finally in the third stage, for the pathogen to spread, spore dissemination must occur, this can be aided by wind, water or by passing animals (Hauelsen and

Stukenbrock, 2016; Sephton-Clark and Voelz, 2018).

iv. The different lifestyles of plant pathogenic filamentous organisms

Pathogen infection strategy largely depends of the lifestyle of the fungus or oomycete, how nutrient uptake is performed (Rodriguez-Moreno et al., 2018). Filamentous pathogens are heterotrophs, meaning they require organic substrate for nutrition and development. Biotrophic fungi such as rusts and powdery mildew, infect living plants and acquire nutrients from living tissue (Helfer, 2014; Hüchelhoven, 2005; Tang et al., 2018), often feeding off sugars taken up from living cells via intracellular hyphae or specialized feeding structures called haustoria (Haueisen and Stukenbrock, 2016) (Figure 4). Necrotrophs, such as *B. cinerea* or *Parastagonospora nodorum*, kill host cells upon or shortly after infection and feed off dead or dying tissue (Liu et al., 2016; Petrasch et al., 2019; Shao et al., 2021) (Figure 4) degrading polysaccharides such as hemicellulose and pectin (Haueisen and Stukenbrock, 2016). Hemibiotrophs like *P. infestans*, *Colletotrichum lindemuthianum* or *L. maculans*, combine both previous strategies. Initial infection is followed by an asymptomatic biotrophic phase linked to fungal growth and host colonization. Then a switch occurs towards the end of the infection cycle to a necrotrophic strategy, whereupon host-cell death occurs (O'Connell and Bailey, 1991; Mendgen and Hahn, 2002; Lee and Rose, 2010; Horbach et al., 2011; Becker et al., 2017) (Figure 4).

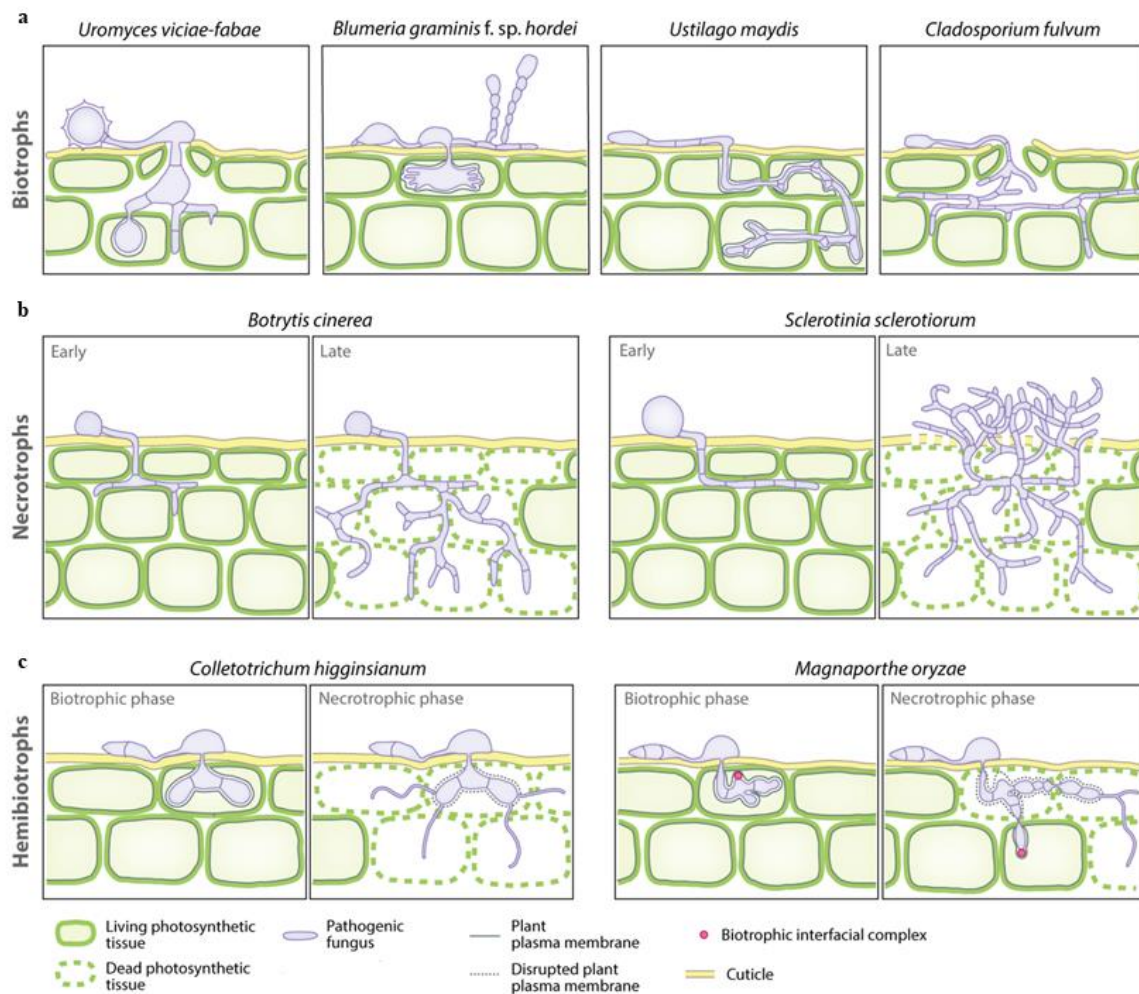


Figure 4: The different lifestyles of plant pathogenic fungi

a. Obligate biotrophs like *Uromyces viciae-fabae*, *Blumeria graminis* f. sp. *hordei* and *Ustilago maydis* develop in living host tissue in a non-destructive manner and form intracellular feeding organs such as haustoria. Another biotroph, but that is not obligate, is *Cladosporium fulvum* which is particular in that it does not invade the intracellular space, but feeds off apoplastic sugars (Thomma et al., 2005). **b.** Necrotrophs such as *Botrytis cinerea* and *Sclerotinia sclerotiorum* penetrate host tissues and shortly after degrade them provoking cell death, the fungus then feeds off dead or dying tissue. **c.** Hemibiotrophs like *Colletotrichum higginsianum* or *Magnaporthe oryzae* go through an initial biotrophic phase during which they colonize host tissues asymptotically, then follows a necrotrophic phase whereupon host cell death occurs. Though the two represented fungi invade host cells, apoplastic hemibiotrophs also exist in nature, such as *Zymoseptoria tritici*. Adapted from Lo Presti et al. (2015).

B. THE PLANT IMMUNE SYSTEM AND THE ZIG-ZAG MODEL

Plants cannot physically remove themselves from an outside attack, and unlike

mammals or fish for instance (Fischer et al., 2006), they do not possess a “sophisticated” adaptive immune system involving antigen receptors expressed by mobile specialized immune cells like leucocytes (Spoel and Dong, 2012). Instead, each plant cell is thought to be equipped with the means to retaliate against an unwelcome pathogen (Ausubel, 2005). This innate immune system can be divided into what has been described as two branches, tiers, even flavours, which serve to perceive and respond to pathogen infection (Boller and Felix, 2009; Han, 2019; Jones and Dangl, 2006). One branch enrolls transmembrane pattern recognition receptors (PRRs) which serve to recognize external elicitors, characteristic of a pathogenic attack known as MAMPS (microbe-associated molecular patterns), PAMPs (pathogen-associated molecular patterns) or DAMPs (damage-associated molecular patterns) (Haueisen and Stukenbrock, 2016). PRR stimulation leads to PAMP-triggered immunity (PTI). PTI is widely regarded as being a relatively non-specific and broad-spectrum line of defence involved in non-host resistance (incompatible interaction) and potentially quantitative resistance (compatible interaction) also (Martin et al., 2020). In the context of a compatible interaction, adapted pathogens are capable of circumventing PTI by producing molecules known as effectors, which can act on an intracellular or extracellular level. The molecular dialog that ensues leads to effector-triggered susceptibility (ETS) or effector-triggered immunity (ETI). ETI is associated with highly specific gene-for-gene interactions for which the groundwork was carried out by Flor who worked with the flax/flax rust pathosystem (Flor, 1956). Since this work, the notions surrounding gene-for-gene interactions have been studied in a plethora of pathosystems involving fungi and oomycetes (de Wit, 1995; Jia et al., 2000; Gout et al., 2006b; Stukenbrock and McDonald, 2009; Hall et al., 2009). The gene-for-gene interaction is between an *R* major resistance gene which recognizes the product of an *Avr* avirulence gene, often an effector, thus inducing a hypersensitive response (HR), a type of programmed cell death (Niks et al., 2019). The zig-zag model proposed by Jones and Dangl (2006) links PTI, ETS and ETI (Figure 5). Four phases constitute this model. In the first phase, PTI is activated by MAMPs, PAMPs or DAMPs and can stop colonization by a non-adapted pathogen. If the pathogen is adapted, the second phase takes place: pathogens deploy effectors, which can interfere with PTI resulting in ETS. If an *R*-gene product recognizes an *Avr* effector, ETI takes place, this is the third phase. In the fourth phase, through natural selection, pathogens evolve to adapt to ETI by loss or diversification of the recognized effector gene, or by generating novel effectors to suppress ETI. Natural selection will also influence the host, which will develop new *R* specificities to trigger ETI once more. The cyclical iterations of ETI and ETS represent the arms race caused by the constant coevolution of the host and pathogen.

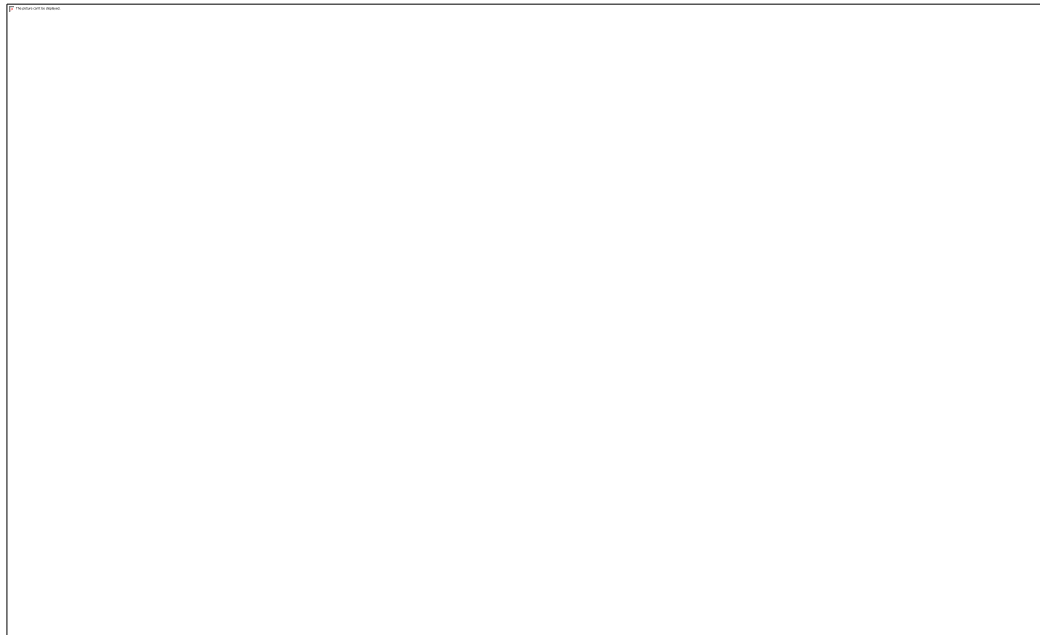


Figure 5: The zig-zag model of plant-pathogen interactions

The zig-zag model is divided into different phases. The first phase occurs when pathogen-associated molecular patterns (PAMPs) are recognized by the host triggering PAMP-triggered immunity (PTI). Pathogen effectors can modulate this form of innate immunity by bypassing PTI thus triggering effector-triggered susceptibility (ETS). Certain R resistance proteins are capable of recognizing corresponding effectors, which will then be referred to as avirulence factors (Avr) due to an *Avr-R* gene-for-gene interaction. This recognition induces resistance to the disease with effector-triggered immunity (ETI). A mutation through natural selection in an *Avr* gene can render the effector undetectable by the R protein, reinstating ETS. The alternation between ETS and ETI trace the “zig-zag” pattern which illustrates the coevolution of the plant and pathogen who are involved in a veritable arms race. Adapted from Jones and Dangl (2006).

C. MOLECULES INVOLVED IN PTI

PRRs are conserved molecules that can recognize PAMPs and MAMPs. PRRs are surface-localized receptor-like kinases (RLKs) or receptor-like proteins (RLPs), both types of protein contain an extracellular domain which is responsible for ligand binding (He and Wu, 2016), they can therefore exclusively recognize foreign molecules on an extracellular level. These extracellular domains can be LRR (leucine rich repeat), LysM (domain carrying lysine motifs), or GDPD (glycerophosphoryldiester phosphodiesterase) for example (Monaghan and Zipfel, 2012; Schwessinger and Ronald, 2012). Both RLKs and RLPs have a transmembrane domain rooting them to the

cell membrane but RLKs have a cytoplasmic kinase domain while RLPs simply have a short cytoplasmic tail (Lu and Tsuda, 2021). The nature of the ectodomain of RLKs and RLPs defines the PAMPs and MAMPs that they can recognize. PAMPs and MAMPs are known as general elicitors, they are highly conserved like PRRs and are widely distributed among microbial species. They carry out an essential function, such as being part of the cell wall for example, and are absent in the host species (Ingle et al., 2006). They can be fungal cell structure molecules such as glucans, chitins, cerebrosides or ergosterol, enzymes such as xylanase, which can degrade plant cell walls, they can also be linked to pathogenic activity like elicitors and NLPs (necrosis and ethylene-inducing protein 1 (Nep1)-like proteins) (Nürnberg and Kemmerling, 2009). Recognition of any of these molecules by a PRR triggers PTI. The earliest physiological response to PAMPs/MAMPs is a rapid change in ion flux. An increased influx of H^+ and Ca^{2+} and an efflux of K^+ lead to depolarization of the plasma membrane and extracellular alkalisation of plant tissues, making them less hospitable to the pathogenic invader (Bigeard et al., 2015; Chinchilla and Boller, 2011). Moreover, Ca^{2+} is an intracellular second messenger involved in many signalling pathways regulating defence processes (Chinchilla and Boller, 2011). PAMP and MAMP recognition is also associated with an oxidative burst, the rapid production of reactive oxygen species (ROS). These are highly toxic reduced forms of oxygen such as hydrogen peroxide, which can act as an antibiotic agent, and are linked to stomatal closure, limiting the pathogens' entry points (Chinchilla and Boller, 2011), this is also linked to the Ca^{2+} influx (Thor et al., 2020). More pathways such as mitogen-activated protein kinase (MAPK) cascades, cytoplasmic kinases and Ca^{2+} -dependent kinases are then activated, leading to gene expression regulation (Schwessinger and Ronald, 2012). Hormone changes are also linked to PAMP/MAMP recognition. The three most studied hormones are salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). SA is associated with defence against biotrophic and hemi-biotrophic pathogens, while JA and ET are more generally associated with necrotrophs (Bari and Jones, 2009). These hormones are involved in PRR expression (Chinchilla and Boller, 2011) and have also been linked to plant cell death (Tsuda and Katagiri, 2010). PAMP and MAMP recognition can also lead to the synthesis of callose. Callose deposition at the site of the pathogen attack between the plasma membrane and the cell wall or at plasmodesmata blocking these potential cell-entry sites and contributing to limiting the spread of the pathogen structures (Wang et al., 2021).

D. MOLECULES INVOLVED IN ETS

ETS is induced when adapted pathogens are able to overcome PTI by secreting effectors that can manipulate the host cell and particularly can manipulate or block

PTI-associated mechanisms.

i. Defining effectors

Effectors are molecules that manipulate host immunity, be it in terms of cell structure or function, to enable parasitic infection (Kamoun, 2006). Effectors are able to target various signalling, metabolic and defence pathways in the host (Pradhan et al., 2021). In plant pathogenic fungi and oomycetes, the major part of known effector proteins are cysteine-rich, non-enzymatic, small, less than 300 amino acid residues in length, and carry a signal peptide for secretion (Houterman et al., 2009; Oliva et al., 2010; Rocafort et al., 2020; Stergiopoulos and de Wit, 2009). They show low homologies with other species making their biological function difficult to predict (Outram et al., 2021; Plissonneau et al., 2017). They are among the most polymorphic genes found in pathogen genomes (Win et al., 2012) and can be found, though not exclusively, in highly plastic transposable element (TE)-rich regions of the genome (Fouché et al., 2018; Ma et al., 2010; Plissonneau et al., 2018; Soyer et al., 2014). Though small secreted proteins (SSP) represent the majority, some larger proteins, enzymes and non-conventionally secreted proteins can also function as effectors (Rocafort et al., 2020). Moreover, some small RNAs have been characterized as effectors (Jian and Liang, 2019; Rodriguez-Moreno et al., 2018; Zhu et al., 2019), along with some secondary metabolites including toxins (Collemare et al., 2019; Rodriguez-Moreno et al., 2018; Valent and Khang, 2010).

ii. Effector delivery into the host

Effectors are by definition secreted and pathogens produce an arsenal or cocktail of effectors during infection. While apoplastic effectors are secreted into the apoplast, cytoplasmic effectors must be translocated inside the plant cell beyond the membrane and nuclear effector must penetrate the nucleus. Moreover, other cellular compartments can be targeted such as the endoplasmic reticulum, chloroplasts, mitochondria or the Golgi apparatus (Figuroa et al., 2021) (Figure 6). The effector delivery system for a given filamentous pathogen is dependent on this pathogens' lifestyle (Selin et al., 2016), for example a haustoria forming pathogen will secrete effectors into the space between the haustorium and the host cell membrane at very close contact, while a strictly apoplastic pathogen will not possess these structures. For many plant pathogenic bacteria, *Pseudomonas syringae* for example, effector delivery has been well characterized, involving a type three secretion system (T3SS) which creates a structure to directly inject effectors into the infected plant cells (Coburn et al., 2007). Fungi do not have the ability to produce such structures (Chaudhari et al., 2014). In oomycetes, translocation of effectors into plant cells has been linked to RxLR motifs

that are conserved N-terminal amino-acid sequences on certain effector proteins. Oomycete haustoria that have invaginated the host cell membrane secrete RxLR effectors which are then translocated into plant cells (Wawra et al., 2017), though the exact mechanism remains unknown (Bi et al., 2021). For fungal plant pathogens, effector uptake into host cells has been discussed, and mechanisms similar to what is found in oomycetes have been hypothesized, but no formal proof has been found leaving the debate open (Lo Presti et al., 2017; Rafiqi et al., 2012; Uhse and Djamei, 2018; Van den Ackerveken, 2017).

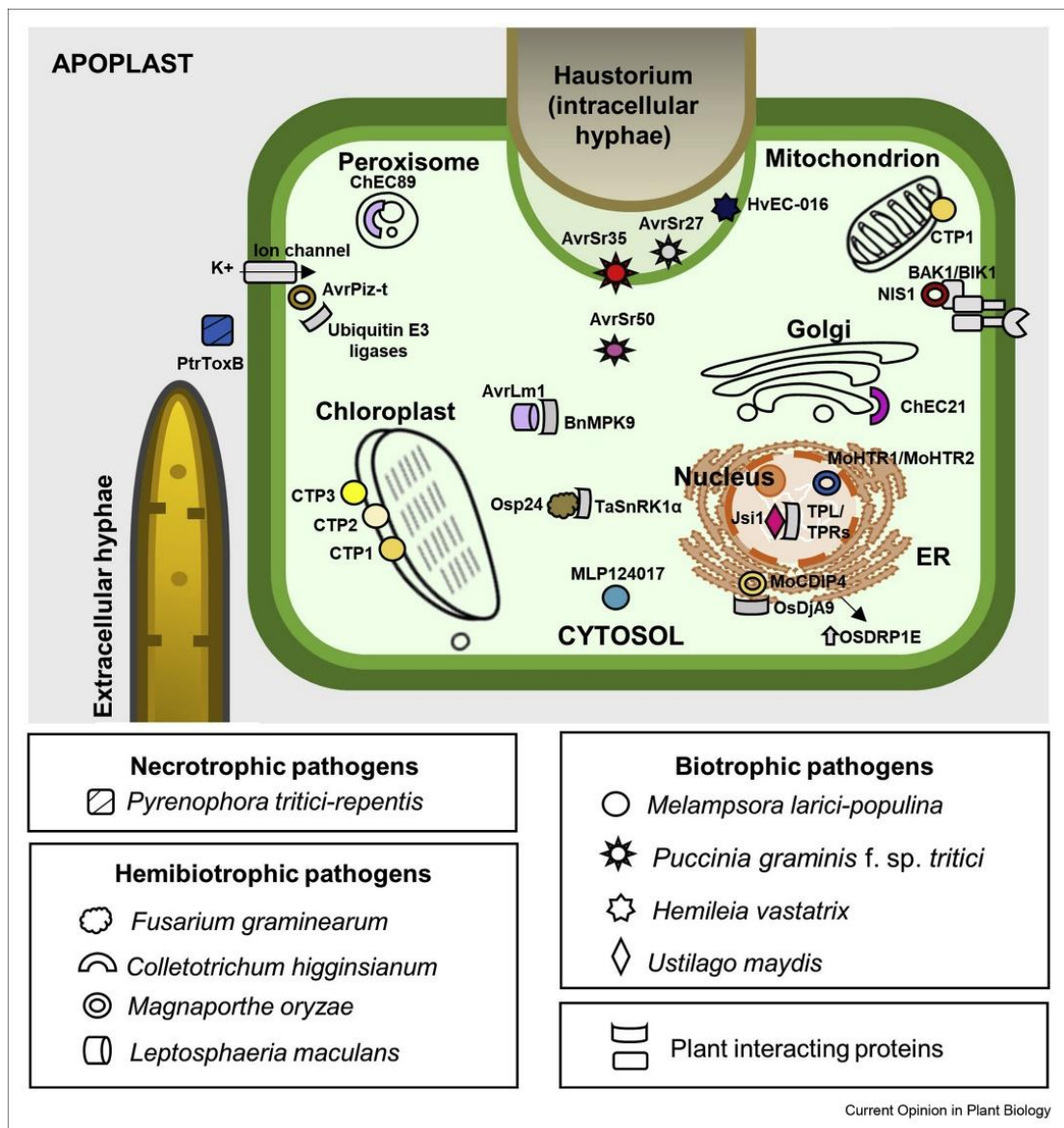


Figure 6: Effectors can target different cellular compartments

Fungal effector proteins can target different cellular compartments. Here a few examples illustrate this diversity, and the diversity that is observable within a trophic strategy be it necrotrophic, biotrophic or hemibiotrophic. All examples presented are

of effectors that are translocated into the intracellular space, though some known effectors are strictly apoplastic. Adapted from Figueroa et al. (2021).

iii. How effectors can modulate host cells

Effectors can silence host defences involved in PTI or promote infection by altering plant metabolism or can have toxic ability (Hauelsen and Stukenbrock, 2016). As effectors, targets and modes of action are extremely diverse throughout fungal and oomycete species, I will present some examples with no claims to exhaustiveness.

Necrosis-inducing protein 1 (NSI1) is a necrosis-inducing secreted protein which is highly conserved in pathogenic fungi and oomycetes. It has the ability to bind to PRR-associated kinases BAK1 and BIK1. NSI1 is able to inhibit the kinase activity of these proteins, halting any potential downstream signalling. Upon targeting BIK1 in particular, NSI1 suppresses ROS generation associated PTI (Irieda et al., 2019).

The effector protein AvrLm1 is secreted and translocated to the host cytosol during the infection of *Brassica napus* by *L. maculans*, which causes blackleg disease. This effector is capable of suppressing SA and JA signalling by specifically binding with BnMPK9, a MAP kinase, thus facilitating *L. maculans* infection. AvrLm1 is therefore able to modulate host immunity pathways (Ma et al., 2018).

Osp24 is a cytoplasmic effector secreted by *F. graminearum* the causal agent of Fusarium head blight (FHB) in wheat, barley and oat. Osp24 acts by binding to TaSNRK1 α (*Triticum aestivum* sucrose non-fermenting-1 related protein kinase 1 catalytic subunit α), directly competing with TaFROG (*Triticum aestivum* Fusarium Resistance Orphan Gene), an FHB resistance gene that bind to the same region of TaSNRK1 α (C., Jiang et al., 2020).

VdSCP41 is a secreted protein of *Verticillium dahliae*, a pathogen that can infect a broad range of hosts including cotton and *Arabidopsis thaliana*, causing *Verticillium* wilt. VdSCP41 acts as an intracellular effector by binding to the immune regulator CBP60g, interfering with its transcription factor ability to bind to the promoters of SA synthesis controlling genes. SARD1, another protein with transcription factor ability is also targeted by VdSCP1 (Qin et al., 2018).

MoCDIP4 is a cellulose-growth-specific secreted protein of the fungal pathogen *M. oryzae* which causes rice blast disease. This effector acts on mitochondrial dynamics in rice thus reducing defence responses. MoCDPI4 interacts with OsDjA9, a protein that outside of *M. oryzae* infection forms a complex with OsDRP1E. The OsDjA9-OsDRP1E

complex is known to modulate mitochondrial dynamics for plant growth and defence. Through competition with MoCDIP4 for OsDJA9 binding, OsDRP1E accumulates promoting mitochondrial fission, reducing mitochondrial size and reducing ROS generation. For this effector, the presented system is a working model that is not yet fully validated (Xu et al., 2020).

Tin2 is an effector of *U. maydis*, the fungus responsible for the tumour generating disease corn smut. During infection, Tin2 translocates into plant cells and targets the cytoplasmic protein kinase ZmTTK1, which is responsible for the activation of genes in the anthocyanin biosynthesis pathway (Tanaka et al., 2019). Anthocyanins are pigmented health-promoting secondary metabolites that are involved in protecting plants from stresses (Lin et al., 2021). In the case of *U. maydis* however, the effector stabilizes ZmTTK1 leading to anthocyanin formation, thus reducing lignification and facilitation the spread of fungal hyphae. The production of anthocyanin therefore becomes beneficial for the fungus rather than the host. Involvement in tumour formation has also been suggested (Tanaka et al., 2019).

PtrToxA was the first proteinaceous host selective toxin (HST) to be identified and is produced by several fungal pathogens among which is *Pyrenophora tritici repentis* (*Ptr*) responsible for tan spot in wheat (Ciuffetti et al., 2010). PtrToxA leads to a light-dependant accumulation of ROS and subsequent cell death, presumably by inducing changes in the proteins of photosystems I and II (PSI and PSII) in chloroplasts thus affecting photosynthetic electron transport (Manning et al., 2009).

These different examples show the extreme diversity in modes of action of effectors, even when only looking into fungal secreted protein effectors, diversity made even greater by the existence of other effector types.

E. MOLECULES INVOLVED IN ETI

ETI is established when R proteins are able to recognise pathogen effectors through an R/Avr gene-for-gene relationship.

i. Principal types of R proteins

These R proteins are of two types, intracellular or extracellular. Inside the cell, characterized R proteins are generally nucleotide-binding site leucine-rich repeat immunoreceptors (NLRs also known as NB-LRRs). Outside the cell, the proteins called upon are generally plasma-membrane localised receptors RLPs or RLKs, which are similar to PRRs (Figueroa et al., 2021). Additionally, some R genes can encode

detoxification enzymes (Nelson et al., 2018). NLRs typically contain a C-terminal LRR (Leucine-rich repeat) domain and a central NB-ARC (nucleotide-binding adaptor). The N-terminal domain of the protein is generally one of two types, either an N-terminal coiled coil domain (CC) or an N-terminal Toll/interleukin-1 receptor domain (TIR) (Qi and Innes, 2013); another type of C-terminal domain was identified to confer resistance to powdery mildew 8 named RPW8, which can confer specific or broad resistance (Barragan et al., 2019) (Figure 7). RLPs and RLKs, much as in PTI have a transmembrane domain and an extracellular LRR domain, with the difference that RLKs have an intracellular kinase domain and RLPs have a short cytoplasmic tail (Figure 7). Other types of *R* genes that encode proteins that carry no LRR or NB domains and have enzymatic activity have also been identified (Gururani et al., 2012).

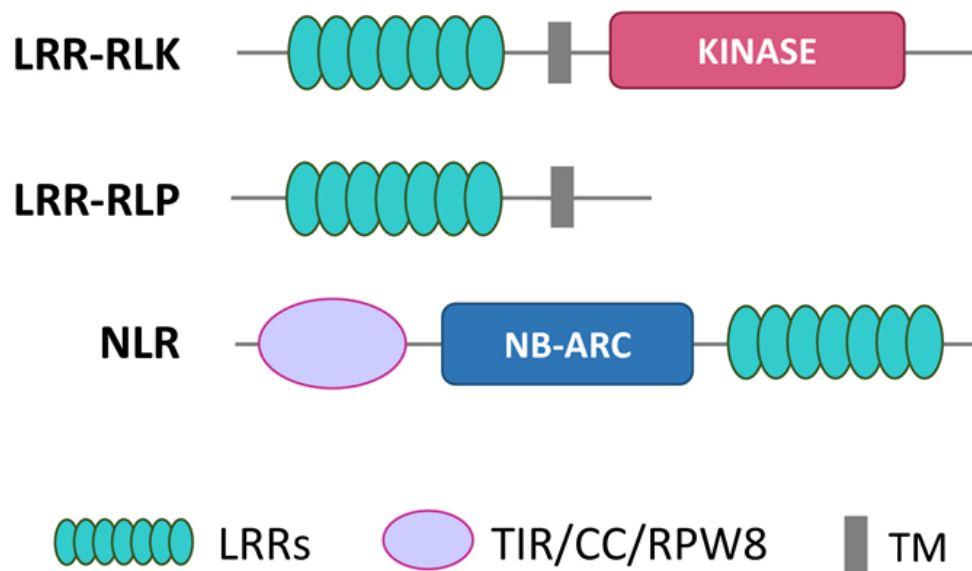


Figure 7: Main classes of *R* gene encoded receptors

LRR: Leucine-rich repeat; RLK: receptor-like kinase; RLP: receptor-like protein; NLR: nucleotide-binding leucine-rich repeat receptor; NB-ARC: nucleotide-binding adaptor; TIR: toll-interleukin receptor; CC: coiled coil domain; RPW8: resistance to powdery mildew 8 domain; TM: transmembrane domain. Adapted from Gottin et al. (2021)

ii. Effector recognition strategies and *R* gene mechanisms

Different models for the perception of *Avr* gene products by *R* proteins have been proposed, these can have different effects on pathogen fitness, defined as the organism's capacity to survive and reproduce. The first and simplest is the direct recognition of an effector by a receptor, wherein the effector acts as a ligand (Figure 8). The ligation of both *Avr* and *R* elements triggers host immunity responses

associated with ETI, leading for example to HR (Dangl and McDowell, 2006). The second model is the “guard” model and corresponds to indirect association of the effector and R protein. In this model, the R protein acts as a guard for the effector target and in the event of a modification of the target, it will induce defence responses (Figure 8). This indirect activation of the R protein means that multiple effectors with the same target can be indiscriminately recognized by a single guard protein, contrary to direct recognition of the effector, where a single mutation in the Avr can render it undetectable (Dangl and McDowell, 2006). A variation on the guard model is the “decoy” model, where specific proteins will mimic the effector target and R proteins will recognize modifications in the decoy thus inducing a defence response (Kroj et al., 2016) (Figure 8). The decoy strategy brings advantage to the host plant as the decoy itself has no function in host defence or susceptibility in the absence of the associated R guard protein, meaning that its modification by an effector will not facilitate infection (van der Hoorn and Kamoun, 2008). An improvement on the decoy strategy is the integrated decoy model in which R proteins carry an integrated decoy domain, an NLR-ID domain (Cesari, 2018), other than the classic TIR, CC or RPW8 (Moffett, 2016). The decoy domain acts as bait for the effector within the R protein structure, in a way cutting out the middle man (Figure 8). R proteins can therefore directly perceive the effector while it targets the integrated decoy domain (Kroj et al., 2016). In a variant of this model, a second R protein, or helper protein, constituting a sensor/helper pair with the R protein that serves to detect the effector, can complete the “effector trap” by signalling for an immune response leading to HR (Cesari et al., 2014). Helper proteins or helper NLR do however not seem to be directly involved in effector recognition, but serve to trigger the downstream pathways. One particular clade of these proteins are NRCs (NLR required for cell death) which have been studied in Solanaceae (Wu et al., 2017). The direct and indirect models are valid for RLPs, RLKs and NLRs, though there are more examples based on work with NLRs, while the integrated decoy model seems specific to intracellular receptors (Kourelis and van der Hoorn, 2018).

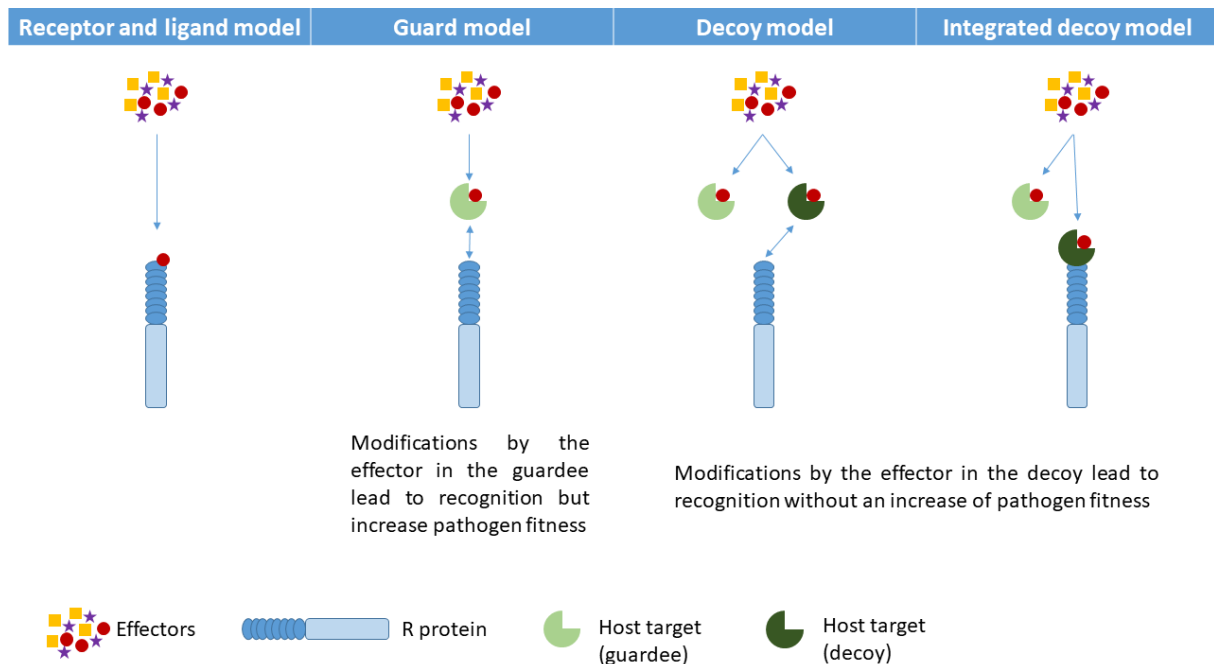


Figure 8: Four main models for effector recognition by an R protein

In the **receptor-ligand model**, the R protein directly recognizes an effector which acts as a ligand. In the **guard model**, the effector induces modifications in the guardee, these modifications are perceived by the guard R protein. The modifications in the guardee come at a price for the host, as they increase pathogen fitness. In the **decoy model**, the effector targets both its intended target and a decoy target that has no other purpose in the host than interaction with the effector, leading to the modifications in the decoy having no cost for the host, the R protein recognizes modifications in the decoy. In the **integrated decoy model**, the decoy is part of the R protein and mimics the intended target of the effector, again, the interaction between the decoy and effector does not lead to increased pathogen fitness. In all scenarios, direct or indirect recognition of the effector leads to ETI. Drawn up with information from Dangl and McDowell (2006), Kroj et al. (2016), van der Hoorn and Kamoun (2008), Głowacki et al. (2010) and Cesari (2018).

A different form of R gene strategy has evolved in plants to specifically target nuclear effectors. Originally identified with *Xanthomonas* plant pathogenic bacteria on rice, R genes called executor genes take advantage of transcription activator-like effectors (TALEs) by functioning as promoter traps, mimicking the promoter regions targeted by TALEs during infection (Kourelis and van der Hoorn, 2018). Nuclear effectors in oomycetes have also been studied, for example *Phytophthora* Crinkler (CRN) effectors (Stam et al., 2013). In comparison, advances in fungal nuclear effectors have lagged, however, candidate genes have been identified in fungi, and two nuclear effectors have

been identified and characterized in *M. oryzae* (Kim et al., 2020). A similar scenario to what is observed with executor genes specific to bacterial infections could happen in relation to fungal pathogens.

R proteins systems can be more complex than just a single R protein somehow leading to a resistance response, as previously described with the guard and decoy models and also the sensor/helper NLR pairs. In rice for example, some R proteins function by heterodimerization of two homologs, these are Pikp-1 and Pikp-2, which together are then able to interact with a single *M. oryzae* effector AVR-PikD triggering cell death, a feat they are unable to perform when expressed alone (Zdrzałek et al., 2020).

iii. Downstream responses after effector recognition

After the presence of the adapted pathogen has been detected by R proteins, the downstream immune responses are similar to those involved in PTI, including MAPK cascades, calcium flux, ROS burst and hormone signalling (Yuan et al., 2021). In ETI however, the response is thought to be accelerated and amplified in comparison with PTI (Jones and Dangl, 2006). It has also been suggested that rather than being two separate immune pathways, PTI and ETI interact. In the context of a compatible interaction, effectors secreted by the pathogen act to suppress PTI, but recognition by R proteins in an ETI context leads to upregulation of PTI pathways, thus compensating the suppression. ETI mechanisms then act as a sort of PTI amplification module or “booster”. ETS and ETI then correspond the suppression and potentiation of PTI respectively (Yuan et al., 2021).

iv. Resistance through loss of susceptibility without effector recognition

Rather than recognizing pathogen effectors, *R* genes can be linked to a “loss of susceptibility” strategy, through active (compensation) or inactive (loss of recognition) mechanisms.

Active loss of susceptibility has been demonstrated in plant responses to toxin effectors (Kourelis and van der Hoorn, 2018). For example, *Cochliobolus carbonum* race 1 is a fungus that causes ear rot and Northern leaf spot in maize and secretes a toxin called HC-toxin. Specific resistance to this fungus was linked to detoxification by an NADPH-dependant reductase encoded by *Hm1*. The encoded enzyme inactivates HC-toxin thus stopping infection (Johal and Briggs, 1992; Meeley et al., 1992).

Passive loss of susceptibility is often associated with resistance to viral diseases and the *R* genes involved are often recessive (Kourelis and van der Hoorn, 2018). The intended

effector target can be subject to a single mutation rendering interaction with the effector impossible, but without loss of function of the target. Examples for interactions with fungi are however not available to my knowledge. Furthermore, resistance by “host reprogramming” occurs when the host resists by loss of function in a susceptibility gene (*S*), thus bypassing the effector’s intended effect. The prime example for this is the *mlo* (*mildew locus o*) gene in barley. Recessive mutations in the *Mlo* gene, leading to an *mlo* genotype, confer broad, non-race-specific, resistance to powdery mildew, disease caused by *Blumeria graminis* f. sp. *hordei*. *Mlo* is considered as an *S* gene to powdery mildew as it negatively regulates plant defence responses, while *mlo*, the loss-of function allele, carrying plants demonstrate rapid formation of large cell wall appositions in epidermal cells which correlate with resistance to the pathogen (Ge et al., 2016; Jørgensen, 1992). Another example of loss of function resistance has been identified in cucumber for resistance to several pathogens including fungi and oomycetes (Yuhui Wang et al., 2019). A single nucleotide polymorphism (SNP) in the *STAYGREEN* (*CsSGR*) gene led differential expression in the chlorophyll degradation pathway between cucumbers carrying the mutation and cucumbers without the mutation. The recessive SNP mutation was associated with broad-range disease resistance, although the exact mechanisms remain under investigation (Yuhui Wang et al., 2019).

F. ADAPTATION BY THE PATHOGEN TO ETI, THE CYCLE GOES ON

If a pathogen can evolve to adapt to ETI by overcoming the defence conferred by *R* genes, ETS is reinstated, illustrating the evolutionary arms race between host and pathogen. The stratagems employed by pathogens for adaptation to *R* genes is very similar to the stratagems employed by hosts to adapt to specific effectors.

Just as *mlo* can suppress ETS by loss of function (Ge et al., 2016), pathogens can suppress ETI by loss of gene function. Loss or inactivation of an effector gene can be due to the insertion of TE in the promoter region or into the coding sequence. TE can also trigger epigenetic silencing of an effector gene. Finally, loss of function can be due to the leakage of repeat-induced point mutations (RIP) which disrupt the gene sequence (Fouché et al., 2018). An example of TE insertion leading to the inactivation of an effector and subsequent loss of avirulence is *UhAvr1*, an effector gene of *Ustilago hordei*, causal agent of covered smut in barley. A comparison between virulent and avirulent isolates of *U. hordei* revealed that the insertion of TE sequences in the promoter of *UhAvr1* changed its expression leading to a virulent phenotype (Ali et al., 2014). RIP induced mutations and subsequent disruption of avirulence genes has been observed in *L. maculans*, with RIP affected isolates being able to infect rapeseed

cultivars that were previously resistant (Van de Wouw et al., 2019).

A mutation in an effector that is an avirulence factor can also lead to adaptation to ETI. For example, some known effectors secreted by *P. infestans* during infection in potato are in the IPI-O family. The majority of these effectors are recognized by the potato *RB* gene which encodes a CC domain carrying NLR. IPI-O1 is a member of this effector family, which when recognized by the RB protein, activates programmed cell death. An IPI-O1 mutant with a single amino-acid mutation suppressed RB-mediated cell death (Chen et al., 2012), thus overcoming *R* gene mediated resistance. Additionally, another IPI-O family member, IPI-O4, which led to a virulent phenotype and directly interacted with the RB CC domain, was able to block the recognition of wild-type IPI-O1 by RB, demonstrating the possibility of the suppression of the recognition of an Avr by another effector with physical interaction between the would-be (or rather has-been) R protein and effector (Chen et al., 2012). Another example of suppression of R gene function by an effector has also been observed with effectors of *F. oxysporum* f. sp. *lycopersici* in interaction with tomato (Houterman et al., 2008). Highly specific gene-for-gene relationships between *R* and *Avr* genes are the drivers of this type of adaptation.

Finally, adaptation to ETI can be achieved by hijacking of the response pathways, and notably by suppressing HR. This has been studied with the bacterium *P. syringae* which is able to produce effectors which inhibit Bax, a proapoptotic programmed cell death inducing protein (Jamir et al., 2004). I could however not find any similar occurrences in fungi or oomycetes.

IV. THE DICHOTOMY OF VOCABULARY LINKED TO DISEASE IN PLANTS

A. QUANTITATIVE VS. QUALITATIVE

i. Vocabulary relating to resistance

Pathogen borne disease resistance in plants has for a very long time been subject to a strict dichotomy, a separation of two worlds (Poland et al., 2009), which classically separates "basal"/"horizontal"/"partial"/"broad-spectrum" resistance from "vertical"/"total"/"specific" resistance (Figure 9). Van Der Plank described horizontal resistance as a polygenic, non-specific, minor-gene resistance akin to field resistance or tolerance. Vertical resistance on the other hand was described as monogenic or oligogenic (governed by a small number of genes), major gene resistance, *R*-gene resistance, racial resistance (meaning race or strain-specific) or HR inducing resistance

(Van Der Plank, 1966). This separation survives to this day. The terms now in use are generally quantitative rather than horizontal, and qualitative rather than vertical. The chosen words reference the distribution observed in phenotypes, quantitative for a continuous distribution, a gradient, shades of grey, and qualitative for a discrete distribution into two categories, resistant or non-resistant, black or white (Niks et al., 2015; Poland et al., 2009; St Clair, 2010). In general, qualitative resistance is used in reference to *R* genes while quantitative resistance is used in reference to smaller effect quantitative trait loci (QTL). The aforementioned gene-for-gene interaction is generally associated to qualitative or major-gene resistance, while the mechanisms underlying quantitative resistance remain relatively poorly understood. They usually considered to be involved in broad-range defence and to be linked to pathogen fitness (Cowger and Brown, 2019). PTI is thought to contribute to quantitative resistance, while ETI and the corresponding gene-for-gene interaction between *R* and *Avr* genes form the basis of qualitative resistance (Nelson et al., 2018).

ii. Vocabulary relating to pathogenicity

On the pathogen side of the argument, there seems to have been less diversity in the vocabulary employed, with the very general term pathogenicity being widely used, along with the term virulence. One major issue with “pathogenicity” and “virulence” is their use in several disciplines, with varying definitions. In invertebrate pathology, medicine, evolutionary biology and microbiology, definitions vary (Shapiro-Ilan et al., 2005; Thomas and Elkinton, 2004) but follow a general theme in which pathogenicity relates to the ability of a pathogen to produce disease in a host or several hosts, while virulence is used to qualify “the degree of pathogenicity within a group or species” (Shapiro-Ilan et al., 2005). Pathogenicity is then the pathogen equivalent of qualitative resistance plant side, a pathogen can either cause disease or it cannot, and virulence is the quantitative resistance equivalent, corresponding to the degrees of variation in pathogenicity, a pathogen can cause more or less damage.

In plant pathology the definitions seem less clear, despite several authors attempting to review or redefine terms over the last decades (Bos and Parlevliet, 1995; Sacristán and García-Arenal, 2008; Shaner et al., 1992; Shapiro-Ilan et al., 2005; Surico, 2013). In this particular discipline, more words that comes into the already confused mix are “avirulent” and “aggressiveness” (Pariaud et al., 2009; Shaner et al., 1992).

In 1992, Shaner *et al.* summarize the different meanings they have found for these words as follows:

“Pathogenicity: 1. the capability to cause disease; 2. the amount of damage caused to

the host.

Avirulence: 1. the specific inability of a pathogen race to establish a compatible (disease) interaction in a host cultivar with genetic resistance (R gene) in which other races of that pathogen may establish compatible interactions; antonym of virulence; 2. non-pathogenic; antonym of pathogenicity (usage 1); 3. relative damage to the host caused by one strain of the pathogen that is less than the damage caused by the most virulent strain of the pathogen known.

Virulence: 1. synonym of aggressiveness (usage 1); 2. antonym of avirulence (usage 1); the genetic ability of a pathogen race to overcome genetically determined host resistance, which is effective against other races of that pathogen, and cause a compatible (disease) interaction; 3. synonym of pathogenicity (usage 2); the relative amount of damage caused to a host by a given pathogen; 4. the relative ability of a pathogen with a given genotype to cause damage on a specific host with a given genotype under certain environmental conditions; 5. the ability of a pathogen race to multiply on a host cultivar containing genes conferring resistance to other races of that pathogen.

Aggressiveness: 1. synonym for virulence (usage 1); a property of the pathogen reflecting the relative amount of damage caused to the host without regard to resistance genes; 2. amount of replication in the host without regard to resistance genes; 3. amount of disease caused by a pathogen upon a host that contains only race nonspecific (minor gene or "horizontal") resistance to pathogenesis; e.g. races of pathogens that do not interact differentially with host cultivars having R genes (major, race-specific, or "vertical" resistance) vary in aggressiveness; 4. description of the rate at which an amount of disease is reached; more aggressive pathogens reach that amount faster; 5. the reproductive fitness of a pathogen while growing on a host."

In 2013, Surico, a teacher of plant pathology proposes:

"Pathogenicity: the ability of a micro-organism to cause disease on a particular host (i.e., a quality of a micro-organism that enables it to cause disease in a particular host).

Non-virulent (avirulent) pathogenic micro-organism: a pathogenic micro-organism that does not cause measurable damage to the host; or (in an incompatible interaction), any pathogen that harbours an effector gene whose product (a non-pathogenic factor) is recognized by a host that harbours the complementary R gene.

Virulence: the measurable degree of damage caused by a pathogenic, virulent micro-organism to a host plant."

No definition of aggressiveness is given.

Two lists of definitions of terms relating to plant pathology written 20 years apart have similar issues, which are the absence of anything clear-cut and sometimes opposing definitions given within the same discipline.

Given that there is a plethora of definitions for these words and that no consensus seems possible, I choose to revert to the safety and comfort of a dictionary, the Chambers dictionary (Robinson and Davidson, 1998) gives the following definitions:

Pathogenicity: "...the quality of producing or the ability to produce disease..."

Virulence: "...the quality of being extremely infectious, highly poisonous or malignant..."

Avirulence and aggressiveness are not defined. What is however interesting with this particular definition of virulence is the insistence on its extreme quality. It then makes sense that virulence would be the pathogen's equivalent of qualitative resistance, with avirulence as its antonym. While any form of pathogenicity, now used as an umbrella term, that is not extreme, i.e. there is not a clear division of phenotypes into two groups that are virulent and avirulent, but rather phenotypes with a continuous distribution, will fall into a category equivalent to quantitative resistance plant side, for which the word aggressiveness seems well adapted. These definitions integrate the plant side and pathogen side into one bi-partite model which opposes quantitative and qualitative interactions.

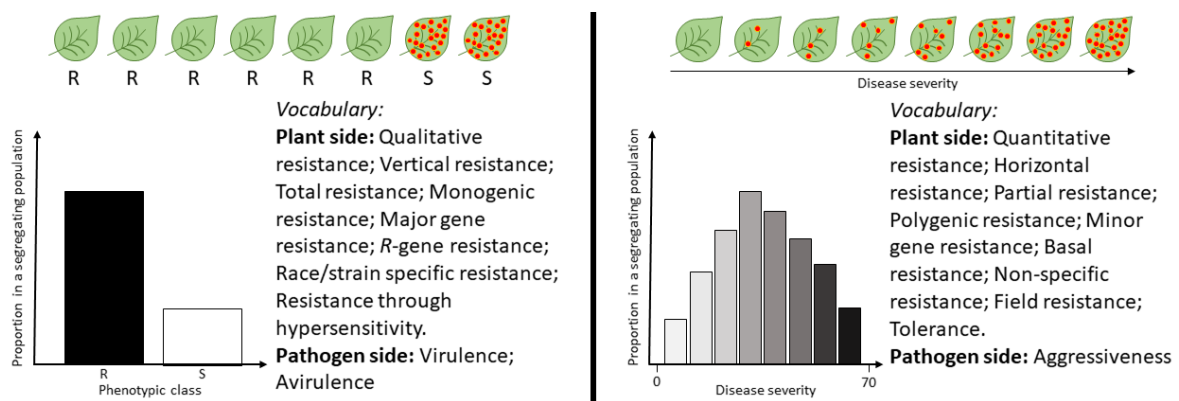


Figure 9: Dichotomy of quantitative and qualitative forms of disease resistance

iii. Possible molecular mechanisms involved in quantitative resistance

For the most part, the mechanisms underlying quantitative resistance have not been well documented and thus remain largely unknown or poorly understood (Cowger and Brown, 2019; Kou and Wang, 2010; Mundt, 2014; Poland et al., 2009; St Clair, 2010).

Some quantitative resistance genes and many resistance QTL have however been identified in a great many pathosystems involving all types of pathogens (Pilet-Nayel et al., 2017). Particularities have been noted for QTL which were for example only identified in certain environmental conditions or at certain stages in disease development or plant development, suggesting differential expression of the genes involved. QTL identified at specific stages of plant development led to the designation of adult plant resistance (APR), a particular branch of quantitative resistance, in which genes or loci identified confer durable, polygenic, broad and partial resistance to adult plants, with most loci identified in field conditions (Aghnoum et al., 2010; Chen, 2013; Li et al., 2014; Ren et al., 2012; Singh and Rajaram, 1992; L., Wang et al., 2010; Zhang et al., 2017). Interestingly the genes involved can be named according to the same nomenclature as major resistance genes (Gervais, 2017; Herrera-Foessel et al., 2014). Despite the definitions previously presented, no clear rule seems to appear in experimental data as to if resistance QTL have additive or epistatic effects or isolate-race-pathogen specificities (Pilet-Nayel et al., 2017). Pilet-Nayel et al. (2017) reported the cloning of 15 genes with partial effects on disease resistance in a variety of pathosystems. Of these 15, two had NB-LRR domains and five had kinase domains, while the remaining eight had more original domains including different types of transferases, heavy-metal transport detoxification, transporters or pore-forming toxin-like. Since then, four more were cloned, three of which encoded kinase domain carrying proteins (Dmochowska-Boguta et al., 2020; Gadaleta et al., 2019; Yajun Wang et al., 2019) while the other encoded an NBS-ARC gene, a type of NLR (G., Jiang et al., 2020). A review by Poland *et al.* (2009) proposed a variety of possibilities for the mechanisms underlying quantitative resistance. These included host plant development or morphology regulating genes, mutations in basal defence genes, detoxification mechanisms, defence signal transduction or partially altered weaker forms of R genes. Their last suggestion was that the genes underlying resistance QTL could be a unique set of previously unidentified genes. The domains that the few cloned quantitative resistance conferring genes carry, notably kinases and NB-LRR, seem to suggest that the before last option may be close to the truth, while other types of genes can also be involved. Types of genes involved in quantitative and qualitative resistance are therefore not exclusive to one form of resistance but can overlap.

B. A STRICT DICHOTOMY AT THE ROOT OF ALL CONFUSION

i. Contradictions in the zig-zag model

It is practical and elegant to conceptualize plant-pathogen interactions and to place their different components into boxes labelled quantitative and qualitative or ETI and

PTI or even PAMP and effector. However, as in any other experimental discipline, biological models are made to evolve and grow, incorporating new findings. Several papers suggest that the previously presented strict dichotomy in may be an oversimplification. Some PAMPs have effector-like qualities, such as being species specific, or being present only in certain pathogen species or even by contributing to the virulence of a pathogen (Naveed et al., 2020; Thomma et al., 2011). Additionally, some effectors have very PAMP-like properties, such as being widely distributed across the fungal kingdom and even in oomycetes and bacteria (Thomma et al., 2011), despite effectors generally showing low homologies with other species (Outram et al., 2021; Plissonneau et al., 2017). If PAMPs are mainly involved in pathogen fitness and host colonization while effectors are specifically linked to host immunity manipulation, there are overlaps. Another notable overlap on a molecular level is the structural similarities between PRRs and *R* gene encoded RLPs and RLKs. These surface receptors are triggered by PAMPs or effectors respectively but due to their similarities, most likely activate similar signalling pathways, causing some scientists to reject the differentiation between the corresponding host defences (Kanyuka and Rudd, 2019). These overlaps lead to certain secreted proteins identified as effectors being recognized by receptors classified as PRRs (de Wit, 2016). Moreover, some identified intracellular *R* proteins show high sequence conservation, similar to PRRs (Kanyuka and Rudd, 2019), two notable examples are the barley stem rust-resistance gene *Rpg1* which encodes a receptor kinase-like protein with two tandem kinase domains but does not have a transmembrane domain (Brueggeman et al., 2002), and *Yr36* a wheat stripe rust resistance gene which has a kinase domain and a START lipid-binding domain (Fu et al., 2009; Huang et al., 2016). These two proteins are particularly interesting, because despite being classified as *R* proteins, they confer broad resistance, meaning that they are not strain/race/pathotype specific, and additionally are considered durable (Brueggeman et al., 2002; Huang et al., 2016), attributes which do not quite fit the classic gene-for-gene relationship checklist. *Yr36* in particular has been shown to lead to phenotypes characteristic of *R/Avr* interactions, which is why it is included here, but also to intermediate “quantitative” phenotypes (Huang et al., 2016), which I will look into further in the next section. To avoid confusion, some propose to update the zig-zag model. Rather than ETI and PTI being thought of as layers or branches, they are presented as an integrated system wherein ETI acts as a sort of PTI “booster” (Yuan et al., 2021), the non-separation of ETI and PTI has also been described as a continuum in *Phytophthora* oomycetes (Naveed et al., 2020). Others suggest the implementation of adapted terms in specific cases where the classic zig-zag model does not quite work. Effector-triggered defence (ETD) for example has been suggested to account for *R* proteins interacting with extracellular effectors, in an attempt to solve the PRRs and *R*

gene encoded RLPs and RLKs confusion (Stotz et al., 2014). Yet more, suggest that rather than complicating an already complex model, terms could be simplified and categorizations erased. A "Spatial Invasion model" is proposed where separation is not based on PAMPs and effectors or on PTI and ETI, but simply on where exactly the action takes place, i.e. inside the cell or in the apoplast (Kanyuka and Rudd, 2019). This relates back to the conclusion that Thomma et al. (2011) came to: "microbe sensing and plant immune activation is determined by any type of plant receptor that recognizes appropriate ligands to activate defence", and this regardless of the PAMP, effector, PRR, RLP, RLK or NLR status of the molecules involved.

ii. Quantitative and qualitative overlaps

Quantitative and qualitative resistances have also been subject to partial overlaps (Roux et al., 2014). Some very good examples of *R* genes that seem to hover somewhere between quantitative and qualitative are the aforementioned resistance genes *Mlo* (Piffanelli et al., 2002), *Rpg1* (Brueggeman et al., 2002), *Yr36* (Huang et al., 2016), a fourth is *Stb16q* which is a wheat cysteine-rich RLK conferring resistance to *Z. tritici* (Saintenac et al., 2021). These four *R* genes are very particular in that they are broad-resistance and considered durable. *Mlo* is a special case in that it is the recessive *mlo* that confers resistance, and it is not a recognition of an effector system, but a loss-of function in a susceptibility gene, hence the associated durability (Gorash et al., 2021). The other three genes however are dominant. *Rpg1* displayed remarkable durability in broad-range resistance being widely effective in North America for over 50 years (Ayliffe et al., 2008) dating back to the 1940s (Solanki et al., 2019b). An R/Avr relationship with an unknown effector was speculated on, but not demonstrated, and the indispensable nature and consequent conservation of the Avr encoding effector in rust strains was proposed as an explanation for the extreme durability of the resistance (Ayliffe et al., 2008). This theory has however been put into question, with suggestions that the resistance conferred by *Rpg1* simply does not fit into the classic R/Avr model corresponding more to broad-spectrum incomplete resistances (Solanki et al., 2019b) which according to previously presented definitions fit into what is termed quantitative. In the case of *Stb16q*, it was suggested that its broad-spectrum resistance could be explained by its absence in most cultivated wheat varieties prior to 2016, limiting any selection in pathogen populations (Saintenac et al., 2021). The resistance gene was already incorporated into wheat cultivars in 2012, when the 'q' was added to the locus name as there was a possibility of several genes underlying the corresponding QTL, suggesting a quantitative locus rather than a classic *R* gene (Ghaffary et al., 2012). Despite the subsequent cloning of a single explicative gene (Saintenac et al., 2021) the 'q' was conserved in the name. Moreover, breakdown of this broad resistance has been

observed in the last years (Kildea et al., 2020; Orellana-Torrejon et al., 2021) putting into question the purported durability of this resistance gene. Probably has this gene been cloned after the emergence of virulent strains, there would be no doubt as to an R/Avr interaction, and the broad-spectrum appellation would not have been used. This example is marking as it seems to prove right the slightly irate seeming conclusion of a paper dating back to 1975, where the author states: "*Careful analysis in several laboratories has continued to accumulate evidence that field resistance (or horizontal resistance, or non-specific resistance, or whatever you want to call it) is controlled by the same kinds of genes, the same kinds of genetic interactions, as genes controlling infection type. [...] The results of numerous detailed studies in several laboratories over the past few years has lent greater credence to the statement that non-specific resistance (field, horizontal, generalized, etc.) is that resistance which hasn't yet been shown to be specific.*" (Ellingboe, 1975). The debate on a clear separation of quantitative and qualitative into two very distinct boxes therefore is not new to the 21st century, but has somehow persisted despite many exceptions to the rule. The last *R* gene we will comment on here is *Yr36* also known as *WKS1* and in particular the haplotype diversity that has been observed for this gene (Huang et al., 2016). A study of haplotype diversity showed that depending on the *WKS1* allele carried by a given cultivar of *Triticum dicoccoides*, the phenotypes observed on seven different cultivars inoculated with the same fungal strain could be susceptible or resistant (HR) or intermediate, which the authors linked to quantitative resistance (Huang et al., 2016). This shows that for a same resistance gene, phenotypes can be qualitative or quantitative depending on the allele, suggesting that possibly, what is perceived as qualitative is merely the extreme phenotypes observed in so-called quantitative interactions, further nullifying any justification of dichotomy.

iii. Gene-for-gene interactions involved in quantitative resistance, more than a hypothesis?

If quantitative and qualitative interactions can be based on the same types of genes and similar plant-pathogen interaction systems, it seems strange that gene-for-gene relationships should be valid only for what has been termed qualitative. And indeed, examples exist of gene-for-gene interactions outside of classic qualitative phenotype yielding pathosystems. In quantitative resistance of potato to the oomycete *P. infestans*, QTL mapping revealed a race-specific locus among other non-specific loci (Leonards-Schippers et al., 1994). Isolate-specific and broad spectrum QTL with varying contributions to the overall phenotype were shown to contribute to polygenic resistance in interactions between pepper (*Capsicum annuum* L.) and potyviruses (Caranta et al., 1997). The isolate specificities in both cases suggest underlying gene-

for-gene interactions in overall quantitative interactions. Minor gene-for-minor gene interactions have been suggested in the *P. hordei*-barley pathosystem due to the mapping of QTL for partial resistance that showed isolate specificities (Marcel et al., 2008; Qi et al., 1999). In the *Pyrenophora graminea*-barley pathosystem, partial resistance to the associated disease leaf stripe was shown to be governed by several loci including isolate-specific loci, once again suggesting minor gene-for-minor gene interactions (Arru et al., 2003). Such interactions had been previously hypothesized but not demonstrated (Parlevliet and Zadoks, 1977). Another study on *P. hordei* not only showed more evidence of minor gene-for-minor gene interactions in partial or quantitative resistance but also suggested that the resistance genes involved were not NLRs (González et al., 2012). With all of these examples, it is surprising that very often, when the mechanisms underlying quantitative resistance are mentioned in articles, they are more often than not said to be “poorly understood” and surrounded by question marks, when several studies strongly suggest that the underlying mechanisms are the same as in qualitative interactions, but lead to “partial” or “incomplete” phenotypes. One issue seems to be that though the resistance loci are identified, the potential corresponding effectors remain elusive.

Two rather recent articles seem to mark a breakthrough however. Working with the *Z. tritici*-wheat pathosystem, Meile et al. (2018) identified Avr3D1 a fungal avirulence factor that triggers partial resistance, meaning a strong defence response but allowing pathogen infection and reproduction, in wheat cultivars that carry *Stb7* and likely *Stb12* also, the proposed candidates for the corresponding resistance gene. Both of these resistance genes had been previously identified in the same region of the wheat chromosome 4A as major resistance genes in wheat to *Z. tritici* infection (Brown et al., 2015; Chartrain et al., 2005a). *Avr3D1* lies in a highly plastic, TE-rich region of the *Z. tritici* genome, is subject to high sequence polymorphism in field populations of the fungus and encodes an SSP. The quantitative aspect of the interaction is justified by the fact that avirulent strains are able to produce symptoms on *Stb7* harbouring cultivars but to a lesser degree than their virulent counterparts (Meile et al., 2018). This is an example of a gene-for-gene interaction identified with a known effector involved in quantitative resistance mechanisms. Another example was proposed within the *L. maculans*-rapeseed pathosystem. A gene-for-gene interaction was demonstrated between LmSTEE98, a ‘late effector’ encoded by *AvrLmSTEE98*, and RlmSTEE98 (Jiquel et al., 2021), encoded by the corresponding resistance gene that had previously been fine-mapped in rapeseed (Gervais, 2017). As a ‘late effector’, *AvrLmSTEE98* is expressed during the part of the long *L. maculans* life cycle when stem colonization occurs in adult plants (Gay et al., 2021; Rouxel and Balesdent, 2005). As previously presented with APR,

stem colonization in adult plants is considered to be polygenic and quantitative (Zhang et al., 2017). It then follows that a 'late effector' would be involved in quantitative resistance mechanisms. With the high variability observed in quantitative plant-pathogen interactions, and the effect of the environment on disease development, assessing adult plants for quantitative traits in the *L. maculans*-rapeseed pathosystem has proved complicated (Jestin et al., 2015). Jiquel et al. (2021) circumvent this issue by inducing the expression of *AvrLmSTEE98* at the cotyledon stage by promoter manipulation, which interestingly led to a clear HR. The authors suggest that this could be due to *RlmSTEE98* interacting with *LmSTEE98* at the cotyledon stage and having a differential expression throughout the infection as has been previously observed in other pathosystems with stage-specific expression patterns of resistance genes (Krattinger et al., 2009). I would argue however, that if, when the genes involved plant-side and pathogen-side are co-expressed, HR is observed and that the lack of HR is thought to be due to the absence of one of the genes in the mix, it isn't any different to any classic R/Avr interaction aside from issues due to being in the right place at the right time. One similar point in both the *Avr3D1* and *AvrLmSTEE98* studies is that the polygenic aspect of quantitative resistance is not accounted for. Regardless however, both studies highlight the overlaps in the reality of quantitative and qualitative interactions, going against the long upheld definitions and the associated strict dichotomy.

iv. Durability of genetic resistance to pathogens

Durable resistance can be defined as that which remains effective while being extensively used in agriculture for a long period in an environment favourable to the disease (Johnson, 1979; Johnson, 1984). For illustration, *mlo* can be considered truly durable, shown to be efficient still now despite having been discovered in the 1930s and largely deployed since the late 1970s at the latest (Kusch and Panstruga, 2017). *Stb16q* on the other hand is not durable as its recent widespread introduction into cultivars, notably 'Cellule', has led to a breakdown of the resistance (Kildea et al., 2020; Orellana-Torrejón et al., 2021).

The evolutionary arms race between host and pathogen is due to constant coevolution between both parties. As pathogen populations can evolve to adapt to resistances, durability can be maximized by reducing the probability of adaptation. The first major aspect of this is selective pressure. Indeed, lower selective pressure means lower probability of selecting the most pathogenic strains over others. Qualitative resistance and the phenotypes associated are not considered durable because of the strong selective pressure imposed by HR inducing gene-for-gene interactions of major genes

on the plant and pathogen sides (Niks et al., 2019). Quantitative resistance on the other hand is considered more durable because of leading to partial phenotypes, thus imposing lower selective pressure on pathogen populations (Cowger and Brown, 2019).

Polygenic resistance can contribute to diluting selective pressure. The polygenic nature of quantitative resistance renders adaptation more complex than in an R/Avr interaction as adaptation must be effective on several fronts (Parlevliet, 2002). In viral plant pathogens, the probability of adaptation is negatively correlated with the number of mutations required for virulence, i.e. polygenic resistance is less likely overall to be broken down than monogenic resistance (Harrison, 2002; Lindhout, 2002; Palloix et al., 2009). In the *Capsicum annuum*/*Potato virus Y* pathosystem, polygenic resistance breakdown is slower than monogenic, experiments showed that the virus requires a step-by-step selection for virulence, first towards major resistance genes, then towards the QTL and major gene combinations (Palloix et al., 2009; Quenouille et al., 2014). Polygenicity however is not exclusive to what is quantitative. Indeed efforts in improving the durability of major resistance genes involve gene pyramiding, wherein several major resistance genes are introgressed into an agronomically interesting cultivar. This similarly dilutes selective pressure between these genes. Pyramids can also involve a combination of *R* genes and resistance QTL (Mundt, 2018; REX Consortium, 2016).

A third argument for durability can be diversity in resistance mechanisms, necessitating an equivalent diversity in breakdown mechanisms. In effector recognition-based qualitative resistance, the mechanisms are seemingly mostly based on the action of NLRs, RLKs or RLPs. However, durable qualitative resistance has been observed with more original mechanisms such as loss of susceptibility with *mlo*. Quantitative resistance is thought to be based on more diverse mechanisms than its qualitative counterpart, additionally the few cloned genes involved in quantitative resistance do present with diverse domains (Pilet-Nayel et al., 2017; Poland et al., 2009). Moreover, the plant-stage specificities of quantitative resistance such as in APR and indeed specificities linked to stages in the pathogen life cycle, add more complexity to possible adaptation (Mundt, 2014; Palloix et al., 2009; Pilet-Nayel et al., 2017).

Overall, though strategies can be employed to increase the durability of qualitative resistance, in literature it does present as being less durable than qualitative resistance as it performs less well when it comes to selective pressure and underlying mechanism diversity.

There have however been reported cases of quantitative resistance breakdown by

pathogen populations (Caffier et al., 2014; Delmotte et al., 2014; Lehman and Shaner, 1997; Montarry et al., 2012). This not only means that quantitative resistances can be overcome, but also that additional measures should probably be included for their potential use in breeding. Some have suggested pyramiding broad-spectrum factors, using cultivars carrying narrow-spectrum resistance QTL to minimize resistance erosion or combining specific QTL that complement each other thus slowing the selection of one particular pathogen strain (Van et al., 2013), cultivar mixtures have also been suggested as an extra barrier to pathogen adaptation (Montarry et al., 2012).

V. AN INTERESTING PATHOSYSTEM TO STUDY THE RESISTANCE DICHOTOMY: *TRITICUM AESTIVUM/ZYMOSEPTORIA TRITICI*

A. WHEAT IN BRIEF

i. Economic importance

Wheat is the most widely-grown crop in the world in terms of surface area and the most important cereal crop in Europe, and second most important, after rice, on a global scale (Fones and Gurr, 2015). As a staple crop for one third of the ever increasing human population (Paux et al., 2022), it represents a highly valuable food source, be it by direct consumption as bread or other baked goods, but also by indirect consumption as animal feed. In human nutrition, 20% of total food calories and protein are attributed to wheat (Gupta et al., 2008). It is farmed on every continent, with Asia and Europe producing 44.3% and 33.1% of the world's wheat respectively over the 2010-2020 period (<https://www.fao.org/faostat/en/#data>). In Europe, the biggest wheat producers are France followed by Germany and then Ukraine (<https://www.fao.org/faostat/en/#data>). In France alone during the 2010-2020 period, 36 million tonnes were harvested each year on average from 5 million hectares of cultivated area on average, while production for maize, the first runner up in terms of cereal production in France, yielded 14 million tonnes each year on average (<https://www.fao.org/faostat/en/#data>). The domestication of wheat dates back to the Neolithic about 10,000 years ago (Shewry, 2009) and plays a large part in the history of agriculture (Venske et al., 2019). *T. aestivum* represents 95% of cultivated wheat, the remaining 5% mainly comprise durum wheat *T. turgidum* (Venske et al., 2019).

ii. Principal agronomic characteristics

T. aestivum belongs to the Poaceae family (Snape and Pánková, 2013) and is self-pollinating (autogamy) presenting with essentially cleistogamous florets (Zajączkowska et al., 2021). Three main categories of wheat exist, these are winter wheat, spring wheat

and alternative wheat (Doré and Varoquaux, 2006). The names can vary between countries, but the main characteristics are the same (Crofts, 1989). In Europe, winter wheat is sown in the autumn. It necessitates a vernalisation period, corresponding to temperatures below 8°C during eight weeks or so, in order to grow. Spring wheat does not need vernalisation and is sown in the spring (Doré and Varoquaux, 2006). The wheat cultivation cycle, from sowing to harvesting, lasts around ten to eleven months for winter wheat and six to seven months for spring wheat. A great number of different cultivars are farmed in France with varying properties relating to yield, height, abiotic (temperature, drought, lodging) and biotic (diseases, pests, weeds) resistance or tolerance factors, and industrial qualities such as grain hardness, protein content and value in bread-making.

iii. The *T. aestivum* genome

T. aestivum is allohexaploid ($2n=6x=42$) and has a genome of about 17 gigabases in size, one of the largest in higher plants (Gardiner, 2020). This sizeable genome consists of three interrelated diploid 'sub-genomes' A, B and D (Chen et al., 2022). These genomes find their origins firstly in a natural hybridization of diploid grass species that occurred between *T. urartu* (A genome) and most likely *Aegilops speltoides* (B genome) (Nielsen et al., 2014). Secondly, after initial domestication, the hexaploid subspecies *T. dicoccum* (AABB) intercrossed with *A. tauschii* (D genome) thus producing hexaploid wheat (Nielsen et al., 2014). Each of the three homologous sub-genomes is composed of seven chromosome pairs (Marcussen et al., 2014). Overall the genome hosts around 80 to 90% of repetitive sequences, this has led to some difficulty in analyses (Gardiner, 2020). Despite these difficulties, a fully annotated reference genome is available and comprises 107,891 high-confidence genes. The cultivar Chinese Spring is the reference genome in *T. aestivum* (Guan et al., 2020; IWGSC et al., 2018). Recently, other cultivars have also been assembled at a chromosomal level. Karioga is a South African *T. aestivum* cultivar and its assembly has been used in studies on stripe rust resistance (Athiyannan et al., 2022). The French wheat cultivar Renan has also been very recently assembled at a complete chromosome scale (Aury et al., 2022).

B. *Z. TRITICI* THE CAUSAL AGENT OF SEPTORIA LEAF BLOTCH

i. Economic cost of STB

Septoria tritici blotch (STB), caused by the fungus *Z. tritici*, is one of the most devastating diseases of wheat in Europe (Fones and Gurr, 2015). It is responsible for high yield losses worldwide, 30 to 50% loss when environmental conditions are favourable to the disease's development (Eyal et al., 1987; Fones and Gurr, 2015).

Controlling this disease is generally dependant on fungicide application and STB alone is responsible for 70% of fungicide use on cereals in Europe with an annual cost of over 400 million euros (O'Driscoll et al., 2014). Active substances deployed act as curative solutions but also as prophylactics. Different compounds such as methyl benzimidazole carbamates (MBCs), quinone outside inhibitors (QoIs), demethylase inhibitors (DMIs) or succinate dehydrogenase inhibitors (SDHI) have however been subject to ever-increasing pathogen resistance or insensitivity from *Z. tritici* strains. This renders the durability of the chemical control of disease more than questionable (O'Driscoll et al., 2014; Orton et al., 2011; Torriani et al., 2015). The second most important aspect of disease control is the use of disease resistant or tolerant cultivars (Orellana-Torrejon et al., 2021). Alternative measures of control can include the implementation of culture rotations, as crop residue is the ideal nesting place for ascospores, which then constitute primary inoculum for the next epidemic (Suffert et al., 2015). It is also possible to consider cultivar mixtures in the event of reasonably low disease pressure (O'Driscoll et al., 2014) or indeed late sowing (Palmer and Skinner, 2002).

ii. Classification of *Z. tritici*

Z. tritici, previously known as *Mycosphaerella graminicola*, is a phytopathogenic fungus which can infect bread wheat, durum wheat and triticale. It is an ascomycete fungus belonging to the Dothideomycetes class and the Mycosphaerellaceae family (Orton et al., 2011; Suffert et al., 2015). It is a dimorphic fungus, meaning that it can exist both in a yeast-like form and a mycelium form. Under its yeast-like form the cell type is the macrospore and is well-adapted to being grown in laboratory conditions outside of a host-plant interaction on a rich medium (Steinberg, 2015). The mycelium form is mostly found *in planta* but can be generated *in vitro* on a nutrient-poor culture medium (Kema and Annone, 1991). Reproduction can be sexual or asexual. Sexual reproduction necessitates the presence of both mating-types MAT1-1 and MAT1-2 as the fungus is heterothallic (Waalwijk et al., 2002). Having mostly been considered as a hemibiotroph due to the asymptomatic and necrotrophic phases in its development *in planta*, it has been proposed that *Z. tritici* be described as a latent necrotroph (Sánchez-Vallet et al., 2015).

iii. Origins and geographical distribution

The centre of diversity of *Z. tritici* coincides with *T. aestivum* in the middle East and fertile crescent (Banke et al., 2004; Palmer and Skinner, 2002; Stukenbrock et al., 2007). Nowadays, it is found worldwide (Kellner et al., 2014), and in Europe it flourishes in the European and Mediterranean Plant Protection Organization's (EPPO) "Maritime Zone" under a humid climate; this area includes Northern France, Germany and the United

Kingdom (Fones and Gurr, 2015).

iv. Life cycle

The *Z. tritici* infection cycle in wheat is characterized by two infection phases over the cropping season. Primary infection starts with sexual ascospores which are wind-dispersed from wheat debris, secondary infection corresponds to the subsequent asexual reproduction cycles that take place on growing plants producing asexual pycnidiospores which are dispersed by splashing (Suffert et al., 2015) (Figure 10). In general, 4 to 6 asexual embedded infection cycles take place over the course of the cropping season (Suffert et al., 2015). In the winter, *Z. tritici* survives as mycelium, pycnidia or pseudothecia on wheat debris or on other hosts (O'Driscoll et al., 2014; Suffert et al., 2015).

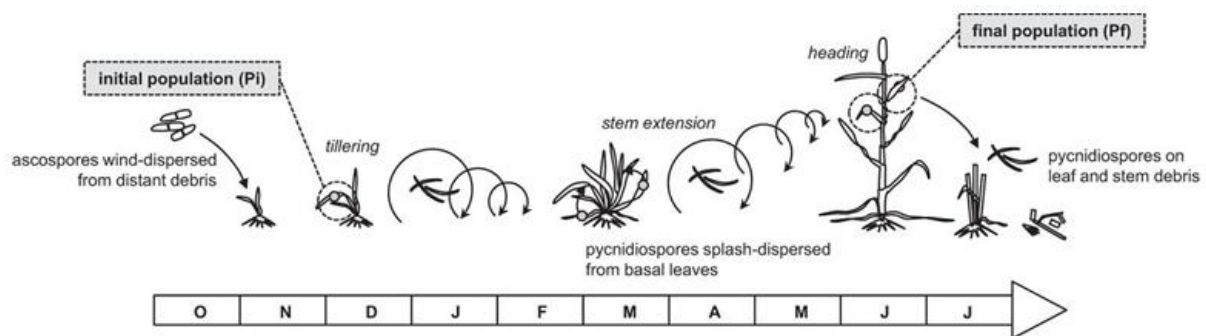


Figure 10: Developmental stages of an STB epidemic over a cropping season

Adapted from Suffert et al. (2015)

v. Infectious process

In the leaf, infection takes place in the same way for ascospores and pycnidiospores. A first asymptomatic phase, sometimes called a biotrophic phase although the term in this particular case is debatable (Sánchez-Vallet et al., 2015), occurs upon spore germination on the leaf surface. Germination is dependent on high humidity conditions (Boixel et al., 2019b). Hyphae enter the leaf tissue through stomatal openings and invade the mesophyll while remaining in the apoplast, no intracellular hyphae or haustoria are formed (Steinberg, 2015). Nutrient uptake by *Z. tritici* during the initial phase remains mysterious (Sánchez-Vallet et al., 2015), although the metabolism of lipids and fatty acids and aspartic proteases could be involved (Palma-Guerrero et al., 2016; Rudd et al., 2015), however minimal utilization of host-derived nutrients could merely be a subterfuge engineered by the pathogen during early infection to avoid detection while colonizing (Rudd et al., 2015). Once hyphae have successfully colonized sub-stomatal cavities and the mesophyll, at about 9-10 days post infection (dpi),

symptoms start to appear such as chlorosis and necrosis of foliar tissue (Figure 11), demonstrating apoptosis and cell death characteristic of the necrotrophic phase of disease development (Palmer and Skinner, 2002; Steinberg, 2015). After another 14 to 21 dpi, what at a glance look like black or brown dots will appear at the stomata (Figure 11), these are pycnidia (asexual form) or pseudothecia (sexual form) (Steinberg, 2015). In high humidity conditions, these will release pycnidiospores or ascospores respectively, around $4 \cdot 10^3$ pycnidiospores are released by each mature pycnidium, 40 to 60% of which at first contact with water (Eyal, 1971; Palmer and Skinner, 2002). *Z. tritici* takes around 20 days to asexually generate a new generation (Fones and Gurr, 2015). Figure 12 presents the stages of *Z. tritici* infection on plant leaves.



Figure 11: Observable STB symptoms on the leaf surface in wheat

Adapted from an image by Arvalis Institut du végétal (<http://www.fiches.arvalis-infos.fr/>)

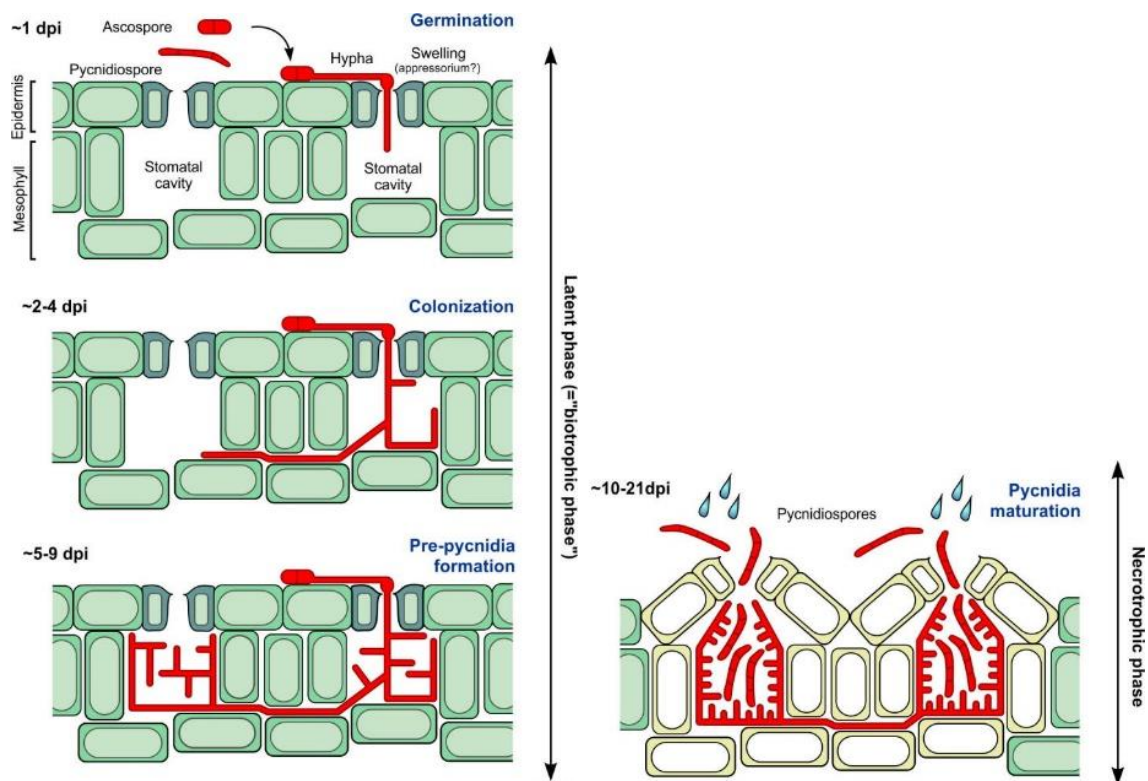


Figure 12: Summary of the host infection phases of *Z. tritici*

Plant cells are represented in green and fungal cells are represented in red. Adapted from Steinberg (2015).

vi. The *Z. tritici* genome

The *Z. tritici* genome is a lot smaller than the wheat genome at a mere 39.7 Mb. It is a haploid genome and has been fully sequenced (Goodwin et al., 2011). The reference genome for *Z. tritici* is that of the Dutch field strain IPO323, isolated from the cultivar Arminda in 1984 (Goodwin et al., 2011). This genome comprises 21 chromosomes, 13 of which are core-chromosomes and are present in every known strain, the remaining 8 are dispensable chromosomes and are subject to presence-absence polymorphisms in strains. No strain identified has complete absence of dispensable chromosomes however, suggesting that they play an important role in the fungus (Perez-Nadales et al., 2014). Additionally, a large number of genes identified on dispensable chromosomes appear to be duplications of genes found in core-chromosomes (Goodwin et al., 2011). IPO323 is considered to be one of the best-assembled eukaryotic genomes with 11,839 predicted genes, 43% of which have an assigned function, and well annotated TE which represent 18.6% of the genome (Grandaubert et al., 2015). A pangenome analysis of *Z. tritici* showed that through high polymorphism and between strains and genome plasticity, the study of IPO323 underestimates the number of genes in the species, estimating that number closer to 15,000 (Plissonneau

et al., 2018). Of the annotated genes in *Z. tritici*, 441 were predicted to encode SSPs in 2015, and an earlier study had identified 50 predicted proteins with over 5% of cysteines, less than 200aa in length and no functional annotation, strongly suggesting fungal effectors (Do Amaral et al., 2012; Grandaubert et al., 2015).

C. KNOWN SOURCES OF RESISTANCE TO STB IN WHEAT AND FUNGAL PATHOGENICITY FACTORS

Resistance and pathogenicity in the wheat-*Z. tritici* pathosystem have qualitative and quantitative components, with the identification of classic gene-for-gene interaction in at least one case. Overall however, the interaction is considered primarily quantitative due to high variation and continuous distribution observed in phenotypes (Jlibene et al., 1994; G., H., Kema et al., 1996; Stewart et al., 2016; Stewart and McDonald, 2014; Zhan et al., 2007).

i. Resistance factors to STB in wheat

To date, 22 major resistance genes in bread wheat have been described, the most recent being *Stb19* (Brown et al., 2015; Yang et al., 2018). In controlled conditions as in the field, resistance to STB manifests itself as being quantitative, largely additive and with varying heritability (Stewart et al., 2017). Brown *et al.* (2015) counted 89 STB resistance QTL carrying regions, for a total of 167 individual QTL. Among these 89 regions are 62 QTL and 27 meta-QTL. Brown *et al.* (2015) describe QTL as showing lower plant stage specificity than major resistance genes. These were not all detected at the same developmental stage, 27 at a seedling stage, 48 in adult plants and 14 for both of these stages. These regions are located on all wheat chromosomes but chromosome 5D, while chromosomes 3B, 6B and 7B are more represented than others and several identified QTL colocalize with major resistance genes (Brown et al., 2015). Moreover, several recent studies have reported additional QTL for resistance to STB including on chromosome 5D (Gerard et al., 2017; Karlstedt et al., 2019; Langlands-Perry et al., 2022; Odilbekov et al., 2019; Riaz et al., 2020; Vagndorf et al., 2017; Yates et al., 2019). Figure 13 summarizes the known sources of resistance to STB in hexaploid wheat as of 2018. These different studies show that STB resistance in wheat is complex, due to the combination of a large number of QTL with varying effects on phenotypes. No STB quantitative resistance genes have been cloned. Major resistance gene *Stb6*, however, was the first cloned gene specifying resistance to STB (Saintenac et al., 2018). It encodes a wall-associated kinase-like protein, which detects the presence of a matching apoplastic effector. A second major resistance gene *STB16q* was cloned in 2021, which confers broad-spectrum resistance against *Z. tritici* and encodes a

cysteine-rich receptor-like kinase (Saintenac et al., 2021). There is a gene-for-gene relationship between *Stb6* and *AvrStb6* (Zhong et al., 2017), the corresponding avirulence gene, which encodes a small secreted protein, the aforementioned apoplastic effector. This relationship is a classic R/Avr interaction as previously described. Another gene-for-gene interaction was identified in this pathosystem, involving major resistance genes *Stb7* and/or *Stb12* and the matching effector *Avr3D1* (Meile et al., 2018). Unlike with *Stb6/AvrStb6*, the recognition of *Avr3D1* does not lead to total resistance, strains carrying the avirulent allele lead to significantly reduced symptoms in comparison with strains carrying the virulent allele, these are quantitative phenotypes (Meile et al., 2018).

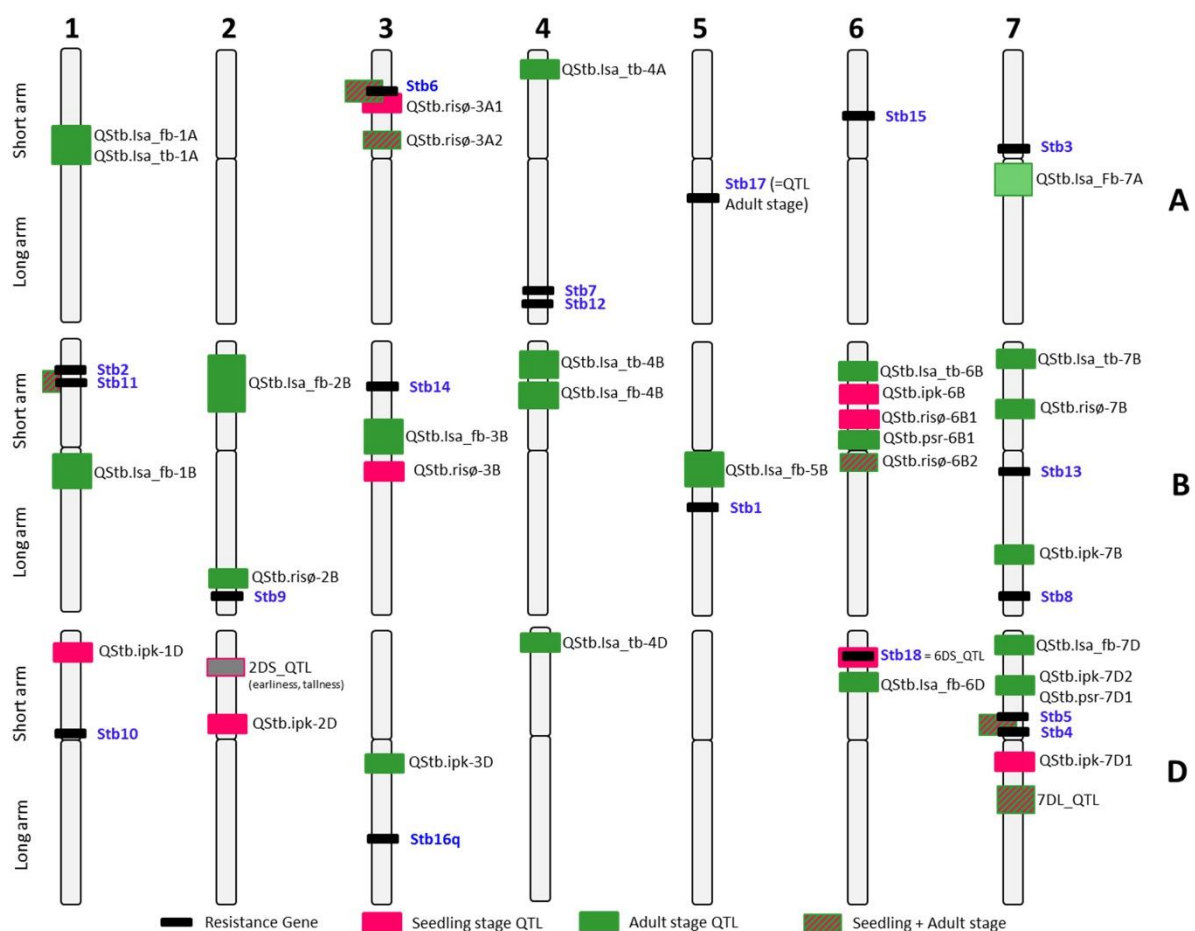


Figure 13: Location in the *T. aestivum* genome of the known major genes (*Stb*) and QTL conferring resistance to STB as of 2018. Adapted from Rothamsted Research (<https://www.rothamsted.ac.uk/news/another-blow-fungal-infection>).

ii. Pathogenicity factors in *Z. tritici*

Aside from *AvrStb6* which resides on chromosome 5 and *Avr3D1* which resides on chromosome 7, no other avirulence factors in *Z. tritici* has been cloned and validated.

Both of these effectors are cysteine-rich SSPs with 14% and 11% of their sequence represented by cysteines respectively, additionally, neither carries any known functional domains. Both effectors are located in highly polymorphic, TE-rich regions of the *Z. tritici* genome (Meile et al., 2018; Zhong et al., 2017). *AvrStb6* is highly expressed at 12 dpi and *Avr3D1* is expressed during the asymptomatic phase but downregulated upon the onset of the necrotic phase, suggesting that both of these effectors are involved in host colonization (Meile et al., 2018; Zhong et al., 2017).

Other identified genes that contribute to virulence in *Z. tritici* have been identified. *MgLysM* on chromosome 11 encodes a chitin-binding protein which blocks the elicitation of chitin-induced defences (Marshall et al., 2011). *Zt103264* and *Zt80707*, both located on chromosome 5, were shown to be involved in virulence, but the underlying mechanism are not known (Poppe et al., 2015). *Zt_8_609* on chromosome 8 encodes an SSP thought to be an avirulence gene, but for which the corresponding wheat resistance gene has yet to be identified (Hartmann et al., 2017). A secreted phytotoxic ribonuclease called Zt6 has been identified in *Z. tritici* and is thought to contribute to inducing necrosis, while also playing a role in antimicrobial competition (Kettles et al., 2018). Very recently, two genes designated *MYCO5* and *MYCO6* were identified and shown to play a role in dimorphic switch, which is the transition from the yeast form to the mycelium form, and pathogenicity in the fungus (Yemelin et al., 2022). Gohari et al. (2015) analysed the expression profiles of 156 different SSPs from *Z. tritici*, these were not however validated to be involved in pathogenicity.

Studies to identify pathogenicity QTL in *Z. tritici* have been carried out. QTL for the leaf area covered by pycnidia on chromosomes 2, 3, 4, 5, 6, 7 and 13 were identified (Gohari et al., 2015). Using two different mapping populations, Stewart et al. (2017) detected four QTL for virulence traits on chromosomes 3, 5, 7 and 9, leading to the identification of 918 candidate genes with emphasis on cysteine-rich SSPs. No pathogenicity QTL have been identified in dispensable chromosomes, however they do seem to play an as of yet undefined role in infection processes (Habig et al., 2017).

Mating types have been found to influence strain pathogenicity (Zhan et al., 2007). A link between melanisation and pathogenicity has also been shown (Lendenmann et al., 2014).

VI. SCOPE OF THE THESIS

A strict dichotomy between quantitative and qualitative resistances has governed how plant-pathogen interactions are perceived and studied since the 1960s at least. On the

one hand, qualitative resistance is effective but is not durable due to gene-for-gene interactions between *R* and *Avr* genes. On the other hand quantitative resistance is considered more durable as it is linked to complex traits governed by several small effect genes with varying effects on the overall phenotype. However a growing number of cases seem to contradict this dichotomy, putting into question the validity of the bi-partite model. Additionally, though the mechanisms underlying quantitative resistance are not well known, the looming possibility of a gene-for-gene system explaining quantitative interactions potentially limits the durability of this type of resistance. Finally, to our knowledge, no study has previously looked into quantitative interactions without the involvement of previously identified major resistance genes in this pathosystem.

In this thesis, we propose to study quantitative interactions between *Z. tritici* and its host *T. aestivum* to elucidate their genetic architecture and underlying mechanisms and to understand how these mechanisms may impact the durability of quantitative resistance. A better understanding of these interactions will contribute to their implementation in future breeding programs with a focus on maximizing durability.

To this effect, we worked with the cultivar Renan, a four-way hybrid resulting from a complex cross between cultivars Courtot, VPMxMoisson, Maris Huntsman and Mironovskaia-808, created by INRA Rennes in 1989 (Doré and Varoquaux, 2006). This cultivar, which is still used in organic farming today has very good baking quality, a high tolerance to the cold, is relatively short, preventing lodging and has reasonably good competitive value against weeds (Rolland et al., 2017). Renan has also displayed remarkable resistance to cereal rusts, eyespot, septoria leaf blotch and FHB. These resistances result in part from the introgression of two chromosomal fragments from *Aegilops ventricosa* which carry the resistance gene to eyespot *Pch1*, on chromosome 7D, and resistance genes to rusts *Lr37*, *Yr17* et *Sr38* (Dedryver et al., 2009; Doussinault et al., 2001; Hanzalova et al., 2007) on chromosome 2A. Until the end of the 1990s, Renan had a good level of resistance to septoria leaf blotch and today, virulent strains remain scarce in natural populations. The second cultivar we worked with was Chinese Spring, a Sichuan landrace (Liu et al., 2018). As the first fully sequenced and assembled wheat genome (IWGSC et al., 2018; Zhu et al., 2021), it is the reference genome for *T. aestivum* and remains the most contiguous wheat assembly to date (Uauy, 2017), and has been used in a great number of different studies (Brenchley et al., 2012). Additionally, it is susceptible to a wide array of biotic and abiotic stresses, including STB, making it ideal as a susceptible parent for a bi-parental population with a resistant parent such as Renan (Sears and Miller, 1985). Such a bi-parental population was

available to us.

We also worked with a bi-parental *Z. tritici* population obtained from a cross between two strains that were similarly pathogenic on Chinese Spring and differentially adapted to Renan, with one of the strains producing significantly more symptoms than the other on Renan, in identical inoculation and growth conditions.

Two preliminary studies carried out during master's degree internships validated these populations as good material to study quantitative interactions. First in 2017, Renan was shown to carry resistance QTL towards both *Z. tritici* parental strains, with strain specificities, these had varying contributions to the overall phenotype (Murielle Cuenin, Marster's internship 2017). Secondly, in 2018, preliminary results using only half the fungal population, showed that both parental *Z. tritici* strains carry pathogenicity QTL towards Renan, demonstrating a polygenic and quantitative architecture of pathogenicity in these strains (Camilla Langlands-Perry, Master's internship 2018).

Linkage analyses leading to QTL detections are the main methods used in this thesis, for the detection of resistance QTL wheat-side, and pathogenicity QTL fungus-side. With the identification of pathogenicity QTL, we hope to identify candidate genes, which will then be cloned and a potential gene-for-gene interaction validated. Will also be looked into, the allelic variability of the pathogenicity genes identified, to evidence any adaptation to resistance in Renan, and therefore adaptation to quantitative resistance.

The work carried out over the last three and a bit years is summarized in three chapters organized as articles as follows:

I/ Resistance of the wheat cultivar 'Renan' to Septoria leaf blotch explained by a combination of strain specific and strain non-specific QTL mapped on an ultra-dense genetic map

This chapter, which was published as an article in *Genes* on the 31st of December 2021, produced an ultra-dense genetic map for *T. aestivum* using genotyping data from two different SNP (single nucleotide polymorphism) arrays, TaBW410K (Danguy des Déserts et al., 2021; Kitt et al., 2021) and iSelect90K (Wang et al., 2014). It demonstrated that the resistance of Renan to both *Z. tritici* strains used in this project is polygenic, quantitative and strain specific, and enabled the identification of a novel STB resistance locus on wheat chromosome 5D, designated *Stb20q*.

II/ Quantitative and qualitative plant-pathogen interactions call upon similar

pathogenicity genes with a spectrum of effects

In this second chapter, we investigated the genetic architecture of pathogenicity towards Renan in the fungal population. This led to the detection of three pathogenicity QTL, for one of which, the pathogenic allele was carried by the least pathogenic of the fungal parental strains. We identified and cloned two candidate genes with conclusive results for one. This showed that genes fungus-side underlying quantitative interactions can be similar to genes identified in classic R/Avr interactions, that is to say SSP. For the validated SSP, we also looked into haplotype distribution using two *Z. tritici* populations from different time periods. This analysis showed that despite some signatures of diversifying selection, the SSP is highly conserved, suggesting relatively slow evolution of an effector-like gene. We will submit this chapter to *New Phytologist*.

III/ Gene-for-gene interactions between host and pathogen blur the lines between qualitative and quantitative definitions of resistance

This final scientific chapter used linkage analyses to validate QTL-for-QTL interactions between two pairs of coupled resistance QTL and pathogenicity QTL. Using the same methods, this chapter also validates the interaction between *Stb20q* and the previously cloned and validated gene encoding an SSP, prompting its designation as *AvrStb20q*. This interaction is a first in this pathosystem as it does not involve any previously identified major resistance genes. A final experiment was carried out, inoculating a panel of diverse strains on a specific Renan×Chinese Spring progeny which carried the resistance allele only for *Stb20q*. It enabled us to show that the diverse isoforms of *AvrStb20q* all led to quantitative phenotypes on a cultivar carrying *Stb20q*. These phenotypes were distributed into two groups, with different levels of pathogenicity. These two groups were distinguished by the presence or absence of TE insertions in the 3' UTR of *AvrStb20q*, strongly suggesting the involvement of TE in quantitative interaction adaptation mechanisms.

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CHAPTER 2: RESISTANCE OF THE WHEAT CULTIVAR 'RENAN' TO SEPTORIA LEAF BLOTCH EXPLAINED BY A COMBINATION OF STRAIN SPECIFIC AND STRAIN NON-SPECIFIC QTL MAPPED ON AN ULTRA-DENSE GENETIC MAP

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Article

Resistance of the Wheat Cultivar 'Renan' to Septoria Leaf Blotch Explained by a Combination of Strain Specific and Strain Non-Specific QTL Mapped on an Ultra-Dense Genetic Map

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Abstract: Quantitative resistance is considered more durable than qualitative resistance as it does not involve major resistance genes that can be easily overcome by pathogen populations, but rather a combination of genes with a lower individual effect. This durability means that quantitative resistance could be an interesting tool for breeding crops that would not systematically require phytosanitary products. Quantitative resistance has yet to reveal all of its intricacies. Here, we delve into the case of the wheat/Septoria tritici blotch (STB) pathosystem. Using a population resulting from a cross between French cultivar Renan, generally resistant to STB, and Chinese Spring, a cultivar susceptible to the disease, we built an ultra-dense genetic map that carries 148,820 single nucleotide polymorphism (SNP) markers. Phenotyping the interaction was done with two different *Zymoseptoria tritici* strains with contrasted pathogenicities on Renan. A linkage analysis led to the detection of three quantitative trait loci (QTL) related to resistance in Renan. These QTL, on chromosomes 7B, 1D, and 5D, present with an interesting diversity as that on 7B was detected with both fungal strains, while those on 1D and 5D were strain-specific. The resistance on 7B was located in the region of *Stb8* and the resistance on 1D colocalized with *Stb19*. However, the resistance on 5D was new, so further designated *Stb20q*. Several wall-associated kinases (WAK), nucleotide-binding and leucine-rich repeats (NB-LRR) type, and kinase domain carrying genes were present in the QTL regions, and some of them were expressed during the infection. These results advocate for a role of *Stb* genes in quantitative resistance and for resistance in the wheat/STB pathosystem being as a whole quantitative and polygenic.

Keywords: bread wheat; Septoria tritici blotch; quantitative trait loci; *Stb20q*; strain specificity; resistance durability

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of *Stb8* and the resistance on 1D colocalized with *Stb19*. However, the resistance on 5D was new, so further designated *Stb20q*. Several wall-associated kinases (WAK), nucleotide-binding and leucine-rich repeats (NB-LRR) type, and kinase domain carrying genes were present in the QTL regions, and some of them were expressed during the infection. These results advocate for a role of *Stb* genes in quantitative resistance and for resistance in the wheat/STB pathosystem being as a whole quantitative and polygenic.

Keywords: bread wheat; *Septoria tritici* blotch; quantitative trait loci; *Stb20q*; strain specificity; resistance durability

Introduction

Bread wheat (*Triticum aestivum*) is a staple food in many countries worldwide and is an economically important crop. *Septoria tritici* blotch (STB) is the most common disease of wheat and is caused by the ascomycete fungus *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*). It is responsible for high yield losses worldwide, ranging from 30 to 50% loss when environmental conditions are favourable to the disease's development (Eyal et al., 1987; Fones and Gurr, 2015). Disease control is generally undertaken with the application of fungicide treatments and/or cultivation of varieties carrying major resistance (*R*) genes. Neither of these methods, nor indeed their combination, are considered to be durable, thus necessitating constant renewal and research. Indeed, with ever-increasing restrictions on the use of chemical treatments in crops, a fully chemical approach does not seem to be the way to go. Another factor which encourages reducing the use of chemical treatments for this disease is their cost, which comes to more than 400 million euros a year in Europe (O'Driscoll et al., 2014). *R* genes and their pyramiding appear as an excellent alternative. However, due to the existence of a gene-for-gene interaction between *R* genes and *Avr* avirulence genes in the fungus, the former are often overcome. Indeed, a single mutation in the *Avr* gene sequence can suppress recognition by the plant, stopping resistance mechanisms' action (Niks et al., 2015). Quantitative resistance is considered to be polygenic and to have a smaller effect than that due to *R* genes. It therefore imposes less selection pressure on fungal populations, making it more durable. Very few quantitative trait loci (QTL) or quantitative genes for resistance have been cloned, and the few that have are varied in terms of gene families and underlying mechanisms with cases of specificity and non-specificity (Dmochowska-Boguta et al., 2020; French et al., 2016; Fu et al.,

2009; Fukuoka et al., 2009; Fukuoka and Okuno, 2001; Gadaleta et al., 2019; G., Jiang et al., 2020; Krattinger et al., 2009; Manosalva et al., 2009; Niks et al., 2015; Yajun Wang et al., 2019). The *T. aestivum*-*Z. tritici* pathosystem is considered to be primarily quantitative, though 22 major resistance genes in bread wheat have been described (Brown et al., 2015), the most recent being *Stb19* (Yang et al., 2018). In controlled conditions as in the field, resistance to STB manifests itself as being quantitative, largely additive and with varying heritability. Brown et al. (2015) counted 89 STB resistance QTL carrying regions, for a total of 167 individual QTL. Among these 89 regions are 62 QTL and 27 meta-QTL. Brown et al. (2015) describe QTL as showing lower plant stage specificity than major resistance genes. These were not all detected at the same developmental stage, 27 at a seedling stage, 48 in adult plants and 14 for both of these stages. These regions are located on all wheat chromosomes but chromosome 5D, while chromosomes 3B, 6B, and 7B are more represented than others (Brown et al., 2015). Moreover, several recent studies have reported additional QTL for resistance to STB (Gerard et al., 2017; Karlstedt et al., 2019; Odilbekov et al., 2019; Riaz et al., 2020; Vagndorf et al., 2017; Yates et al., 2019), with r^2 values ranging from 3.3% to 29.52%. Notably, Vagndorf et al. (2017) detected QTL on chromosomes 1B, 2A, 5D, and 7A, with the QTL on chromosome 5D being the most effective of all four. These different studies show that STB resistance in wheat is complex, due to the combination of a large number of QTL with varying effects on phenotypes. No STB quantitative resistance genes have been cloned. However, major resistance gene *Stb6* was the first cloned gene specifying resistance to STB (Saintenac et al., 2018). It encodes a wall-associated kinase-like protein, which detects the presence of a matching apoplastic effector. A second major resistance gene *Stb16q* was cloned in 2021, which confers broad-spectrum resistance against *Z. tritici* and encodes a cysteine-rich receptor-like kinase (Saintenac et al., 2021). There is a gene-for-gene relationship between *Stb6* and *AvrStb6* (Zhong et al., 2017), which is the avirulence gene which encodes a small secreted protein, the aforementioned apoplastic effector. This relationship entails specificity between cultivars carrying *Stb6* and strains carrying *AvrStb6*. Created in 1989 by INRA Rennes, Renan is a four-way hybrid resulting from a complex cross between cultivars Courtot, VPMxMoisson, Maris Huntsman, and Mironovskaia-808 (Doré and Varoquaux, 2006). Renan has the advantage of having very good baking quality and a high tolerance to the cold. Renan is resistant to a number of diseases such as cereal rusts, eyespot, septoria leaf blotch, and fusarium. These resistances result in part from the introgression of two chromosomal fragments from *Aegilops ventricosa* which carry the resistance gene to eyespot *Pch1*, on chromosome 7D, and resistance genes to rusts *Lr37*, *Yr17*, and *Sr38* (Dedryver et al., 2009; Doussinault et al., 2001; Hanzalova et al., 2007) on chromosome 2A. Until the end of the 1990s, Renan had a good level of

resistance to septoria leaf blotch. However, this resistance has been overcome by certain strains, even if Renan's global disease resistance level remains reasonably good. Due to its disease resistance qualities, relative shortness and reasonably good competitive value against weeds, Renan is a popular choice for organic farming (Rolland et al., 2017). Chinese Spring is the reference genome for wheat and has been used in a great number of studies (Brenchley et al., 2012). It was the first wheat to have its genome fully sequenced and assembled (IWGSC et al., 2018; Zhu et al., 2021), and that sequence remains the most contiguous wheat assembly to date (Uauy, 2017). Moreover, it is sensitive to a wide array of biotic and abiotic stresses, including STB. This means that it is ideal as a sensitive parent for a bi-parental population with a resistant parent such as Renan (Sears and Miller, 1985). One thing to bear in mind when working with Chinese Spring is that it carries resistance gene *Stb6* and is therefore resistant to isolates carrying the corresponding avirulence gene *AvrStb6* (Zhong et al., 2017). The aim of this study is to better understand quantitative resistance to STB and the isolate-specificities of QTL. We hypothesize that these specificities could be due to minor gene-for-minor gene interactions, such as those that have already been suggested in other works, in the cases of barley—barley leaf rust (González et al., 2012; Qi et al., 1999), barley—barley leaf stripe (Arru et al., 2003), potato—*Phytophthora infestans* (Leonards-Schippers et al., 1994) or indeed pepper and potyviruses (Caranta et al., 1997). Further knowledge of these interactions would give us better understanding of the durability of quantitative resistance.

Materials and methods

Plant and fungal material

The plant material used in this study consisted in a population of 236 Recombinant Inbred Lines (RILs; F6 generation) obtained from a cross between wheat cultivars Renan and Chinese Spring. Hereafter, this population will be referred to as RxCS. The fungal strains used in this study are INRA09-FS0813 and INRA09-FS0732, which we will refer to hereafter as I05 and I07, respectively. These two strains were isolated from leaves of the cultivar Soissons collected from the same field in 2009 at Thiverval-Grignon in France. They are both virulent on *Stb6*. They were chosen for this study out of a panel of eight different strains as they were both pathogenic on Chinese Spring and showed contrasted pathogenicity on Renan. I05 can infect Renan, while I07 cannot.

Experimental setup and procedure for pathology assays

Data sets

Due to the size of the population, it could not be phenotyped in its entirety over one experiment. It was therefore divided into two equal sets, which were tested in 2017 with two replications. All phenotypic traits were evaluated for this set. To corroborate results, 148 individuals were chosen randomly out of the original 236, and tested over three replications in 2018. For this data set, PYC and NBS (explained later on) were not evaluated. For every experiment, Renan and Chinese Spring were used as controls.

Culture conditions

For each individual in the population, five seeds were sown per pot filled with Floradur B (Floradur Pot Medium) potting soil (NPK 14, 16, 18 kg.m⁻³) (Floragard Vertriebs-GmbH, Oldenburg, Germany). The pots were split into trays with 15 pots per tray, for a total of 8 trays per experiment. Before inoculation, plants were cultivated in a climate chamber with a 16h photoperiod, hygrometry fixed at 70% and temperatures at 16 °C night and 20 °C day. Post-inoculation, plants were placed in a climate chamber with a 16h photoperiod, hygrometry fixed at 90% by day, 80% by night and temperatures of 22 °C during daytime and 18 °C at night-time. In the climate chambers, light conditions were maintained at 300 μmol.m⁻².s⁻¹ with eight neon tubes (Osram Lumilux L58W/830) placed 40 cm above the trays (OSRAM GmbH, Munich, Germany).

Inoculum preparation

Inocula were prepared from strains conserved at -80 °C as spores in a 70% water and 30% glycerol mix. The strains are precultured 10 days prior to inoculation in 10 mL of a YPD liquid culture medium (1% yeast, 2% bacto-peptone, 2% glucose). The precultures were kept in a growth chamber for 6 days at 17 °C and a hygrometry of 70% under agitation (140 rpm). Each preculture was then grown in a petri dish (Ø 90) on a PDA (potato dextrose agar) solid culture medium with 50 to 80 μL of inoculum. The day of the inoculation, 150 mL of inoculum were prepared from these cultures, each with a concentration of $1.10^6 \pm 1.10^5$ spores.mL⁻¹. Lastly, one drop of Tween 20 was added per 15 mL of inoculum to insure adherence of the inoculum to the leaf surface.

Inoculation

Plants were inoculated 16 days after sowing. The day before the inoculation, only three plants out of a maximum of five were kept per pot. On the first true leaf (generally 3 to

5 cm from the base) of each plant, a surface of 7.5 cm in length was marked out with two black felt tip lines. The inoculum was spread out on this surface using a square-tipped flat paintbrush in six passages (3 times back and forth). Once all the pots of a tray were inoculated, the procedure was carried out a second time. The paintbrush was dipped into the inoculum before each set of six passages. After inoculation, each tray was watered and covered with transparent polyethylene bags. The bags create a water-saturated atmosphere, which encourages infection (Boixel et al., 2019b; Shaw, 1991). The bags were removed after three days, a 72 h incubation period being the time it takes for the fungus to reach the mesophyll, which is necessary to the rest of the colonisation process (G., H., J., Kema et al., 1996). To optimise conditions for the survival of the inoculated leaf and to homogenise the quantity of light received by each leaf, new leaves were cut 2 to 3 cm above the first node 10 days post-inoculation (dpi).

Evaluation of phenotypic traits

Visual evaluation of symptoms

The leaf area marked out with black felt tip was visually evaluated at 14, 20, and 26 dpi. The percentage of the surface which was green, necrotic, and sporulating was evaluated. The sporulating area is defined here as the area which presents pycnidia, regardless of density, colour, or size. The values for sporulating area at 14, 20, and 26 dpi were used as phenotypic traits in linkage analyses and are referred to as S14, S20 and S26. The chlorotic area was not evaluated because it is deductible from the green and necrotic areas. These notations were used to calculate AUDPCs (Area Under the Disease Progress Curve) for the green, necrotic, and sporulating areas (AUDPCG, AUDPCN, and AUDPCS, respectively) (Ouaja et al., 2020). The formula for calculating an AUDPC is as follows:

$$\text{AUDPC} = \frac{\sum[(t_{i+1} - t_i) \times (y_i + y_{i+1})]}{2}$$

With:

$t_{i+1} - t_i$: number of days between two notations (6 days)

y_i : percentage of green, necrotic, or sporulating area at day i (for AUDPCG, AUDPCN and AUDPCS respectively)

y_{i+1} : percentage of green, necrotic, or sporulating area at day $i+1$ (for AUDPCG, AUDPCN and AUDPCS respectively).

Pycnidia counting by image analysis

The following procedure and macros used were developed by Stewart and McDonald (2014) and improved by Stewart et al. (2016). Images were obtained by scanning the inoculated part of the leaf. Firstly, for sample identification, A4 pages are generated using a Linux supported macro. The page is divided into eight sections, each carrying a QR code which is specific to a sample. For each sample the three leaves are glued inside the corresponding section. Pages are then scanned using a CanoScan 9000F MarkII scanner (resolution = 1200 dpi, luminosity = contrast = 0) and the resulting images are saved as .tiff files. Pycnidia density was evaluated using a macro in ImageJ (Schneider et al., 2012). This macro is able to quantify several traits which are the percentage of the leaf surface covered by lesions or by necrosis only (PLACL or PLACN respectively), the total number of pycnidia per sample, the size of the pycnidia and the grey value of the pycnidia. For this study, only the total number of pycnidia per sample was taken into account (one sample being the three inoculated leaves for each strain). Parameters within ImageJ were adjusted for each image as it was not possible to use the exact same parameters for each sample, depending on the colours of the leaves.

Pycnidia density was calculated for each sample using the following formula:

$$PYC = \frac{n_{pycnidia\ total}}{N_{total}}$$

With:

$n_{pycnidia\ total}$: total number of pycnidia in the sample.

N_{total} : total necrotic surface of the sample.

Quantification of sporulation

Sporulation was quantified with the use of the particle size & shape analyser Occhio Flowcell FC200S+HR (Occhio s.a., Angleur, Belgium). This tool is controlled by a computer which, with the help of image analysis, can precisely count particles while evaluating their size and shape. It is used alongside the Callisto software which controls the quality of the analysis. The user interface allows one to control various parameters such as light, image resolution and particle selection settings (Table 1). The latter is particularly important as it means that any particle that is not a pycnidiospore can be excluded from the analysis. Preparation of the samples for analysis is done the day after the last visual notation (27 dpi). The inoculated area of the leaf is cut out. The three leaves from each genotype are placed into a 15 mL Falcon tube containing 0.75 mL of

osmosed water and two pieces of blotting paper (10.5 cm × 0.5 cm). The blotting paper maintains a water-saturated atmosphere within the tube which favours the extrusion of cyrri from pycnidia, and thus sporulation (Palmer and Skinner, 2002). Tubes are then placed in a growth chamber for at least 18 h. On the next day, 5 mL of an 80% water 20% glycerol mix is added to each tube. The tubes are then gently agitated so that the spores present on the surface of the leaves are transferred to the liquid phase. The leaves are then removed from the tubes and mounted on a white paper sheet for scanning. The tubes containing pycnidiospores suspended in 5.75 mL water/glycerol are kept at –20 °C awaiting analysis. Before the analysis, each tube is homogenized after the addition of a drop of Tween 20 to the mix. 0.9 mL of the mix are used for each analysis. As each sample is passed through the particle counter, an image control is used to verify that all particles are counted independently. Over 350,000 particles mL⁻¹, the initial mix is too concentrated in particles thus it becomes necessary to dilute the sample and repeat the procedure. The particle counter is rinsed with osmosed water between each sample.

Table 1. Settings for the Occhio Flowcell FC200S+HR.

Particle counter	Diameter	0–8 µm
	Size	7–85 µm
	Circonference	0–0.70 µm
Grey value		195-205
Luminous intensity		7.5
Spacer thickness		150 µm
Resolution	Magnification	x4
	Calibration	0.47 µm.pixel ⁻¹ (1 pixel=1.67 µm)

For each sample, the total number of pycnidiospores was extracted from data obtained from this particle counter. The total number of pycnidiospores was used to calculate the number of pycnidiospores per pycnidium after image analysis.

The number of pycnidiospores per pycnidium (NBS) was calculated using the formula:

$$\text{NBS} = \frac{n_{\text{pycnidiospores total}}}{n_{\text{pycnidia total}}}$$

With:

$n_{\text{pycnidiospores total}}$: total number of pycnidiospores extracted from the sample

$n_{\text{pycnidia total}}$: total number of pycnidia in the sample

Statistical analysis of phenotypic data

The obtained data sets were analysed with the R software (R Core Team, 2019), for each trait an analysis of variance (ANOVA) was performed with the following model:

$$Y_{ij} = \mu + I_i + r_j + Ir_{ij} + \varepsilon_{ij}$$

With Y_{ij} the trait which is being studied, μ the mean value for this trait, I_i the individual genotype, r_j the replication, Ir_{ij} the interaction and ε_{ij} the residual. For the following analyses, Ir_{ij} was included in the residual.

The following hypotheses were verified after the variance analyses:

$$\varepsilon \sim N(0, \sigma^2) \rightarrow \text{cov}(\varepsilon, \varepsilon') = 0 \quad \text{Homoscedasticity (homogeneity of var}(\varepsilon))$$

Broad sense heritability is defined by the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

With H^2 the heritability, σ_g^2 genotypic variance and σ_e^2 residual variance.

The correlation between traits was also studied using the Bravais–Pearson correlation.

Genotyping R×CS

The wheat population was genotyped on two different single nucleotide polymorphism (SNP) arrays, the Breedwheat Affymetrix Axiom 410K array (Danguy des Déserts et al., 2021; Kitt et al., 2021) and the Illumina Infinium iSelect Wheat 90K array (Wang et al., 2014).

Axiom 410K

In total, 429 individuals from the R×CS population were genotyped on the Axiom 410K array in two sets of 282 and 147. Among these, 236 individuals were randomly selected for further phenotyping. DNA extraction and genotyping were performed by the Gentyane platform (INRAE, Clermont-Ferrand, France).

iSelect 90K

For genotyping on the iSelect 90K array, 159 individuals chosen randomly among R×CS and both parental varieties were genotyped. DNA extraction and genotyping were performed at TraitGenetics GmbH (Gatersleben, Germany).

Genetic analyses

Construction of an ultra-dense genetic map

Markers were re-named so as to be tagged with their SNP array of origin and the chromosome they most likely mapped to as this made following steps more straightforward. There are 13,462 markers in common between the 90K array and the 410K array. These were considered to be distinct for map construction. Out of a total of 409,695 SNP, were kept for the map building file only those that were polymorphic and for which the information from both parents was available, leading to a total of 194,630 SNP. Additionally, only 142 individuals' information was used for map construction as for 17 individuals, genotyping on the 90K had done poorly.

The file comprising all polymorphic markers from the TaBW410K and the iSelect90K arrays was input into the Multipoint ultra-dense software developed by MultiQTL Ltd. at Haifa University in Israel. This software allows ultra-dense genetic maps to be built and is based on the "twin algorithm" (Ronin et al., 2017). A stringent filter for missing data was applied, all SNP with over 5% missing data were filtered out. Then, a filter to correct any potential segregation distortion was applied; it was less stringent, allowing a Chi² up to 9.5. After the first clustering, linkage groups, which belonged to the same chromosome, were merged and the results from Multipoint were transformed with the Kosambi mapping function (Kosambi, 2016). The linkage map was graphically visualized with Mapchart V2.3 (Voorrips, 2002).

Linkage analysis

A linkage analysis was carried out using the R/qtl software (Broman et al., 2003) version 1.42-8. This analysis included for each trait an initial Simple Interval Mapping (SIM), followed by a Composite Interval Mapping (CIM). Analyses were performed replication by replication and set by set (sets only concerning the first lot of phenotypic data). For SIM, 1000 genome-wide permutations were used to calculate the significant logarithm of odds (LOD) threshold. Only QTL that showed p -values < 0.05 were considered significant. The CIM was carried out with the SNP with the highest LOD at QTL peaks used as a covariate. When there were several significant QTL detected, the CIM was recalculated with two covariates, however, this never led to any extra detections. QTL intervals were evaluated with the LOD support interval with a drop in LOD of 1 and the "expandtomarkers" argument as true. QTL effects were calculated with the "effectplot" and "effectscan" functions. Possible epistatic interactions between QTL were looked into using the "addint" function.

QTL gene content

The gene content of the QTL regions was analysed using the IWGSC RefSeq v1.1 annotation, which is anchored on the IWGSC RefSeq v1.0 assembly (both available at <https://wheat-urgi.versailles.inra.fr/>; accessed on 03/12/2021). We specifically searched for the content in wall-associated kinases (WAK), and nucleotide-binding and leucine-rich repeats (NB-LRR) type genes using the IWGSC's 2018 work on manually curated gene families (IWGSC et al., 2018) and we searched through the annotated genes using the keyword "kinase". By using the Wheat Expression Browser powered by expVIP (<http://www.wheat-expression.com/>; accessed on 03/12/2021) (Borrill et al., 2016; Ramírez-González et al., 2018) we were able to access transcriptional data for each of the listed genes in a kinetic of STB infected plants. The data available was for the cultivar Riband at 1, 4, 9, 14, and 21 dpi infected with fungal strain IPO323 (Rudd et al., 2015). This cultivar is regarded as a good positive control for *Z. tritici* infection as it is highly susceptible to the disease, moreover, it does not carry the major resistance gene *Stb6*, unlike Chinese spring for instance (Chartrain et al., 2005b). To compare with known STB resistance genes, we included the expression data for *Stb6* and *Stb16q*, respectively known as *TraesCS3A02G049500* and *TraesCS3D02G500800* in the RefSeq v1.1 annotation.

Results

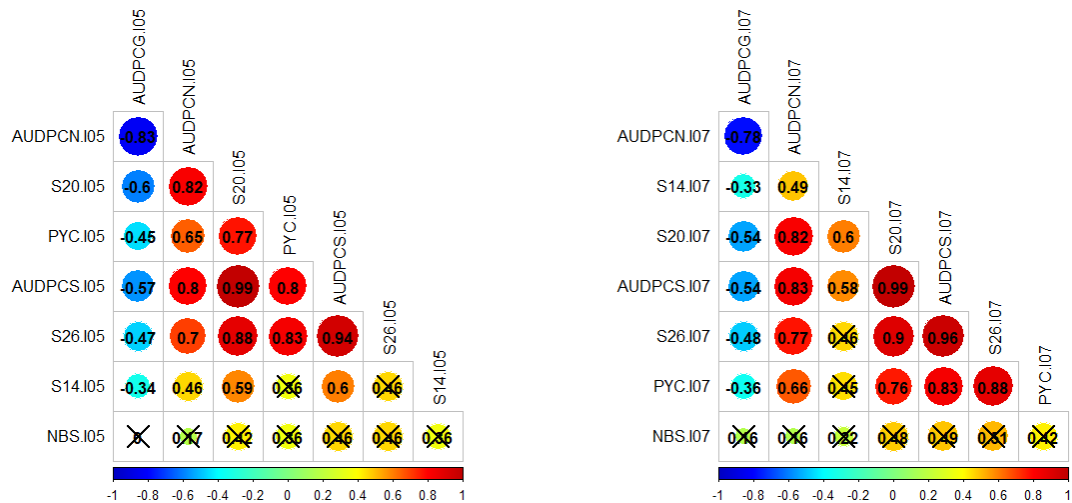
Description of phenotypes

Two phenotypic data sets were acquired on the Renan × Chinese-Spring population for the isolates I05 and I07. The first data set was collected in 2017 on 236 RILs with two replications, and the second data set was collected in 2018 on 148 RILs with three replications. Overall, distributions of phenotypic traits within the population are similar between replications, and Renan is consistently more resistant than Chinese Spring (Figures S1 and S2). The second replication from the 2018 data set of isolate I05 stands out as being quite different from the others, in particular for S26 and AUDPCS. For this replication, the level of infection was lower than for the other replications and heterogeneous, suggesting that infection failed. Therefore, the following analyses were performed excluding the data from this particular replication, leaving only two replications for the 2018 data set of isolate I05. It is also important to notice that distributions are not bi-modal; phenotypes follow a continuous distribution indicating the presence of several genes with quantitative effect. Only for S26 in 2018, for both isolates I05 and I07, does the distribution not look strictly continuous, but rather

skewed towards susceptibility. Finally, the parental phenotypes do not mark the limits of the distribution. Transgressive individuals can be observed for the great majority of studied traits indicating that despite Chinese Spring being susceptible to both fungal strains, it can carry small effect resistance QTL. Correlation coefficients were calculated from mean values between replications using the Bravais–Pearson correlation coefficient (Figure 1). For these analyses, the 2017 sets were analysed together. Overall, AUDPCN, AUDPCS, S20, S26 and PYC (only 2017 data) were all strongly correlated. AUDPCG was poorly correlated to the other traits, except for the 2018 I07 data, where it is strongly negatively correlated to the other traits. Overall, the S14 and NBS traits were poorly correlated to the other traits. This is certainly due to the low values and low variability in S14 data, and the low reproducibility in NBS data. These results suggest a potential common genetic base for all of the studied traits.

2017

I05I07



2018

I05I07

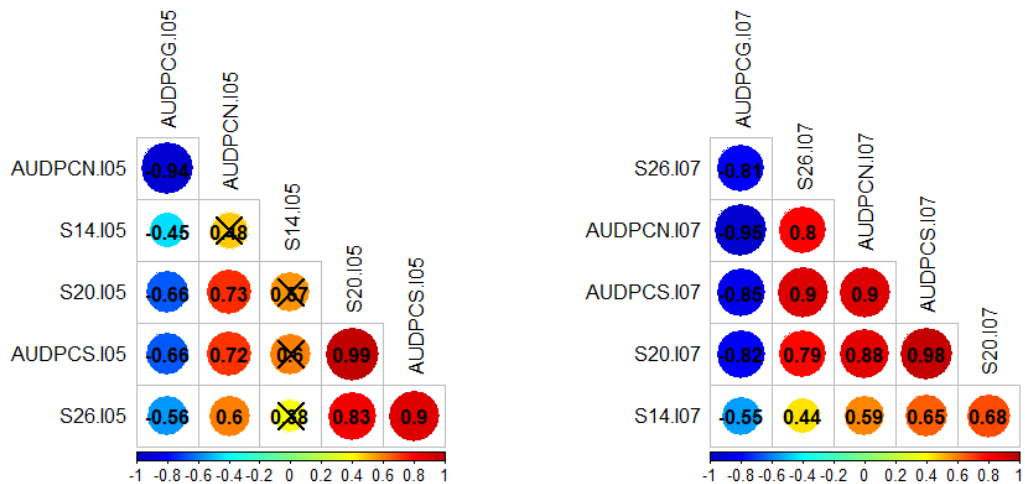


Figure 14. Bravais–Pearson correlograms for the four datasets; crossed out correlation values are not statistically significant (p -value < 0.05). The left column presents the phenotypic trait correlations for RxCS inoculated with I05, the right presents the phenotypic trait correlations for RxCS inoculated with I07. PYC is the pycnidia density. NBS is the number of spores per pycnidiospore. AUDPCG is the area under the disease

progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is the area under the disease progress curve for the sporulating leaf area. S14, S20, and S26 are the sporulating leaf area at 14, 20, and 26 days post-inoculation respectively.

The ANOVA results show that the individual genotype systematically had a significant effect on the phenotype in all cases but I05_2017/1 S14 and NBS, and I07_2017/2 S14 (Table 2). The replication overall had a strong effect on phenotypes, except for the I07_2017/2. Subsequent linkage analyses were therefore carried out replication by replication. Broad-sense heritability was calculated for all traits and was systematically higher for I07 data than for I05 data (Table 2). As the ANOVA assumptions are not respected in all cases, low heritability value does not necessarily indicate that the trait will not lead to the detection of resistance QTL.

Table 2. Statistical analysis of the phenotypic data for each dataset.

Isolate Set	Trait	Statistical Significance of the Genotype ¹	Statistical Significance of the Replication ¹	MSg ²	MSe ³	Broad-Sense Heritability	Shapiro-Wilk Normality Test on Residuals	Independence of Residuals	Homoscedasticity Bartlett Test of Homogeneity of Variances
I05_2017/1	S14		***	27	27	0.00	0.00	no	0.04
	S20	***	***	1094	444	0.42	0.11	yes	0.14
	S26	***	***	877	364	0.41	0.12	yes	0.03
	AUDPCG	*	*	51,787	35,921	0.18	0.92	yes	0.05
	AUDPCN	*	*	77,482	55,907	0.16	0.85	yes	0.31
	AUDPCS	***	***	83,562	32,115	0.44	0.11	yes	0.26
	PYC	***		21,769	9037	0.41	0.29	yes	0.29
	NBS		***	1,003,915	868,109	0.07	0.00	yes	0.05
I07_2017/1	S14		**	2	2	0.04	0.00	no	0.00
	S20	***	***	401	157	0.44	0.00	yes	0.85
	S26	***	***	765	233	0.53	0.40	yes	0.98
	AUDPCG	***		57,879	30,256	0.31	0.00	no	0.00
	AUDPCN	***	***	92,012	25,943	0.56	0.95	yes	0.77
	AUDPCS	***	***	40,045	12,909	0.51	0.00	yes	0.97
	PYC	***		29,153	8834	0.53	0.01	no	0.04
	NBS	***		1,865,055	630,731	0.49	0.00	no	0.00
I05_2017/2	S14	***	*	14	7	0.34	0.00	no	0.00
	S20	**	.	533	344	0.22	0.94	yes	0.54
	S26	**		665	378	0.28	0.40	yes	0.89
	AUDPCG	*	***	38,627	27,765	0.16	0.02	no	0.04
	AUDPCN	*	***	62,573	42,711	0.19	0.93	yes	0.35
	AUDPCS	**	.	44,817	26,387	0.26	0.74	yes	0.79
	PYC	***	***	22,651	12,737	0.28	0.97	yes	0.34

	NBS	**	***	816,528	495,995	0.24	0.00	yes	0.04
I07_2017/2	S14	***		31	13	0.42	0.00	no	0.00
	S20	***		1195	189	0.73	0.00	no	0.01
	S26	***		1411	222	0.73	0.60	yes	0.33
	AUDPCG	***		79,008	28,924	0.46	0.16	yes	0.22
	AUDPCN	***		115,168	23,787	0.66	0.24	yes	0.01
	AUDPCS	***		102,594	13,479	0.77	0.00	no	0.03
	PYC	***	***	59,415	18,267	0.53	0.82	yes	0.65
	NBS	*	*	1,341,693	906,931	0.19	0.00	yes	0.27
I05_2018 only replications 1 and 3	S14	**	***	140	86	0.24	0.00	no	0.00
	S20	***	***	1649	747	0.38	0.28	yes	0.94
	S26	*	***	877	661	0.14	0.00	no	0.82
	AUDPCG	***	***	32,787	17,602	0.30	0.00	no	0.00
	AUDPCN	***	***	58,506	28,592	0.34	0.86	yes	0.26
	AUDPCS	***	***	115,164	50,508	0.39	0.66	yes	0.65
I07_2018	S14	***		43	22	0.25	0.00	no	0.00
	S20	***	**	3061	368	0.71	0.00	no	0.00
	S26	***	***	3270	477	0.66	0.00	no	0.00
	AUDPCG	***		144,099	32,073	0.54	0.32	yes	0.02
	AUDPCN	***	**	170,267	29,795	0.61	0.00	yes	0.00
	AUDPCS	***	***	242,232	25,587	0.74	0.01	yes	0.00

¹ Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1; ² MSg is the mean square value for the individual genotypes output by the ANOVA; ³ MS ϵ is the mean square value for the residuals output by the ANOVA.

An ultra-dense genetic linkage map

We genotyped 159 RILs from the RxCS population with both TaBW410K and iSelect90K arrays. These two arrays share 13,670 common markers, but comparison of parental genotypes between arrays for these common markers revealed an average divergence of 11.1% for both cultivars. Due to these divergences common markers between both arrays were given different names and considered separately for map construction. Overall, only markers which were polymorphic between both parents and presented no missing data, lack of amplification or heterozygosity for either parent were kept, leading to a total of 183,773 usable markers from the TaBW410K array, representing 43.4% of the original data set, and a final list of 10,857 markers for the iSelect 90K array, representing 13.3% of the original data set. Consequently, for this population, the rate of polymorphic markers was more than three times higher on the TaBW410K than on the iSelect90K. The 194,630 SNP markers available for map construction were all re-named to show which array they came from and onto which chromosome they were expected to map based on best BLAST values on the reference genome (Chinese Spring). This enabled us to associate linkage groups and corresponding chromosomes more easily when building the map. From the 159 individuals genotyped with both TaBW410K and iSelect90K arrays, 17 individuals were discarded because of their high number of missing data from the iSelect90K genotypes. Moreover, applied filters deleted 13.4% of the markers, leaving us with a matrix of 142 individuals x 168,522 high quality markers for map construction. After the initial clustering, 25 linkage groups were obtained. Those that corresponded to different parts of the same chromosome were merged. The map we obtained comprised 21 linkage groups, each corresponding to a chromosome (Table 3). It is composed of 5357 genetic bins or unique positions, for a total of 148,820 markers and covers a total genetic distance of 4277 centiMorgans (cM). Table S1 provides the complete map. Of all mapped markers, 3.54% were skeleton markers, each representing a genetic bin. Of the original number of available markers from the SNP arrays, 30% were mapped, and of the markers chosen for mapping after all filters, 78% were mapped. Sub-genomes A and B carry more SNP than sub-genome D. When working with the full set of markers, sub-genome D represents only 16.50% of this latter set, while A and B sub-genomes represent 42.78% and 40.72%, respectively. However, sub-genome B has a shorter genetic length than either of the other two. The great majority of mapped markers are from the Breedwheat Axiom 410K array; indeed, these SNP represent 94.73% of sub-genome A, 94.61% of sub-genome B and 97.91% of sub-genome D. The 90K array is particularly underrepresented on the D sub-genome with markers making up a minimum of 0.84% on chromosome 4 and a maximum of 3.73% on chromosome 6. The distance between consecutive markers

ranges from 0.35 cM to a maximum of 21.54 cM. However, a gap this size is exceptional. Indeed overall, consecutive markers are quite close together, with the average distance between consecutive markers being 0.74 cM in sub-genome A, 0.69 cM in sub-genome B and 1.07 cM in sub-genome D. The third quartile in the distribution of the distance between consecutive markers is 0.72 and 0.71 cM for sub-genomes A and B respectively, while it is slightly higher for sub-genome D with a value of 1.1 cM. In sub-genome A, six gaps in the map are larger than 10 cM on chromosomes 3A, 4A, 5A and 7A. In sub-genome B, only one gap is larger than 10 cM and is on the long arm of chromosome 3B. Moreover, sub-genome D has four gaps larger than 10 cM on chromosomes 1D, 6D (twice), and 7D.

Table 3. Characteristics of the RxCS genetic linkage map

Chromosome	Number of SNP	Number of Genetic Bins	Map Length (cM)	Marker Density (Markers/cM)	
A genome	1	10,479	358	234.44	44.7
	2	11,716	288	189.72	61.8
	3	8413	302	233.28	36.1
	4	8465	203	170.24	49.7
	5	6723	411	312.17	21.5
	6	7816	226	189.03	41.3
	7	10,051	378	273.38	36.7
total A	63,663	2167	1602.26	39.7	
B genome	1	10,503	309	176.4	59.5
	2	9158	298	197.98	46.3
	3	12,016	374	268.03	44.8
	4	5888	180	140.45	41.9
	5	5019	234	192.98	26
	6	10,210	173	109.66	93.1
	7	7803	336	215.35	36.2
total B	60,597	1905	1300.85	46.6	
D genome	1	3609	157	172.81	20.9
	2	4601	134	150.27	30.6
	3	3512	225	225.25	15.6
	4	2131	174	156.99	13.6
	5	2581	203	234.75	11
	6	4180	183	204.6	20.4
	7	3946	211	228.97	17.2
total D	24,560	1287	1373.64	17.9	
Total	148,820	5357	4276.75	34.8	

The comparison between the order of the markers on the genetic map and their assumed physical position shows that, overall, the genetic map follows the assumed physical positions very well (Figure 2). The exception is chromosome 4D, which presents a cluster of markers at the end of the linkage group, which would have been expected to be found in the short arm according to their assumed physical positions (Figure 2). The ratio between genetic and assumed physical positions is also interesting to comment on as it clearly shows the structure of the different chromosomes with SNP distribution following a neat sigmoidal pattern. Indeed, the position of centromeres is marked out by the recombination suppression surrounding them on all chromosomes (Figure 2).

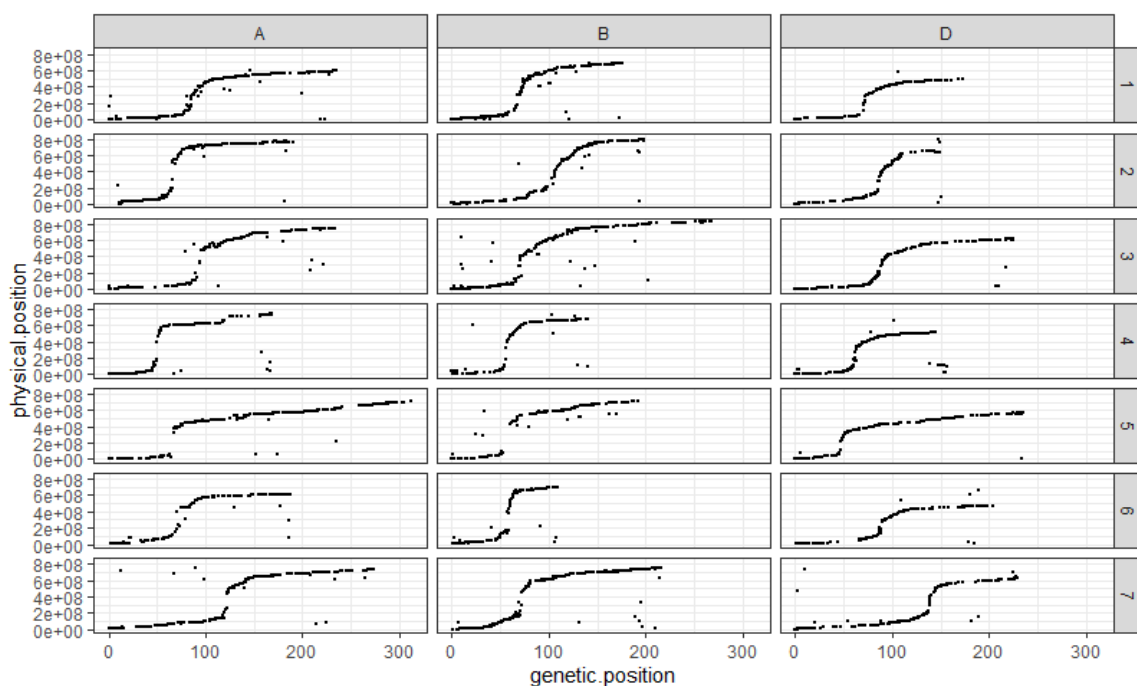


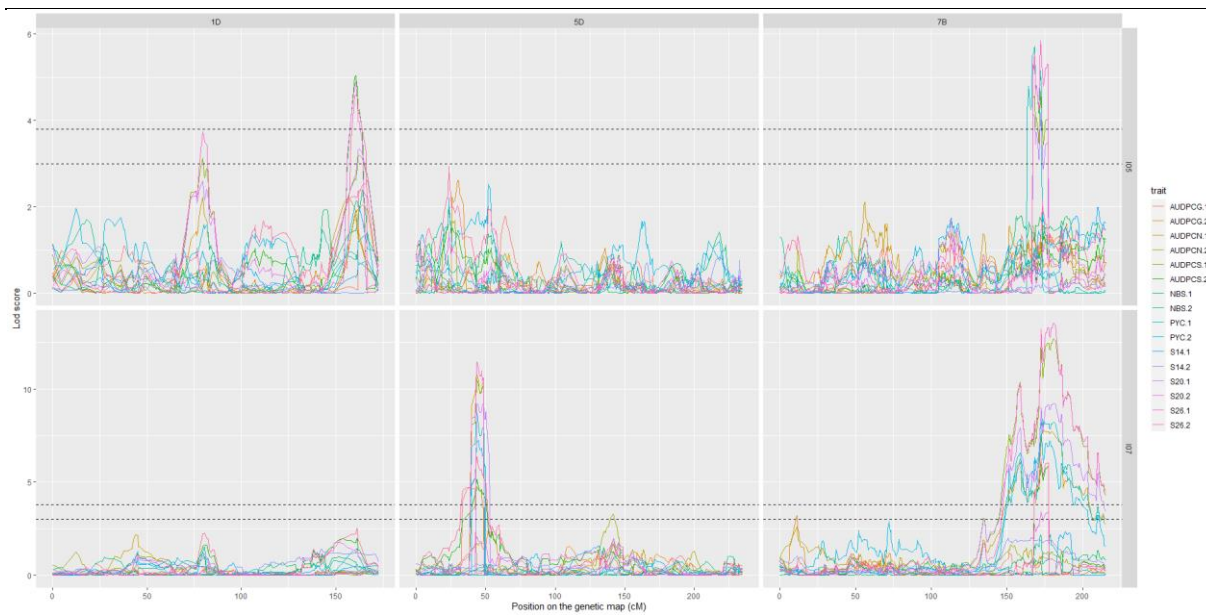
Figure 2. Comparison between markers' genetic and assumed physical positions for each chromosome in each sub-genome. The x-axis corresponds to the genetic position of the mapped SNP on the RxCS genetic map in cM. The y-axis corresponds to the assumed physical position of the SNP on the chromosome they are on in bp. Each column corresponds to a wheat sub-genome, A, B and D respectively. Each line corresponds to a chromosome number; the chromosomes are numbered 1 through 7. Each black dot corresponds to a SNP.

Mapping QTL for resistance

The analyses of the 2017 and 2018 datasets lead to the detection of several QTL on eight different chromosomes. Among these, only three were detected more than once throughout the analyses, we will therefore focus on these three. The complete linkage

analysis results can be found in Table S2. The three robust QTL were detected on chromosomes 1D, 5D and 7B (Tables 4 and 5). The parent carrying the resistant allele is Renan for all three QTL. The QTL however, do not impart resistance to the same strains. Indeed, *Qstb.renan-1D* imparts resistance to I05 while *Qstb.renan-5D* imparts resistance to I07 and *Qstb.renan-7B* is effective against both strains. *Qstb.renan-7B* explained up to 32% of phenotypic variation with a mean r^2 value of 20% when detected with the 2017 datasets and up to 38% with a mean value of 21% when detected with the 2018 datasets. *Qstb.renan-1D* was detected the fewest times out of the three repeatable QTL and explained the least phenotypic variation, between 6 and 13.5% on average, to a maximum of 15%. This QTL was not detected with the 2017/2 dataset (Figure 3B). Finally, *Qstb.renan-5D* explained up to 35.5% of phenotypic variation with a mean r^2 value between 15.5 and 26%. We did not identify any QTL trait specificity in the various linkage analyses, as was to be expected regarding the correlations between traits. The traits that led to the most detections are S26, AUDPCS and PYC. The traits associated with the highest r^2 values overall are AUDPCN, AUDPCS, S26, and PYC. Concatenated results between the 2017 and 2018 data sets result firstly in *Qstb.renan-7B* that is 50.86 Mb long, spanning 64.62 cM. Secondly, *Qstb.renan-1D* is 4.28 Mb long, covering 17.79 cM and carrying 5 SNP with known physical positions. Finally, *Qstb.renan-5D* is 312 Mb long, spanning 28.58 cM. The physical intervals were estimated using markers in the QTL with known positions mapping to the chromosome in question. These numbers take into account every detection of each QTL, and therefore include the least precise detections; this explains, in part, the very large intervals. We however do detect maximum LOD score peaks in the same area throughout the analyses (Figures 3 and 4). No significant interactions were detected between QTL.

A. Set 1



B. Set 2



Figure 3. LOD score profiles for the linkage analyses of the 2017 datasets. **(A)** presents the results for Set 1, **(B)** presents the results for Set 2. In dotted lines are represented the minimal and maximal LOD threshold values obtained in the linkage analyses after 1000 permutations tests. The x-axis represents the position of the markers on the genetic map in cM. The y-axis represents the LOD score associated to the markers. Each column corresponds to a chromosome, chromosomes 1D, 5D and 7B respectively. Each line corresponds to a fungal strain, I05 and I07 respectively. The colours in the graphs correspond to the studied traits for each replication. PYC is the pycnidia density. NBS is the number of spores per pycnidiospore. AUDPCG is the area under the disease

progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. S14, S20 and S26 are the sporulating area at 14, 20 and 26 days post-inoculation respectively. Each trait was studied over two replicates, 1 and 2

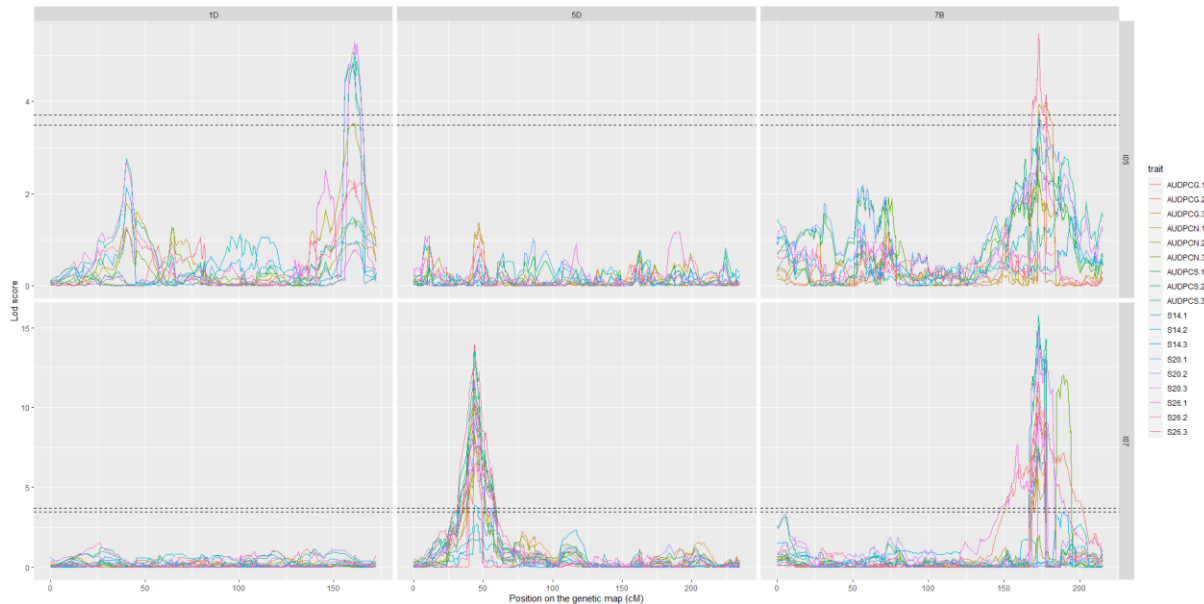


Figure 4. LOD score profiles for the linkage analyses of the 2018 dataset. In dotted lines are represented the minimal and maximal LOD threshold values obtained in the linkage analyses after 1000 permutations tests. The x-axis represents the position of the markers on the genetic map in cM. The y-axis represents the LOD score associated to the markers. Each column corresponds to a chromosome, chromosomes 1D, 5D, and 7B respectively. Each line corresponds to a fungal strain, I05 and I07 respectively. The colours in the graphs correspond to the studied traits for each replication. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. S14, S20, and S26 are the sporulating area at 14, 20, and 26 days post-inoculation respectively. Each trait was studied over three replicates, 1, 2, and 3.

Table 4. QTL for resistance to STB detected with the phenotypic data generated in 2017 and the RxCS genetic map.

QTL.2017	Number of Detections	r² Max (%)	Mean r² (%)	Peak Marker Associated with r² Max	Parent Carrying the Resistance Allele	Traits	Detected with
<i>Qstb.renan-1D</i>	3	7.5	6	cfn1317667_410K_1DS	Renan	S20, S26, AUDPCS	I05
<i>Qstb.renan-5D</i>	22	35.5	26	cfn2823104_410K_5DS	Renan	S20, S26, AUDPCG, AUDPCN, AUDPCS, PYC	I07
<i>Qstb.renan-7B</i>	37	32	20	cfn0449267_410K_7BL	Renan	S14, S20, S26, AUDPCG, AUDPCN, AUDPCS, PYC, I05 and I07 NBS	

Table 5. QTL for resistance to STB detected with the phenotypic data generated in 2018 and the RxCS genetic map.

QTL.2018	Number of Detections	r² Max (%)	Mean r² (%)	Peak Marker Associated with r² max	Parent Carrying the Resistance Allele	Traits	Detected with
<i>Qstb.renan-1D</i>	5	15	13.5	cfn1315024_410K_1DS	Renan	S20, AUDPCN, AUDPCS	I05
<i>Qstb.renan-5D</i>	18	21.5	15.5	cfn2827993_410K_5DS	Renan	S14, S20, S26, AUDPCG, AUDPCN, AUDPCS	I07
<i>Qstb.renan-7B</i>	22	38	21	cfn0916416_410K_7BL	Renan	S14, S20, S26, AUDPCG, AUDPCN, AUDPCS	I05 and I07

Gene content of the QTL

There are two cloned major resistance genes to STB in wheat to date. The first is *Stb6*, which encodes a wall-associated kinase-like protein and detects the presence of a matching apoplastic effector (Saintenac et al., 2018). The second is *Stb16q*, which encodes a cysteine-rich receptor-like kinase and confers broad-spectrum resistance against *Z. tritici* (Saintenac et al., 2021). With this in mind, along with the very large QTL intervals we are working with, we opted to focus on three particular gene families when analysing gene content within the QTL intervals. These families are wall associated kinases (WAK), nucleotide binding leucine-rich receptors (NB-LRR) type genes and genes carrying a kinase domain, all of which have often been associated with disease resistance (Kourelis and van der Hoorn, 2018; Saintenac et al., 2021).

Qstb-renan-1D holds a total of 141 annotated genes. Of the 141, 25 are NB-LRR-type genes, while 5 are WAKs and one has a kinase domain. *Qstb-renan-5D* holds 1981 annotated genes, none of which are NB-LRR-type genes. It holds 12 WAKs and 22 genes with kinase domains, two of which are projected to be receptors or receptor-like, these are *TraesCS5D02G166400* and *TraesCS5D02G181500*. *Qstb-renan-7B* holds 616 annotated genes. This QTL holds no NB-LRR type genes, one WAK, and one gene with a kinase domain. Table 6 presents the details concerning the NB-LRR, WAK and kinase domain carrying genes in the QTL intervals.

Table 6. WAK¹, NB-LRR², and kinase domain carrying genes identified in the three QTL intervals.

QTL	Gene.ID	RefSeq v1.1 ID	Start (bp)	Stop (bp)	Annotation
Qstb-renan-1D	<i>BST_chr1D_nlr_115</i>	<i>TraesCS1D02G015500</i>	7277369	7280463	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_114</i>	<i>TraesCS1D02G016026</i>	7381284	7384806	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_113</i>	<i>TraesCS1D02G016100</i>	7419157	7422949	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_9</i>	<i>TraesCS1D02G016900</i>	7592690	7609204	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_10</i>	<i>TraesCS1D02G016983</i>	7671168	7676063	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_11</i>	<i>TraesCS1D02G016991</i>	7678267	7680938	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_12</i>	<i>TraesCS1D02G017400</i>	7820918	7823449	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_13</i>	<i>TraesCS1D02G017600</i>	7868467	7872465	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_112</i>	<i>TraesCS1D02G018700</i>	8182627	8186066	NB-LRR
Qstb-renan-1D	<i>BST_expressed_pseudo_chr1D_nlr_111</i>	<i>TraesCS1D02G018800</i>	8226547	8230158	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_110</i>	<i>TraesCS1D02G019600</i>	8605145	8610344	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_14</i>	<i>TraesCS1D02G019700</i>	8610887	8616204	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_109</i>	<i>TraesCS1D02G020619</i>	8838803	8842102	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_108</i>	<i>TraesCS1D02G021000</i>	9028322	9037195	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_106</i>	<i>TraesCS1D02G021200</i>	9086119	9091764	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_104</i>	<i>TraesCS1D02G021751</i>	9309301	9328352	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_16</i>	<i>TraesCS1D02G022500</i>	9575753	9593333	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_102</i>	<i>TraesCS1D02G026000</i>	10661025	10664946	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_17</i>	<i>TraesCS1D02G028200</i>	11175841	11182532	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_18</i>	<i>TraesCS1D02G028600</i>	11272449	11278362	NB-LRR
Qstb-renan-1D	<i>BST_expressed_pseudo_chr1D_nlr_19</i>	<i>TraesCS1D02G028700</i>	11287245	11292876	NB-LRR
Qstb-renan-1D	<i>BST_pseudo_chr1D_nlr_20</i>	<i>TraesCS1D02G028736</i>	11319796	11321348	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_21</i>	<i>TraesCS1D02G029000</i>	11408761	11415088	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_22</i>	<i>TraesCS1D02G029100</i>	11451423	11459353	NB-LRR
Qstb-renan-1D	<i>BST_chr1D_nlr_23</i>	<i>TraesCS1D02G029200</i>	11493627	11499140	NB-LRR
Qstb-renan-1D	<i>TaWAK38_1D-gene</i>	<i>TraesCS1D02G016200</i>	7429822	7445013	WAK
Qstb-renan-1D	<i>TaWAK39_1D-gene</i>	<i>TraesCS1D02G016800</i>	7583590	7587977	WAK
Qstb-renan-1D	<i>TaWAK40_1D-gene</i>	<i>TraesCS1D02G017700</i>	7874518	7876881	WAK
Qstb-renan-1D	<i>TaWAK41_1D-gene</i>	<i>TraesCS1D02G017800</i>	7877418	7880329	WAK

QTL	Gene.ID	RefSeq v1.1 ID	Start (bp)	Stop (bp)	Annotation
<i>Qstb-renan-1D</i>	<i>TaWAK42_1D-gene</i>	<i>TraesCS1D02G017900</i>	7896854	7899155	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK349_5D-gene</i>	<i>TraesCS5D02G043400</i>	42925913	42928461	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK350_5D-gene</i>	<i>TraesCS5D02G043500</i>	42930408	42932902	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK351_5D-gene</i>	<i>TraesCS5D02G043532</i>	42944893	42947212	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK352_5D-gene</i>	<i>TraesCS5D02G052500</i>	50569632	50576495	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK353_5D-gene</i>	<i>TraesCS5D02G052800</i>	50635242	50646756	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK354_5D-gene</i>	<i>TraesCS5D02G061800</i>	58138943	58142318	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK355_5D-gene</i>	<i>TraesCS5D02G061900</i>	58143379	58149665	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK356_5D-gene</i>	<i>TraesCS5D02G062100</i>	58151124	58155524	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK357_5D-gene</i>	<i>TraesCS5D02G062200</i>	58226914	58230221	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK358_5D-gene</i>	<i>TraesCS5D02G062600</i>	58419864	58422609	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK359_5D-gene</i>	<i>TraesCS5D02G073900</i>	72901902	72907097	WAK
<i>Qstb-renan-5D</i>	<i>TaWAK360_5D-gene</i>	<i>TraesCS5D02G096200</i>	106519841	106525422	WAK
<i>Qstb-renan-7B</i>	<i>TaWAK556_7B-gene</i>	<i>TraesCS7B02G463200</i>	720131495	720134235	WAK
<i>Qstb-renan-1D</i>	<i>TraesCS1D02G026200</i>	<i>TraesCS1D02G026200</i>	10715309	10722269	Probable serine/threonine-protein kinase WNK3
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G060900</i>	<i>TraesCS5D02G060900</i>	57843934	57851315	Non-specific serine/threonine protein kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G065700</i>	<i>TraesCS5D02G065700</i>	61052683	61060726	Phosphatidylinositol 3-kinase VPS34
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G068700</i>	<i>TraesCS5D02G068700</i>	65753632	65755248	Non-specific serine/threonine protein kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G069700</i>	<i>TraesCS5D02G069700</i>	67578001	67588803	pfkB-like carbohydrate kinase family protein
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G081700</i>	<i>TraesCS5D02G081700</i>	82186877	82189457	Serine/threonine protein kinase%2C Abscisic acid (ABA)-activated protein kinase%2C Hyperosmotic stress response%2C ABA signal transduction
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G089700</i>	<i>TraesCS5D02G089700</i>	97036711	97041067	Diacylglycerol kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G091000</i>	<i>TraesCS5D02G091000</i>	98227410	98230028	L-type lectin-domain containing receptor kinase S.4
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G104600</i>	<i>TraesCS5D02G104600</i>	118455172	118460088	Nucleoside diphosphate kinase

QTL	Gene.ID	RefSeq v1.1 ID	Start (bp)	Stop (bp)	Annotation
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G104900</i>	<i>TraesCS5D02G104900</i>	118834967	118838504	ATP-dependent 6-phosphofructokinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G120500</i>	<i>TraesCS5D02G120500</i>	170376901	170381844	Diacylglycerol kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G138800</i>	<i>TraesCS5D02G138800</i>	221007037	221012985	Pyruvate kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G140700</i>	<i>TraesCS5D02G140700</i>	224325320	224328774	Phosphatidylinositol 4-phosphate 5-kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G144800</i>	<i>TraesCS5D02G144800</i>	231350992	231353762	Non-specific serine/threonine protein kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G145100</i>	<i>TraesCS5D02G145100</i>	231743581	231750603	Mitogen-activated protein kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G166400</i>	<i>TraesCS5D02G166400</i>	259233864	259236422	Receptor like protein kinase S.2
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G181500</i>	<i>TraesCS5D02G181500</i>	282151742	282156543	BR receptor kinase%2C Brassinosteroid (BR) perception in the roo
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G191900</i>	<i>TraesCS5D02G191900</i>	294637785	294639948	NAD(H) kinase 3
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G203600</i>	<i>TraesCS5D02G203600</i>	308863403	308865114	Serine/threonine-protein kinase BLUS1
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G214300</i>	<i>TraesCS5D02G214300</i>	323911872	323914256	Serine/threonine-protein kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G232500</i>	<i>TraesCS5D02G232500</i>	339652596	339654075	Non-specific serine/threonine protein kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G232600</i>	<i>TraesCS5D02G232600</i>	339712134	339713477	Non-specific serine/threonine protein kinase
<i>Qstb-renan-5D</i>	<i>TraesCS5D02G234000</i>	<i>TraesCS5D02G234000</i>	341192646	341202493	ATP-dependent 6-phosphofructokinase
<i>Qstb-renan-7B</i>	<i>TraesCS7B02G466300</i>	<i>TraesCS7B02G466300</i>	723900282	723903056	Serine/threonine-protein kinase

¹ Wall-associated kinases (WAK); ² Nucleotide-binding and leucine-rich repeats (NB-LRR); Genes highlighted are the candidate genes found in common with Yang et al. (2018).

With the data extracted from expVIP (<http://www.wheat-expression.com/>; accessed on 03/12/2021), we were able to see that for a majority of the genes, there was no expression in the inoculated plants. However, for 35 of them, we were able to identify expression patterns over the course of the infection (Figure 5). What we can observe with this data is that for the two known resistance genes *Stb6* and *Stb16q*, expression is maximal at 9dpi, this is also the case for some of the WAK and NB-LRR type genes in the QTL intervals. At 9dpi, the infection is still in the biotrophic phase but at the onset of switching to the necrotrophic phase (Steinberg, 2015). For *Qstb-renan-1D*, these are *BST_chr1D_nlr_113*, *TaWAK40_1D-gene*, *TaWAK41_1D-gene*, *TaWAK42_1D-gene*, and *BST_chr1D_nlr_102*. For *Qstb-renan-5D*, these are *TaWAK355_5D-gene*, *TaWAK356_5D-gene*, *TaWAK358_5D-gene*, and *TraesCS5D02G081700*. For the two receptor or receptor-like kinases in *Qstb-renan-5D*, there was no expression. The only gene with any expression in our list for *Qstb-renan-7B* was *TraesCS7B02G466300*, which had low expression overall, but it was maximal at 1 dpi.

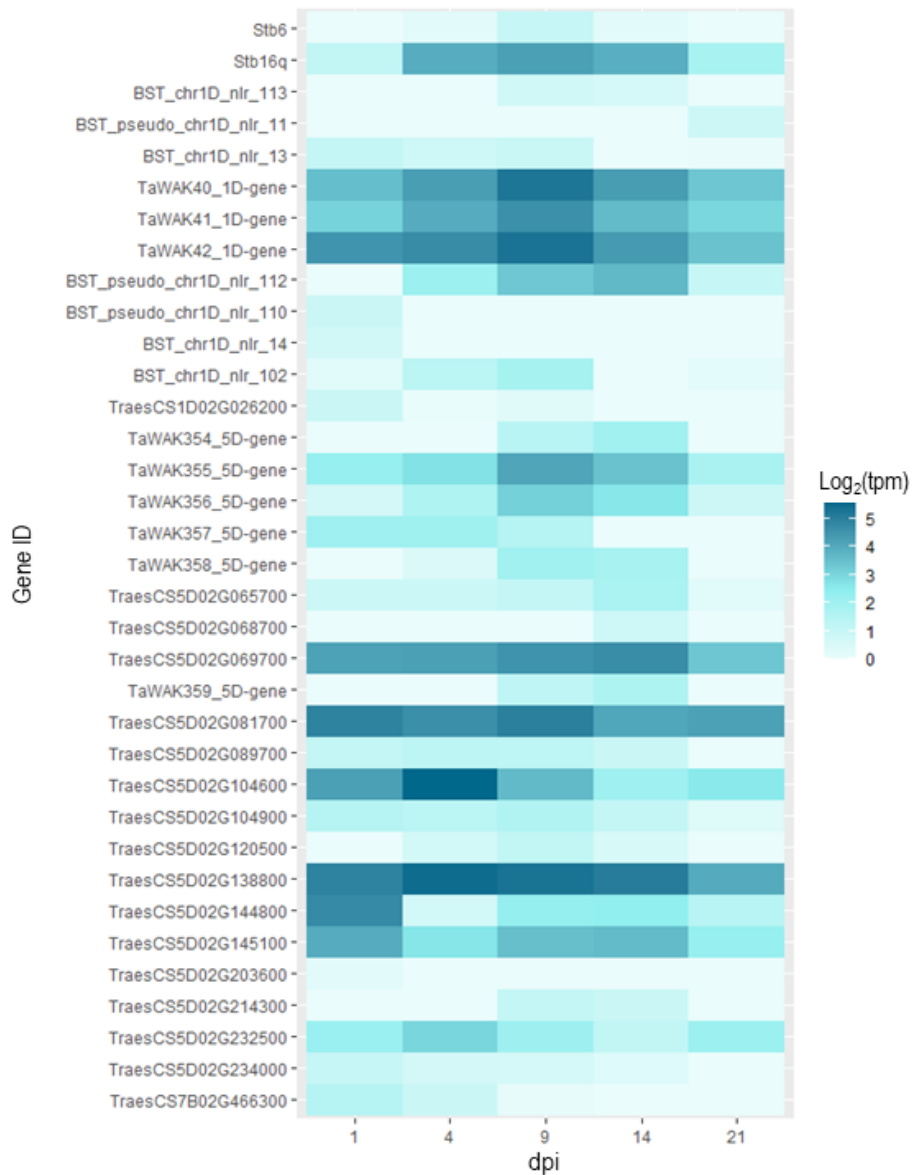


Figure 5. Heatmap representing the expression for each candidate gene where expression values were not equal to zero for the cultivar Riband inoculated with fungal strain IPO323. The x-axis represents post inoculation days, 1, 4, 9, 14, and 20 dpi respectively. The expression data is expressed in $\log_2(\text{tpm})$, with tpm transcripts per million. The first two lines present the data for *Stb6* and *Stb16q* for reference. The subsequent lines represent the candidate genes, which are ordered by physical position, top to bottom. The data represented here was extracted from <http://www.wheat-expression.com/>; accessed on 03/12/2021.

Discussion

An ultra-dense genetic map built from two SNP arrays

In this study, we built an ultra-dense genetic linkage map for bread wheat from the RxCS population. The map we obtained boasts 5357 unique positions for a total of 148,820 mapped markers. At present, this is the most densely marked genetic map built from a single segregating population. It does contain eleven gaps that are larger than 10 cM, which could hinder the detection of QTL in these regions. Moreover, for the most part the assumed physical position of the markers and their order in the genetic map corresponds well. There is a cluster of markers on chromosome 4D that mapped on the long arm of the chromosome while their physical position is assigned to the short arm. This could be due to chromosomal rearrangements between Chinese Spring and Renan, although the D sub-genome is not the most prone of the three to chromosomal rearrangements (Badaeva et al., 2007; O'Rourke, 2014). The Renan–Chinese Spring genetic map built by Rimbart et al. (2018) corroborates the marker ordering we find with this map for chromosome 4D and indeed for the rest of the genome (Figure S3). Finally, a recent refined assembly of Chinese Spring (Zhu et al., 2021) includes several major corrections at the 4D long arm telomere, suggesting that the misplacement is indeed due to errors in the original assembly.

Two studies, which used only Illumina Infinium iSelect 90K markers, provide a good base for comparison with the map we built here. The first is that of Wang et al. (2014) which was the first built with this array. They built a consensus map using six doubled haploid mapping populations; the map contains 40,267 markers distributed in 5564 genetic bins. In the genetic maps that they built for their various mapping populations, the majority of markers were to be found in the A and B sub-genomes while the D sub-genome represented a mere 15% of mapped markers, consistent with what we found in this study. The order of the markers in our map is for the most part consistent with that in Wang et al. (2014) (Figure S4). The second study we used for comparison is that of Wen et al. (2017). They built a consensus map comprising 29,692 markers distributed in 8960 bins; it was built with the maps of four mapping populations genotyped with the Illumina Infinium iSelect 90K array and five maps from previous reports. Firstly, all four of their maps consistently have a lower number of markers on the D sub-genome than on sub-genomes A and B, with the percentage of total markers on the D-sub-genome ranging from 7.64% to 12.8%, this corroborates with our results and other studies which show lower diversity in the D sub-genome (Hussain et al., 2017; Ladejobi et al., 2019; Wen et al., 2017). The total number of markers on each map is 10,986 for 2840 bins, 11,819 for 3242 bins, 9824 for 3198 bins and 14,862 for 3460 bins. The map

presented here carries a total of 148,820 markers for 5357 bins. It is therefore very densely covered and rivals the Wang et al. (2014) consensus map in terms of unique genetic positions despite being a map built from a single mapping population. Its particularity lies in the fact that two different SNP arrays were used to construct it, the TaBW410K Breedwheat array and the Illumina Infinium iSelect Wheat 90K array. The final RxCS map contains 95% of markers from the TaBW410K Breedwheat array and only 5% from the second array, this can in part be explained by the fact that the markers constituting both arrays were not chosen following the same strategies. For the TaBW410K Breedwheat array, a particular effort was made to target polymorphic markers, notably in modern wheat cultivars. Though the Infinium iSelect Wheat 90K array SNP in the RxCS genetic map are fewer compared to those of the Breedwheat array, they are present throughout the map on sub-genomes A and B (Figure S5A). Both arrays therefore contribute to the map's construction and structure (Figure S5). The 13,670 SNP markers, which are supposedly common to both arrays, do not often appear in the same genetic bins, though they do for the most part appear at close genetic positions. Indeed, out of 13,670, only 33 markers appear in the same bin as their counterpart in the other array. The average distance overall between two paired markers is 3.19 cM, while the median value is 2.27 cM. In one exceptional case, that of BS00044983_51, the markers of the pair do not appear on the same chromosome but on the homoeologous chromosomes 2B and 2D. The largest gap between a pair of markers, which are mapped on the same chromosome, is 21.1 cM and is the case for marker BobWhite_c12355_1548. In total, 27 marker pairs are more than 10 cM apart. This could be due to error rates between genotyping experiments, slightly different scoring methods for SNP calling between the arrays or indeed duplications in the sequence. All these results in addition to the very low number of mapped markers from the iSelect Wheat 90K array shows that the TaBW410K Breedwheat array performs better as a base for mapping.

As of yet, this is the only map which carries markers from both of the SNP arrays previously mentioned. As such, this map can be used as a "bridge" to compare maps from studies using only one or the other SNP array.

Phenotypic traits involved in the resistance of Renan to STB

All of the experiments presented here were carried out in controlled conditions at the seedling stage. This simplifies phenotyping as, in field conditions, other diseases with similar symptoms to STB can infect crops. Septoria nodorum blotch caused by fungal pathogen *Parastagonospora nodorum* in particular can lead to confusion (Fagundes et al., 2020). Other external factors such as environmental variations can also greatly

impact field conditions, compromising experiments' success (Riaz and T. Hickey, 2017). Another reason for choosing to work in controlled conditions is that quantitative interactions can be highly variable within and between experiments (Habig et al., 2017; Ouaja et al., 2020; Stewart and McDonald, 2014), controlled conditions allow one to reduce background variation that might exist in field conditions, although it is not completely suppressed. As the values for S26, AUDPC for green area, necrotic area, and sporulating area were all generated using data from visual symptom assessments, it was necessary to reduce background noise as much as possible, this included always having the same person perform the assessments within a triplicated or duplicated experiment. These precautions allowed us to obtain data, which though not necessarily normally distributed or statistically repeatable according to ANOVA results, enabled us to identify reliable QTL when comparing overall results. Precision phenotyping with image analyses has already been used successfully for studying the *Z. tritici*/wheat interaction (Stewart et al., 2016); it is suggested that pycnidia counting and size evaluation could be helpful in evaluating the epidemic potential of fungal strains and that the host can influence these traits. Yates et al. (2019) identified previously undetected loci for quantitative resistance to STB using the ImageJ macro for four phenotypic traits evaluated on a GWAS panel of 335 European winter wheat cultivars infected with a natural highly diverse *Z. tritici* population. In our case, though the PYC trait corresponding to pycnidia density did enable us to detect QTL, these were not different to the ones we were already able to identify using the visual assessment data. Precision phenotyping with a particle counter has also previously been used successfully in the *Z. tritici*/wheat interaction. Boixel et al. (2019a) evaluated the number of spores in a sample and they also evaluated spore size, shape and melanisation. They showed that for spore counting, the particle counter is a good means of gaining in accuracy and with the study of morphological variation between spores, they provide insight into more phenotypic traits that could be accessed by using a particle counter. Particle counters have been used in the study of the *Melampsora larici-populina*/poplar interaction (Maupetit et al., 2018), the *Phytophthora infestans*/potato interaction (Thomas et al., 2018) and the two main species of the ascochyta blight complex of pea/pea interaction (Dutt et al., 2020). In this latter case, the particle counter's image analysis capacities were used to evaluate spore length. This type of phenotyping offers a large diversity of potential phenotypic traits, which could lead to the identification of novel QTL, however, much as for PYC, the sporulation trait NBS did not yield the results we had hoped for, however it did confirm the QTL that we could already identify with our visual assessment data. These traits have previously led to the detection of QTL that were different to those detected with visually acquired data, however this was in the context of a GWAS with a diversified panel of cultivars (Yates et al., 2019).

Quantitative resistance is a multi-layered issue

Our objective was to identify regions in the *T. aestivum* genome, which could carry genes that contribute to quantitative resistance to STB. The continuous distribution of phenotypes that we observed in our data is indeed indicative of a quantitative and polygenic type of resistance (Figures S1 and S2). We were able to identify three robust QTL on chromosomes 1D, 5D, and 7B, each explaining between 6 to 26% of the phenotypic variation on average. These quantitative and polygenic attributes contribute to resistance durability for several reasons. Firstly, quantitative resistance, in theory, exerts less selection pressure on pathogen populations, meaning that the latter are less likely to adapt in the short term (Pilet-Nayel et al., 2017). Secondly, the polygenic nature of quantitative resistance provides more arguments for durability and is illustrated in the present case by the identification of three different QTL. Polygenic resistance is more durable as it is a combination of factors which provides resistance, rendering adaptation to each of these factors more complex (Parlevliet, 2002). This can be illustrated with studied cases of viral plant pathogens where the higher the number of mutations required for virulence is, the lower the probability of adaptation gets, and therefore polygenic resistance is more durable than monogenic (Harrison, 2002; Lindhout, 2002; Palloix et al., 2009). In the case of the *Capsicum anuum*/*Potato virus Y* in particular, it was shown that polygenic resistance breakdown is slower than monogenic, indeed the virus required a step-by-step selection for virulence, first towards major resistance genes, then towards the QTL and major gene combinations (Palloix et al., 2009; Quenouille et al., 2014). Brought back to our study case, as a combination of resistance QTL is efficient against a fungal strain, the adaptation to one of the QTL will not render overall resistance void. This is illustrated by the strain specificities of the identified QTL; i.e., though a strain has adapted to a specific QTL, this same QTL remains effective against the other strain. *Qstb-renan-1D* was detected only with fungal strain I05, while *Qstb-renan-5D* was only detected with fungal strain I07. Finally, *Qstb-renan-7B* did not discriminate between the strains. We therefore have different QTL combinations that are efficient against different fungal strains. This entails no specific strain selection as the selection pressure is in a way diluted between strains (Pilet-Nayel et al., 2017). It has been previously suggested that either pyramiding broad-spectrum factors or using host genotypes carrying narrow-spectrum resistance QTL could minimize resistance erosion (Van et al., 2013) and that combining specific QTL that can complement each other can slow selection of one particular pathogen strain (Van et al., 2013) increasing the durability of quantitative resistance.

Though there are many arguments in favour of durable quantitative resistance, it is not

necessarily the panacea. There are pathosystems where quantitative resistance has been overcome through pathogen adaptation. In the case of the aforementioned pepper/*Potato virus Y*, quantitative resistance alone was not sufficient to halt adaptation, emphasis was put on the possibility of implementing cultivar mixtures (Montarry et al., 2012). In the apple/*Venturia inaequalis* pathosystem use of broad-spectrum quantitative resistance was shown to present a risk of encouraging the emergence of generalist pathogen populations (Caffier et al., 2014). In the wheat/*Puccinia recondita* f. sp. *tritici* pathosystem, it has been shown that partial resistance can be eroded when natural selection is high (Lehman and Shaner, 1997). Another example is the grapevine/*Plasmopara viticola* interaction, where partial host resistance rapidly selected for pathogens with higher virulence (Delmotte et al., 2014). These different examples show that though quantitative resistance is considered to be durable, its use does need to be carefully thought through, with emphasis on diversification of resistance mechanisms, a combination of both broad spectrum and narrow spectrum resistance QTL, and the implementation of certain cultural practices, such as cultivar mixtures for example.

Molecular mechanisms underlying resistance QTL

Identifying the genes underlying the detected QTL would provide more insight into quantitative resistance and reveal the variety of mechanisms potentially involved in resistance to *Z. tritici*. Moreover, the use in breeding of resistance factors with distinct mechanisms could yet more complexify any pathogen adaptation (Pilet-Nayel et al., 2017; Poland et al., 2009).

For the three QTL detected in this study, the parent carrying the resistance allele is the cultivar Renan. Renan carries known resistance genes to a number of diseases, including rust resistance genes *Yr17*, *Lr37*, and *Sr38*, all originating from a chromosomal introgression from *Aegilops ventricosa* on wheat chromosome 2AS (Ambrozkova et al., 2002; Bartoš et al., 2011; Seah et al., 2001), eyespot resistance gene *Pch1* on chromosome 7D (Gallais, 2015; Pasquariello et al., 2017), and powdery mildew resistance gene *Pm4b* on chromosome 2AL (Gallais, 2015; Wu et al., 2018). None of these known resistances to other diseases colocalize with the three QTL detected in this study. *Qstb-renan-7B* was detected on the long arm of chromosome 7B. Two *Stb* genes are found on chromosome 7B (Brown et al., 2015), these are *Stb13* and *Stb8*. *Qstb-renan-7B* could colocalize with *Stb8* as it was detected towards the telomeric region of the long arm of chromosome 7B although this should be validated by using microsatellite markers Xgwm146 and Xgwm577 (Adhikari et al., 2003). Chromosome 1D carries two known *Stb* genes, these are *Stb10* and *Stb19* (Brown et al., 2015; Yang et

al., 2018). *Stb10* is however not found close to the telomere as *Qstb-renan-1D* is, but rather near the centromere (Chartrain et al., 2005a). This QTL does however colocalize with *Stb19* (Yang et al., 2018). The twenty-seven candidate genes in the *Stb19* region identified by Yang et al. (2018) with known *R* gene families are included in the *Qstb-renan-1D* confidence interval. Nine of these are in our NB-LRR type gene list (Table 6) and four of these *BST_chr1D_nlr_13*, *BST_chr1D_nlr_113*, *BST_pseudo_chr1D_nlr_112*, and *BST_chr1D_nlr_14* had expression in the case of a *Z. tritici* infection (Figure 5). Therefore, *Qstb-renan-1D* could be an allele at *Stb19* although this remains to be validated through an allelism test between cultivars Renan and Lorikeet, the cultivar from which *Stb19* was identified (Yang et al., 2018). Chromosome 5D does not carry any known *Stb* genes although STB resistance QTL have been previously identified on the short arm of the chromosome. Bearing in mind that the *Qstb-renan-5D* confidence interval overlaps both the short and long arm, we can therefore not exclude the possibility that it colocalizes with previously identified QTL (Gerard et al., 2017). *Qstb-renan-5D* explained up to 36% of phenotypic variation and is located in a region where no *Stb* gene has been mapped before. As previously discussed, the percentage of explained phenotypic variation cannot be considered as a criterion for the designation of a new *Stb* gene, because this percentage will strongly vary depending on the combination of effective resistances against a particular strain (Saintenac et al., 2021). We therefore propose to designate this locus *Stb20q* in accordance with the *Stb* nomenclature adding a 'q' to indicate the quantitative nature of this locus despite its strong effect on resistance.

The three QTL detected here span overall quite a large interval on the genetic map and contain large numbers of genes, it is therefore impossible to consider studying all of these one by one. It is also possible that the gene or genes underlying the QTL are not present in Chinese Spring, it could therefore be interesting to look at other cultivar annotations. Renan in particular would be pertinent and the data will be available soon (F. Choulet, personal communication). The next best option would be to prune the list of genes to look into. Here, we chose to look into WAK, NB-LRR type and kinase domain carrying genes present in the QTL regions as these are seemingly the most likely gene families involved in quantitative disease resistance (French et al., 2016; Fu et al., 2009; Gadaleta et al., 2019; Yajun Wang et al., 2019) in the context of a potential gene-for-gene interaction (Faris et al., 2010; Saintenac et al., 2018; Saintenac et al., 2021), a hypothesis we are encouraged to support by the strain specificities of the identified QTL. We are able to identify a short-list of genes in these gene families which could explain the detected QTL based on expression data, however, it would be interesting to test these further in the I05/Renan or I07/Renan interactions as the RNAseq data we

have available was acquired from Riband inoculated with IPO323 (Ramírez-González et al., 2018). So far, our results do not exclude that the genes underlying the detected resistance QTL could be based on similar mechanisms as known major *R* genes. However, other as yet unexplored possibilities cannot be ignored, such as those suggested by Poland et al. (2009) who proposed a variety of possibilities, including host plant development or morphology regulating genes, mutations in basal defence genes, detoxification mechanisms, defence signal transduction or partially altered weaker forms of *R* genes. Their last suggestion was that the genes underlying resistance QTL could be a unique set of previously unidentified genes.

Conclusion

This study showed that the resistance in Renan to STB is quantitative and polygenic. In particular, it showed that Renan has a resistance QTL with a small effect, which colocalizes with *Stb19* and a QTL with a strong effect on chromosome 5D, which was designated as *Stb20q*. For breeding, it could be interesting to introgress these regions from Renan into wheat cultivars as they would, in theory, be able to hold up resistance after pyramided major resistance genes are overcome, or even limit the erosion of major resistance genes (Pilet-Nayel et al., 2017). Nevertheless, the QTL were detected with data generated in controlled conditions and at seedling stage, they should be validated in field conditions before any possible implementation in breeding programs. It seems the next step would be to fine-map the QTL intervals, as this would give us a better idea as to which of the identified candidate genes, if any, are responsible for the resistance QTL. Furthermore, we are currently investigating pathogenicity QTL in our strains of interest I05 and I07, in order to evidence potential interactions between known resistance QTL in Renan and fungal QTL, with the aim of deciphering the mechanisms of minor gene-for-minor gene interactions.

Author Contributions: T.C.M. and R.V. conceived the experiments and edited the manuscript. C.L.-P. analysed the data and wrote the manuscript. S.B.K. conducted preliminary experiments that were required to select the most relevant plant and pathogen populations for this study. M.C., C.B., and S.G. collected the phenotypic data sets and carried out the first QTL analyses. P.S. participated in building the genetic linkage map and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The ultra-dense genetic map built from the population Renan x Chinese Spring and used to map resistance QTL is provided as a supplementary data file.

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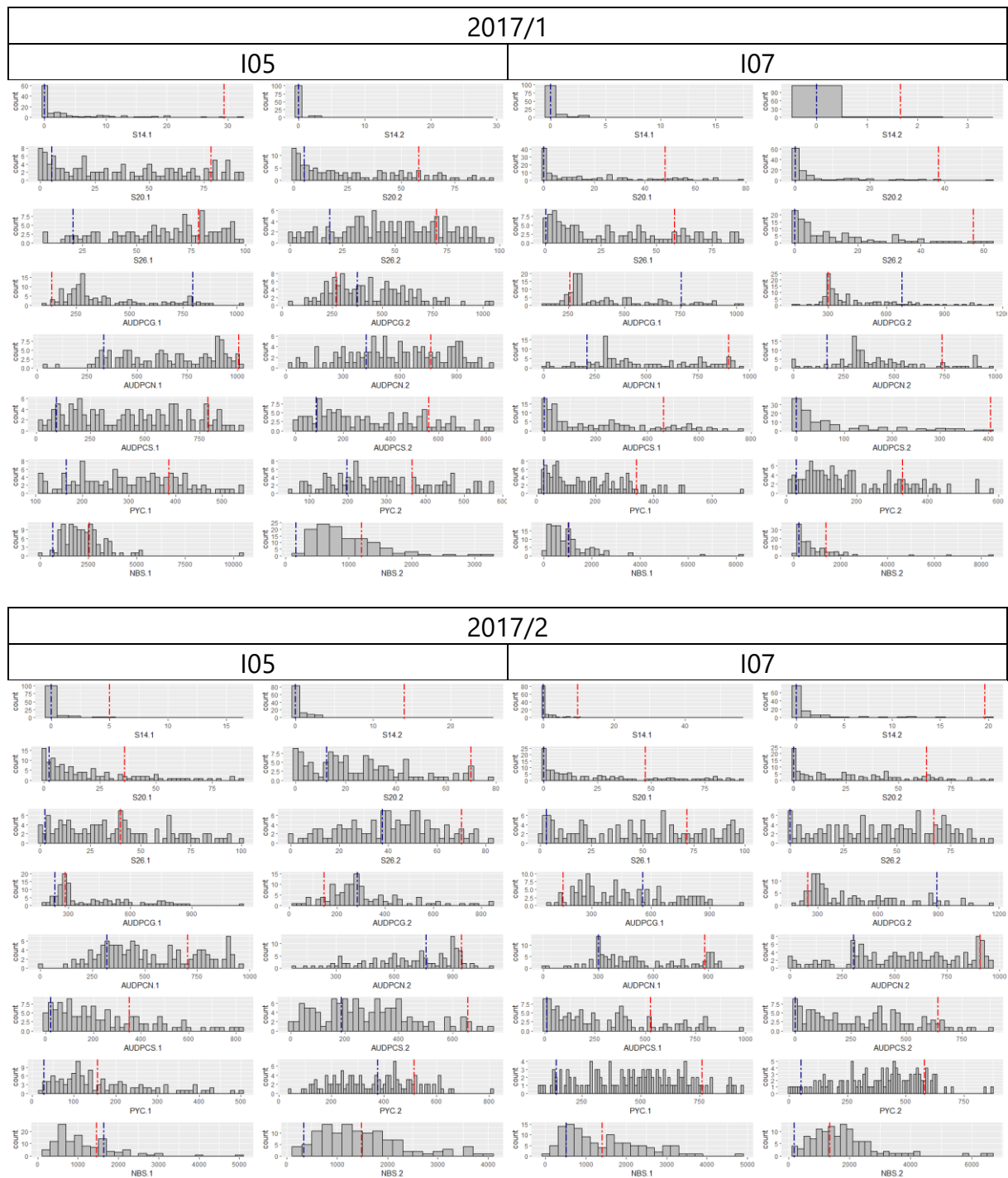
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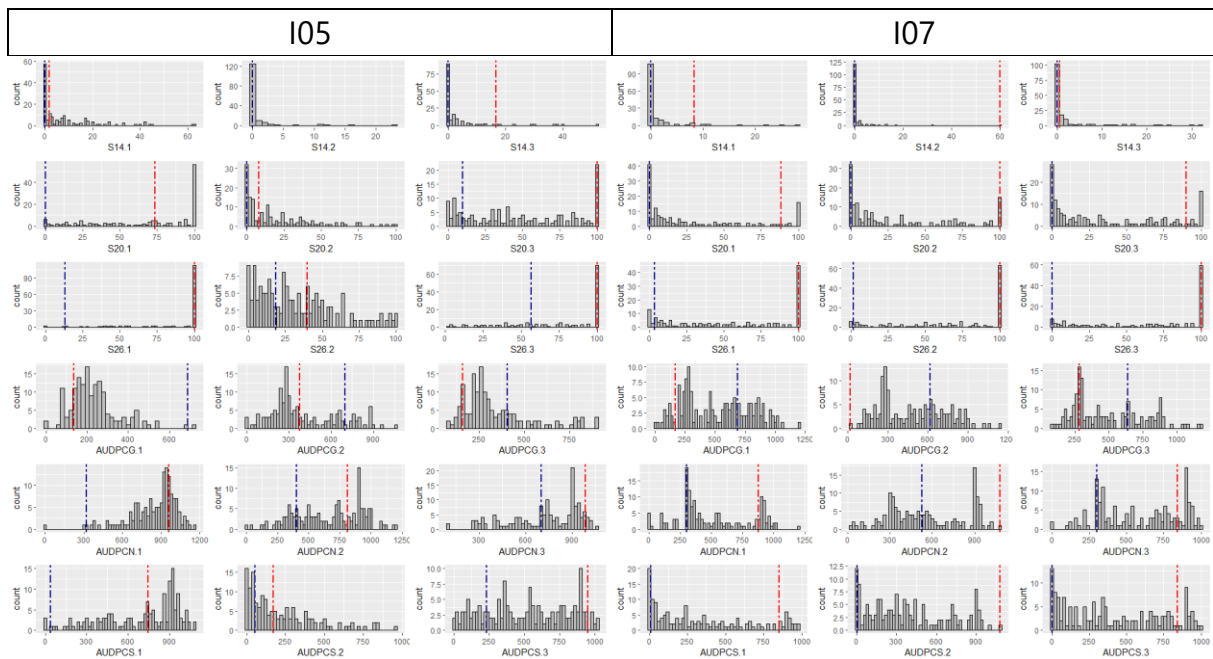
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SUPPLEMENTARY MATERIAL

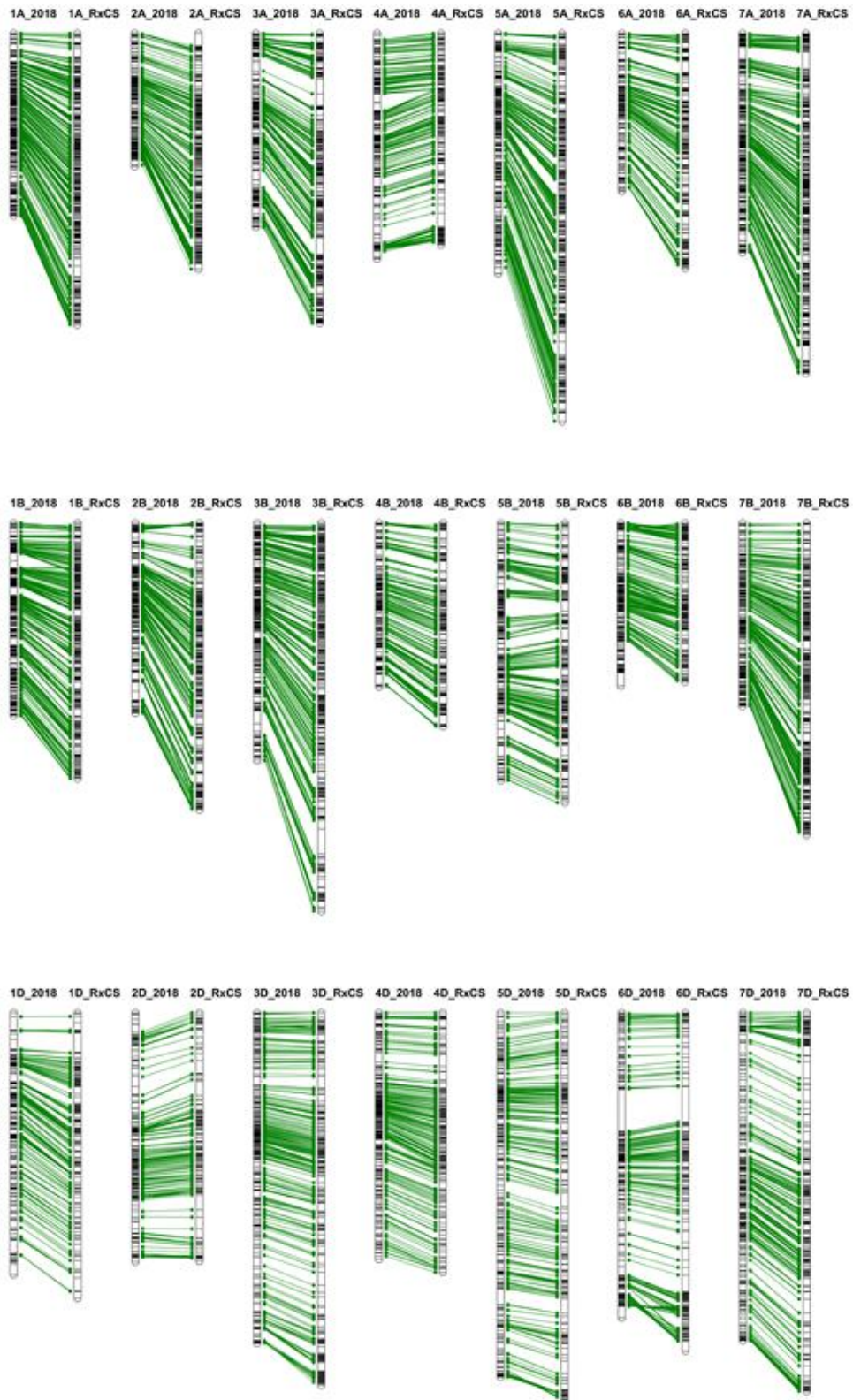


Supplementary material 1. Histograms representing the phenotypic data distribution for the 2017 datasets. For all of the histograms, the X-axis represents the trait values and the Y-axis represents the number of RILs from the RxCS population, which have the corresponding value for the phenotypic trait. 2017/1 corresponds to the data for Set 1, 2017/2 corresponds to the data for Set 2. The two leftmost columns correspond to the data for RxCS inoculated with I05. The two rightmost columns correspond to the data for RxCS inoculated with I07. Each line corresponds to a phenotypic trait. S14, S20

and S26 are the sporulating area at 14, 20 and 26 days respectively and are expressed in percentages. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. PYC is the pycnidia density. NBS is the number of spores per pycnidiospore. Each trait was studied over two replicates, 1 and 2 for both strains. Columns 1 and 3 correspond to replicate 1, columns 2 and 4 corresponds to replicate 2. The blue dotted line corresponds to the trait value for Renan, the red dotted line corresponds to the trait value for Chinese Spring.

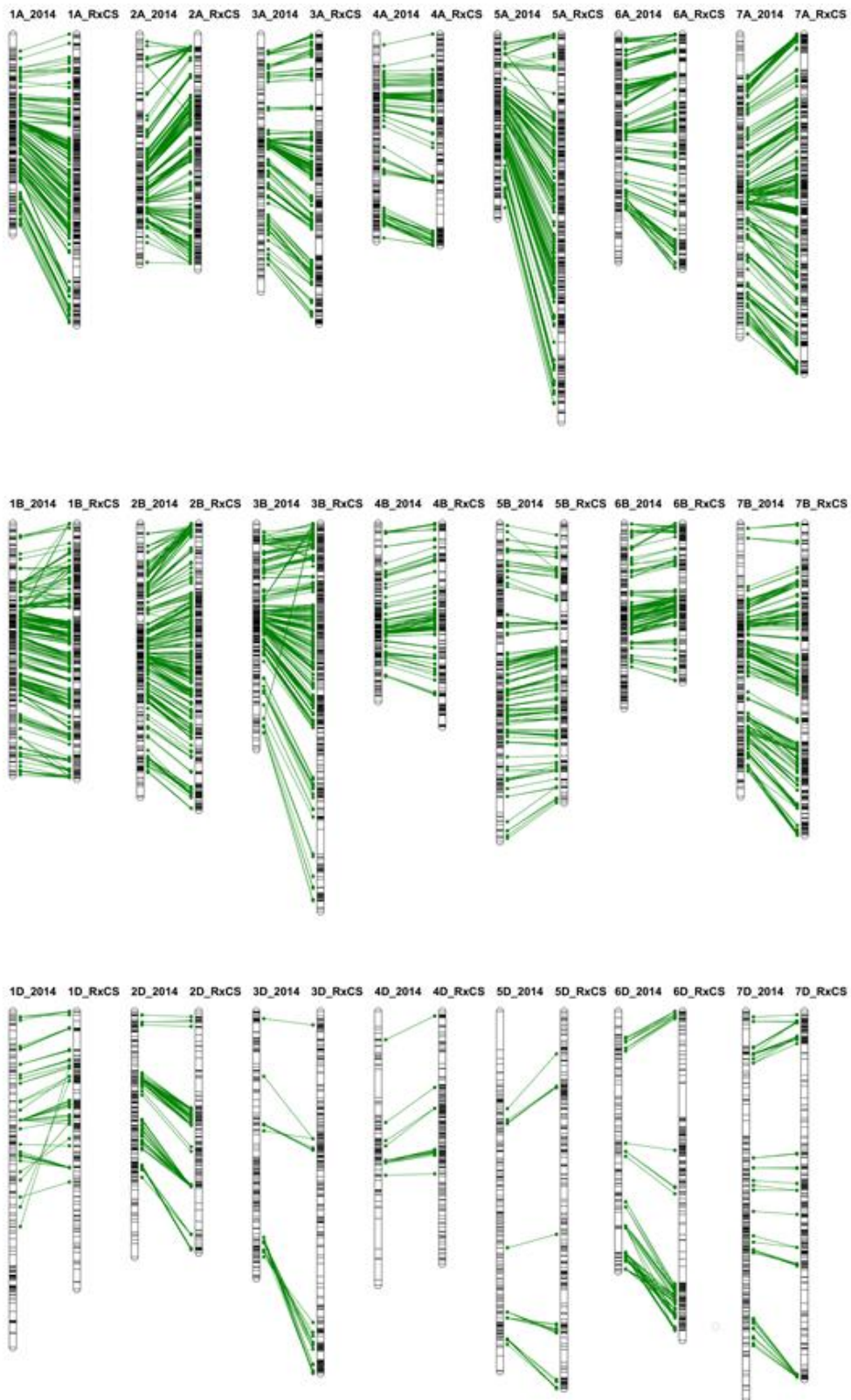


Supplementary material 2. Histograms representing the phenotypic data distribution for the 2018 datasets. For all of the histograms, the X-axis represents the trait values and the Y-axis represents the number of RILs from the RxCS population, which have the corresponding value for the phenotypic trait. The three leftmost columns correspond to the data for RxCS inoculated with I05. The three rightmost columns correspond to the data for RxCS inoculated with I07. Each line corresponds to a phenotypic trait. S14, S20 and S26 are the sporulating area at 14, 20 and 26 days respectively and are expressed in percentages. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. Each trait was studied over three replicates, 1, 2 and 3 for both strains. Columns 1 and 4 correspond to replicate 1, columns 2 and 5 corresponds to replicate 2 and columns 3 and 6 correspond to replicate 3. The blue dotted line corresponds to the trait value for Renan, the red dotted line corresponds to the trait value for Chinese Spring.



Supplementary material 3. Comparison between the RxCS genetic map and the wheat genetic map built by Rimbart *et al.* (2018). Bars represent chromosomes from 1A to 7D. Green lines link identical SNP between chromosomes. The bar on the left of a pairs corresponds to the map for that chromosome in the Rimbart *et al.* (2018)

genetic map. The bar on the right corresponds to the map for that chromosome in the RxCS genetic map.



Supplementary material 4. Comparison between the RxCS genetic map and the RxCS genetic map built by Wang *et al.* (2014). Bars represent chromosomes from 1A to 7D. Green lines link identical SNP between chromosomes. The bar on the left of a pairs corresponds to the map for that chromosome in the Wang *et al.* (2014) genetic map.

The bar on the right corresponds to the map for that chromosome in the RxCS genetic map.

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CHAPTER 3: QUANTITATIVE AND QUALITATIVE PLANT-PATHOGEN INTERACTIONS CALL UPON SIMILAR PATHOGENICITY GENES WITH A SPECTRUM OF EFFECTS

Quantitative and qualitative plant-pathogen interactions call upon similar pathogenicity genes with a spectrum of effects

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Running title: Quantitative pathogenicity of *Zymoseptoria tritici* towards bread wheat

Word count (main text): 7651

Abstract

- Septoria leaf blotch is a foliar wheat disease controlled by a combination of plant genetic resistances and fungicides use. *R*-gene based qualitative resistance durability is limited due to gene-for-gene interactions with fungal avirulence (*Avr*) genes. Quantitative resistance is considered more durable but the mechanisms involved are not well documented. We hypothesize that genes involved in quantitative and qualitative resistances are similar.

- A bi-parental population of *Zymoseptoria tritici* was inoculated on wheat cultivar “Renan” and a linkage analysis performed to map QTL. Candidate genes were identified and cloned by *Agrobacterium tumefaciens* mediated transformation. A pathology test assessed the effect of the mutant strains on “Renan”.
- Three pathogenicity QTL were mapped on chromosomes 1, 6 and 13 in *Z. tritici* and two candidate pathogenicity genes were selected based on their effector-like characteristics. The cloned gene on chromosome 6 was demonstrated to be involved in quantitative pathogenicity while the results for that on chromosome 13 remain inconclusive.
- By cloning a newly annotated quantitative effect gene in *Z. tritici* that is effector-like, we demonstrated that genes underlying pathogenicity QTL can be similar to *Avr* genes. This opens up the previously probed possibility that ‘gene-for-gene’ underlies not only qualitative but also quantitative plant-pathogen interactions in this pathosystem.

Keywords

Quantitative pathogenicity, Quantitative trait loci, Small secreted proteins, Septoria tritici blotch, *Triticum aestivum*

Introduction

Plant-pathogenic microorganisms employ a variety of strategies to successfully infect crops and effectors are very often involved in infection mechanisms (Koeck *et al.*, 2011; Fouché *et al.*, 2018; Shao *et al.*, 2021). Effectors are molecules that manipulate host immunity, be it in terms of cell structure or function, to enable parasitic infection (Kamoun, 2006; Pradhan *et al.*, 2021). They are generally cysteine-rich small secreted proteins (SSP) (Stergiopoulos & de Wit, 2009; Houterman *et al.*, 2009; Oliva *et al.*, 2010) and show low homologies with other species making their biological function difficult to predict (Plissonneau *et al.*, 2017). They are among the most polymorphic genes found in pathogen genomes (Win *et al.*, 2012), often found in highly plastic transposable element-rich regions of the genome (Ma *et al.*, 2010; Soyer *et al.*, 2014; Plissonneau *et al.*, 2018; Fouché *et al.*, 2018). As an answer to the onslaught brought on by effectors, host-plants have evolved *R* resistance genes that encode proteins capable of recognizing effectors, thus triggering a defensive response (Petit-Houdenot & Fudal, 2017). In this context, effectors are referred to as avirulence (*Avr*) genes, and

the *R/Avr* interaction follows a gene-for-gene interaction as defined by Flor (Flor, 1971). Gene-for-gene interactions have been described in many pathosystems (de Wit, 1995; Jia *et al.*, 2000; Gout *et al.*, 2006; Hall *et al.*, 2009; Stukenbrock & McDonald, 2009). Almost exclusively associated with qualitative resistance, these interactions pose an issue for disease resistance durability in crops because of the strong selection pressure they impose on pathogen populations. Indeed, a single mutation in the *Avr* gene is sufficient to overcome the disease resistance provided by the *R* gene as the effector will no longer be recognized by the plant's defence mechanisms (Niks *et al.*, 2015). Quantitative resistance imposes lower selective pressure on populations as it is polygenic, based on a combination of loci with varying effects all contributing to an overall more or less resistant phenotype (Niks *et al.*, 2015). It is therefore widely thought to be able to slow pathogen adaptation and to be more durable than qualitative resistance. Mechanisms underlying quantitative resistance are not well known but have been hypothesized (Poland *et al.*, 2009). Some hypotheses suggest that gene-for-gene interactions similar to those involved in qualitative resistance are in play but it has been shown only in very isolated cases (Leonards-Schippers *et al.*, 1994; Qi *et al.*, 1999; Arru *et al.*, 2003; González *et al.*, 2012; Meile *et al.*, 2018; Jiquel *et al.*, 2021). Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici*, is one of the most devastating diseases of wheat in Europe. It is responsible for high yield losses worldwide, 30 to 50% loss when environmental conditions are favourable to the disease's development (Eyal *et al.*, 1987; Fones & Gurr, 2015). Known sources of resistance in wheat to STB comprise 22 major resistance genes (Brown *et al.*, 2015; Yang *et al.*, 2018) and over 100 resistance quantitative trait loci (QTL) detected genome-wide (Brown *et al.*, 2015; Vagndorf *et al.*, 2017; Gerard *et al.*, 2017; Yates *et al.*, 2019; Karlstedt *et al.*, 2019; Langlands-Perry *et al.*, 2022). Very few genes are known to be involved in pathogenicity for *Z. tritici* (Marshall *et al.*, 2011; Rudd *et al.*, 2015; Poppe *et al.*, 2015; Kettles *et al.*, 2017; Hartmann *et al.*, 2017; Yemelin *et al.*, 2022), and qualitative gene-for-gene interaction has been demonstrated for the *T. aestivum-Z. tritici* pathosystem with the *Stb6/AvrStb6* interaction (Brading *et al.*, 2002; Zhong *et al.*, 2017). Though there are qualitative components to *Z. tritici* pathogenicity, it is regarded as being largely quantitative as phenotypes observed are mostly intermediate and do not correspond to a typically qualitative black or white situation (Stewart *et al.*, 2017; Hartmann *et al.*, 2017). Quantitative components of pathogenicity can be evaluated using different quantitative traits such as infection efficiency, latency period, pycnidia density, spore production, duration of the infectious period and lesion size (Pariaud *et al.*, 2009; Lannou, 2012; Gohari *et al.*, 2015; Stewart *et al.*, 2017). The only other gene-for-gene interaction that has been demonstrated is between *Stb7* or *Stb12* and *Avr3D1* (Meile *et al.*, 2018). This interaction was shown to be linked to quantitative phenotypes despite

the involvement of major resistance genes (Meile *et al.*, 2018), a first for this pathosystem. Both *AvrStb6* and *Avr3D1* encode SSP (Zhong *et al.*, 2017; Meile *et al.*, 2018). While the *Z. tritici* genome is composed of 13 core-chromosomes, present in every known strain, and 8 accessory chromosome, which are subject to presence/absence polymorphisms (Goodwin *et al.*, 2011), to date, no QTL linked to pathogenicity have been identified on accessory chromosomes, though a small effect of these accessory chromosomes on pathogenicity has been detected (Habig *et al.*, 2017). Wheat cultivar Renan displays quantitative resistance to *Z. tritici* strains I05 and I07 with three resistance QTL mapped on chromosomes 1D, 5D and 7B, the first two showing strain specificities between I05 and I07 (Langlands-Perry *et al.*, 2022). We studied the progeny of a cross between the strains I05 and I07, aiming at deciphering the genetic architecture of pathogenicity in quantitative interactions and characterizing the underlying genes.

Materials and methods

Fungal material

The two *Z. tritici* strains "I05" (INRA09-FS0813, Mat1-1) and "I07" (INRA09-FS0732, Mat1-2), sampled in 2010 from STB lesions on wheat cv. Soissons in Grignon, France (48510 N, 1580 E), were crossed by co-inoculating adult plants with an equiproportional suspension of parental blastospores. After ascosporeogenesis, 167 offspring individuals were collected from yeast-like colonies on Petri dishes placed upside down above wheat debris fragments to collect discharged ascospores as described in Suffert *et al.* (2016). The population of the 167 individuals resulting from the cross is hereafter referred to as "I05×I07".

Inoculation procedure

148 strains chosen randomly in I05×I07, and the two parental strains were phenotyped over three replications on 16 day-old seedlings of the wheat cultivar 'Renan' grown in a growth chamber as previously described in Langlands-Perry *et al.* (2022).

The strains were precultured 10 days prior to inoculation, and each preculture was then grown on a PDA solid culture medium (potato dextrose agar), as described in Langlands-Perry *et al.* (2022). 150mL of blastospores were prepared in advance from these cultures, each with a concentration of $10^6 \pm 1.10^5$ spores.mL⁻¹, kept at -80°C between 1 to 3 months to be used for each of the three replicated inoculations. Before

inoculation, a drop of Tween 20® was added per 15mL of inoculum to insure adherence of the inoculum to the leaf.

The day before the inoculation, only three plants were kept per pot. On the first true leaf (about 3cm from the base) of each plant, a surface of 7.5cm in length was marked out with two black felt tip lines. Inoculation was carried out with cotton swabs, one per inoculum, in six passages (3 times back and forth), within the marks. After inoculation, the pot was covered with a plastic bag closed off at the top with a paper clip to not only create a water-saturated atmosphere, which encourages infection (Shaw, 1991; Boixel *et al.*, 2019), but also to isolate the pots from one another to prevent cross contamination. The paper clips were removed after three days, a 72h incubation period being the time it takes for the fungus to reach the mesophyll, which is necessary to the rest of the colonisation process (Kema *et al.*, 1996). To optimise conditions for the survival of the inoculated leaf and to homogenise the quantity of light received by each leaf, new leaves were cut 2 to 3cm above the first node 14 days post-inoculation (dpi).

Evaluation of phenotypic traits

1. Visual evaluation of symptoms

The areas of the 7.5 cm-long inoculated leaf segment which were green, necrotic and sporulating (i.e. presented pycnidia) were assessed as percentages of the total inoculated area at 14 dpi, 20 dpi and 26 dpi. The phenotypic traits S20 and S26 correspond to the percentage of the inoculated area presenting with pycnidia at 20 and 26 dpi respectively. AUDPCs (Area Under the Disease Progress Curve) for the green, necrotic and sporulating areas (AUDPCG, AUDPCN and AUDPCS respectively) were calculated as described by Langlands-Perry *et al.* (2022) using the three assessments realized over the course of the infection.

2. Evaluation of sporulation by image analyses

Inoculated parts of the leaves were scanned and the images analysed with ImageJ, following the method of Stewart & McDonald (2014) and Stewart *et al.* (2016), modified by Langlands-Perry *et al.* (2022). The necrotic leaf surface and the total number of pycnidia were determined and used to calculate pycnidia density.

Sporulation for each three-leaf sample was quantified with the use of the particle size & shape analyser Occhio Flowcell FC200S+HR (www.occhio.be) according to the protocol followed by Langlands-Perry *et al.* (2022) and divided by the number of pycnidia of the three-leaf sample to calculate the number of pycnidiospores per

pycnidium (NBS).

Statistical analysis of phenotypic data

The obtained data sets were analysed with the R software (R Core Team, 2019), for each trait an analysis of variance (ANOVA) was performed with the following model:

$$Y_{ij} = \mu + I_i + r_j + Ir_{ij} + \varepsilon_{ij}$$

With Y_{ijk} the trait which is being studied, μ the mean value for this trait, I_i the individual genotype, r_j the repetition, Ir_{ij} the interaction and ε_{ij} the residual. For the following analyses, Ir_{ij} was included in the residual.

The following hypotheses were verified after the variance analyses.

$$\varepsilon \sim N(0, \sigma^2) \quad cov(\varepsilon, \varepsilon') = 0 \quad \text{Homoscedasticity (homogeneity of } var(\varepsilon))$$

Broad sense heritability is defined by the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

With H^2 the heritability, σ_g^2 genotypic variance and σ_e^2 residual variance.

Two ANOVAs were carried out as for replicate 3, 30 individuals were missing from the experiment. The first ANOVA included the data for all three replicates but with the 30 individuals from replicate 3 removed. The second ANOVA included the data for all individuals but only for the replicates 1 and 2.

The correlation between traits was studied using the Bravais-Pearson correlation.

RAD-sequencing of 105×107

All 150 strains were grown over 7 days in a 250 mL Erlenmeyer flask containing 100 mL of YPD (Yeast extract Peptone Dextrose), composed of 1% yeast extract, 2% peptone, 2% glucose and 95% distilled water inoculated with 30 μ L of inoculum. Growth chambers were fixed at 17 °C, a hygrometry of 70%, constant agitation at 160 rpm and under neon lights (two Osram L 58W/840 Lumilux Cool White tubes). Then, spores were washed and transferred into 50 mL Falcon tubes to be lyophilized for 24 to 30 hours. The dry samples were ground in liquid nitrogen with a mortar and pestle. DNA was extracted following a phenol/chloroform-based protocol adapted from Fagundes *et al.* (2020). After drying, extracted DNA samples were suspended in 250 μ L of Tris buffer at 10mM. Sample purity was verified with a Nanodrop (Desjardins & Conklin, 2010)

measurement while concentrations were measured with a Qubit 2.0 ('The Qubit 2.0 Fluorometer', 2011). All samples were subjected to an electrophoresis to verify that they were not degraded.

Samples were sequenced following the RADseq (Restriction site Associated DNA sequencing) strategy (Etter *et al.*, 2011), on the Plateforme MGX (MGX-Montpellier GenomiX). This type of sequencing enables one to target 1 to 10% of the genome via the use of a restriction enzyme and tagging of digested strands. Sequencing depth per sequenced locus is higher than classic sequencing, while the price is considerably lower. The restriction enzyme used was *Pst1*, following a previous study by Lendenmann *et al.* (2014), corresponding restriction sites were present throughout the IPO323 genome (Figure 1). Sequences were aligned on the reference genome of the strain IPO323 (Goodwin *et al.*, 2011).

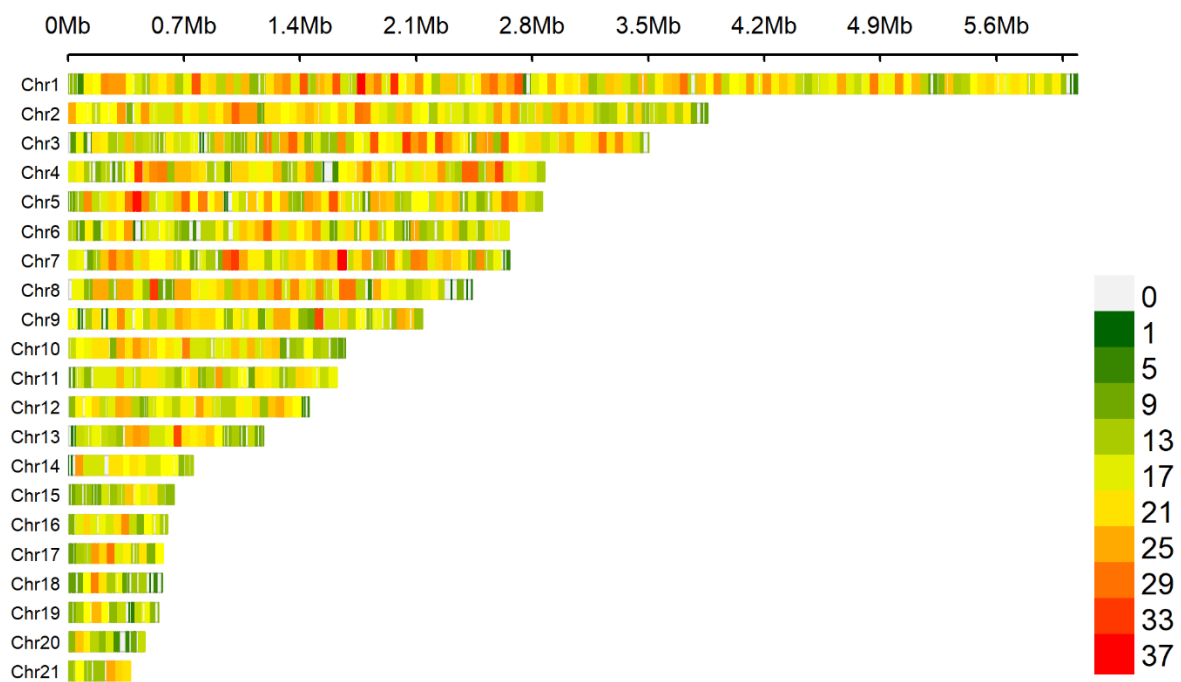


Figure 1. Distribution of *Pst1* restriction sites throughout the IPO323 genome. The colour scale indicates the number restriction sites within a 50kb window. Each line corresponds to a chromosome, 1 through 21.

Genetic analyses

1. SNP identification

The paired-end reads received from the Plateforme MGX were mapped on the reference genome IPO323 with BWAAv0.7.7 using the mem algorithm with default

settings (Li & Durbin, 2009). Reads were filtered using samtools v.0.1.19 and picard tools v1.106 (Li & Durbin, 2009; Broad institute). All secondary alignments and reads with a mapping quality inferior to 30 were filtered out. SNP identification was carried out with Freebayes V.0.9 with the « --report-monomorphic » option (Garrison & Marth, 2012). All positions detected as low complexity regions or transposable elements (TE) were filtered out. Low complexity regions were detected with RepeatMasker (Smit *et al.*, 2013). TE were detected with REPET (Flutre *et al.*, 2011). For each position, SNP with an allelic frequency inferior to 0.9 were filtered to delete falsely heterozygote SNP, as *Z. tritici* is haploid. A coverage depth filter was applied to delete all SNP with a coverage lower than 5 reads.

2. Construction of an ultra-dense genetic map

SNPs were named according to the chromosome and physical position they mapped to, based on the best BLAST values of the reads on IPO323. The genetic linkage map was built with the Multipoint ultra-dense software (MultiQTL Ltd, Haifa University, Israel). Before the first clustering, all SNPs with over 5% missing data were filtered. Then, a filter to correct any potential segregation distortion was applied, allowing a Chi^2 up to 9.5. After the first clustering, linkage groups, which belonged to the same chromosome, were merged and the results from Multipoint were transformed with the Kosambi mapping function (Kosambi, 2016).

3. QTL mapping

A linkage analysis was carried out using the R/qtl software (Broman *et al.*, 2003) version 1.42-8. This analysis included for each trait an initial Simple Interval Mapping (SIM), followed by a Composite Interval Mapping (CIM). Analyses were performed replication by replication. For SIM, 1000 genome-wide permutations were used to calculate the significant logarithm of odds (LOD) threshold. Were considered significant only QTL that showed P-values <0.05. The CIM was carried out with the QTL with the highest LOD used as a covariate. QTL intervals were evaluated with the LOD support interval with a drop in LOD of 1 and the "expandtomarkers" argument as true. QTL effects were calculated with the "effectplot" and "effectscan" functions. Possible epistatic interactions between QTL were looked into using the "addint" function.

Identification of candidate pathogenicity genes

1. QTL gene content

The gene content of the QTL was looked into using the annotation by Grandaubert *et*

al. (2015). Certain reannotations were carried out using data from Haueisen *et al.* (2019) and RepeatMasker (Smit *et al.*, 2013) for TE annotation. Functions of candidate genes were predicted using the translated protein sequences as input for the InterPro database (<http://www.ebi.ac.uk/interpro/search/sequence/>).

2. Structural differences between I05 and I07 at the detected QTL

A *de novo* genome assembly of parental strains I05 and I07 was realized with previously available PE-100 sequences obtained on an Illumina HiSeq-2000 sequencing system with a 70x mean genome coverage (BioProject: PRJNA777581, accessions SRR16762604 and SRR16762605; To be released). Illumina paired-end reads were assembled using a combination of VELVET (Zerbino & Birney, 2008), SOAPDENOV0 and SOAPGAPCLOSER (Luo *et al.*, 2012), as previously described for the assembly of the *Botrytis cinerea* genome (Mercier *et al.*, 2021).

The contigs from the I05 and I07 assemblies covering our regions of interest were identified by BLAST of the assembled genomes on the IPO323 reference genome. We were able to identify polymorphism between I05 and I07 for our candidate genes using these contigs.

A BLAST between the contigs of interest from the I05 and I07 assemblies enabled us to identify TE presence/absence polymorphisms in both strains. These were annotated using RepeatMasker (Smit *et al.*, 2013) and a previously generated TE library for this organism (Grandaubert *et al.*, 2015) according to the TE nomenclature defined by (Wicker *et al.*, 2007).

3. Expression profiles for the top candidate genes

The relative expression of our two top candidate genes was assessed by analysing qPCR data following the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001), the detail for which is in Supporting information 1 and all primers used are referenced in Table S1.

Molecular cloning

A detailed version is presented in Supporting information 2.

1. Bacterial strains and DNA manipulation

For all PCR performed to obtain cloning fragments, the Taq polymerase Phusion® (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used under adapted PCR conditions using primers referenced in Table S2. All DNA assembly manipulations were

conducted with the Gibson Assembly Cloning Kit (New England Biolabs, Ipswich, MA, USA). Plasmids carrying a hygromycin resistance gene flanked by two regions of approximately 1000bp for homologous recombination were generated for knock-out mutants. Plasmids carrying a sulfonyleurea resistance gene and a DNA fragment comprising at least 499bp upstream of the start codon and at least 1kb downstream of the stop codon of the candidate gene were generated for complementation and ectopic integration mutants. NEB 5-alpha Competent *E. coli* (High Efficiency) (New England Biolabs, Ipswich, MA, USA) were transformed by heat shock with the generated plasmids and used for their amplification. Successfully transformed strains were identified by PCR and mini-prepped plasmid constructs validated by Sanger sequencing (Eurofins, Luxembourg). *A. tumefaciens* strain AGL1 was transformed by heat shock with each generated plasmid. Colonies were screened by PCR.

2. *A. tumefaciens* mediated transformation of *Z. tritici*

The *Z. tritici* strains I05 and I07 were transformed by ATMT (Bowler *et al.*, 2010) following the standard protocol to generate knock-out mutants and ectopic integration mutants. This enabled us to obtain I05_ΔG07189, I05_ΔZt.13.364, I07_ΔG07189 and I07_ΔZt.13.364 mutants. I05_ΔG07189 and I05_ΔZt.13.364 mutants were transformed following the same protocol to generate complementation mutants I05_ΔG07189 + G07189_{I05}, I05_ΔG07189 + G07189_{I07}, I05_ΔZt.13.364 + Zt.13.364_{I05} and I05_ΔZt.13.364 + Zt.13.364_{I07} and ectopic integration mutants I05 + G07189_{I07} and I05 + Zt.13.364_{I07}.

Mutant strains were selected by hygromycin or sulfonyleurea screening. Obtained strains were verified by PCR on genomic DNA extracted with the DNeasy® Plant Mini Kit (Qiagen) according to the supplier's protocol.

3. Phenotypic characterization of mutant strains

All generated mutant strains were inoculated on Renan and Chinese Spring (susceptible control) according to the same protocol as the previously described assays. Only visual evaluations were performed. Were tested three clones of each mutant strain type and I05 and I07, three times each per replication. Four replications were carried out. The results obtained were analysed with a Kruskal-Wallis test and a Wilcoxon pairwise comparison.

Diversity and selection analysis of the candidate effector from Qzt-I05-6

In addition to the I05 and I07 *de novo* assemblies, were also available to us the

assemblies of 103 *Z. tritici* strains collected in France in 2009-2010 (BioProject: PRJNA777581; To be released) and 126 strains collected in France in 2018-2019 (To be submitted), enabling us to look into the sequence diversity for our candidate gene for *Qzt-105-6*. We extracted gene sequences from genome assemblies of the 229 *Z. tritici* strains using the ncbi-blast+ software (Camacho *et al.*, 2009). Prior to performing population genetic analysis, we first verified population structure in our dataset, to ascertain that there is no inflation due to population structure. We constructed a protein sequence phylogenetic tree using the RaxML algorithm with the GAMMA JTT model and 100 bootstrap replicates (Stamatakis, 2014). Phylogenetic trees were visualized using iTOL (Letunic & Bork, 2016); <<https://itol.embl.de/>>. We used the R package Popgenome (Pfeifer *et al.*, 2014) to calculate sliding window analyses of nucleotide diversity (π) of the effector gene, including ~500bp upstream and downstream the coding sequence. We used a window length of 20 bp and a step size of 5 bp. To verify whether the candidate effector gene exhibits signatures of positive diversifying selection, we calculated the rates of ω , the ratio of nonsynonymous to synonymous mutational rates using the codon-based selection analysis codeML (Yang *et al.* 2004). The ratio indicates negative purifying selection ($0 < \omega < 1$), neutral evolution ($\omega = 1$), or positive diversifying selection ($\omega > 1$). We compared different evolutionary models and used the statistical likelihood ratio test (LRT) to determine the model that best fitted our data. The Bayes empirical Bayes method (BEB) was then used to evaluate the posterior probability of sites considered to have been positively selected.

Results

An ultra-dense genetic linkage map for Z. tritici

The genetic data from 167 progeny and both parents led to the detection of 72,899 SNP in total. 89% of SNP were detected in the core-chromosomes and the remaining 11% in the dispensable chromosomes (Table S3). Chromosomes 14 and 18 each carried only 14 detected SNP, they were therefore understood to be absent in the parental strains as can be the case for dispensable chromosomes (Goodwin *et al.*, 2011), and the detected SNP we understood to be due to poorly assigned reads. These two chromosomes discounted, we obtain an SNP density of 2.1‰ based on IPO-323 chromosome sizes in bp.

12 individuals were not taken into account to build the linkage map as they carried missing data for over a cut-off of 10,000 SNP. The map carries 18,316 SNP for 1,332

unique positions distributed in 19 linkage groups each corresponding to a chromosome (Table 1), chromosomes 14 and 18 being absent in the map. The core-chromosomes represent together 89% of mapped markers. The least densely covered core-chromosome is chromosome 6 with 6.8 markers per cM and the most densely covered one is chromosome 7 with 14.9 markers per cM. The distance between consecutive markers ranges from a minimum of 0.95 cM for chromosome 2 to a maximum of 2.39 cM for chromosome 20, the average overall is 1.19 cM. Seven gaps in the map are greater than 10 cM. The three largest are just above 20 cM, on chromosomes 1, 6 and 20. A comparison between the order of the markers on the genetic map and their assumed physical position based on best BLAST values of the reads on IPO323 (Figure 2) shows that overall, the genetic map follows the assumed physical positions of the markers. Chromosome 13 denotes from the rest with an inversed bloc of SNP compared with IPO-323.

Table 1. Characteristics of the I05×I07 genetic linkage map

	Chromosome	Total number of markers	Number of bins	Covered genetic distance (cM)	Marker density (markers/cM)
Core-chromosomes	1	2878	218	232.87	12.36
	2	1564	125	117.94	13.26
	3	1648	133	136.35	12.09
	4	1170	103	115.64	10.12
	5	1350	107	124.2	10.87
	6	743	66	109.67	6.77
	7	1330	71	89.19	14.91
	8	1284	91	93.12	13.79
	9	1126	83	92.15	12.22
	10	957	65	82.04	11.67
	11	905	63	87.95	10.29
	12	788	54	68.55	11.5
	13	628	46	63.78	9.85
Subtotal 1		16371	1225	1413.45	11.58
Dispensable chromosomes	15	470	26	26.3	17.87
	16	213	19	30.18	7.06
	17	501	17	18.72	26.76
	19	276	22	23.93	11.53
	20	231	13	28.66	8.06
	21	254	10	17.43	14.57
Subtotal 2		1945	107	145.22	13.39
Total		18316	1332	1558.67	11.75

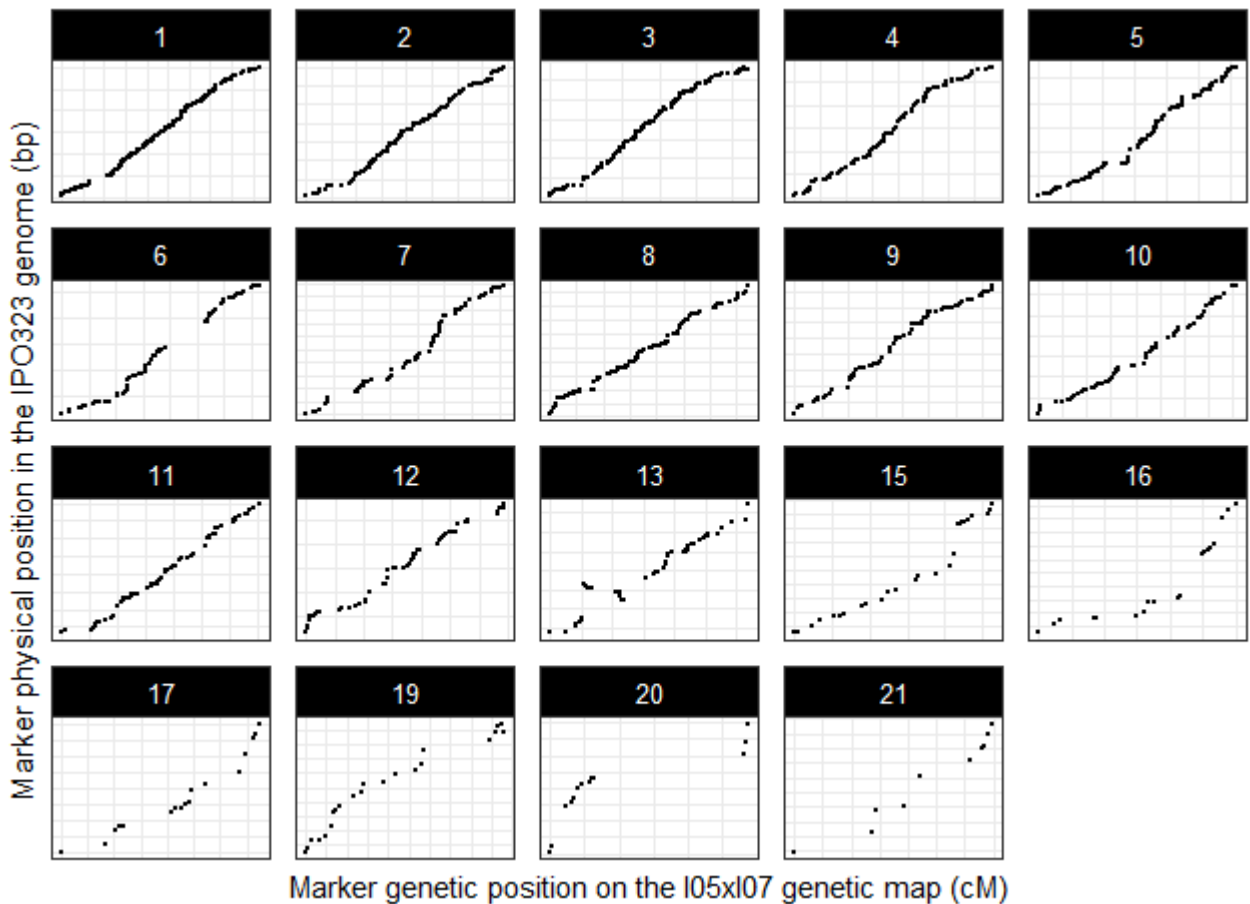


Figure 2. Comparison between markers' genetic and assumed physical positions for each chromosome. The X-axis corresponds to the genetic position of the mapped SNP on the I05×I07 genetic map in cM. The Y-axis corresponds to the assumed physical position of the SNP on the chromosome they are on in bp. Each graph represents a chromosome. Each black dot corresponds to a SNP.

Phenotypic data analysis

A representation of the distribution of the different traits for all three replicates shows that the traits do not follow a normal distribution (Figure S1), as confirmed by a Shapiro-Wilk normality test. For all traits, transgressive individuals are observed.

Bravais-Pearson correlation results show that all traits but NBS were correlated (Figure 3). The most strongly correlated traits were S26 and AUDPCS with a correlation coefficient of 0.96, both traits linked to the sporulating area. AUDPCN and AUDPCG were also strongly negatively correlated with a correlation coefficient of -0.95. PYC was less strongly correlated overall than the other traits, it was however significantly correlated with absolute correlation coefficient values ranging from 0.46 to 0.76 for PYC (NBS values excluded).

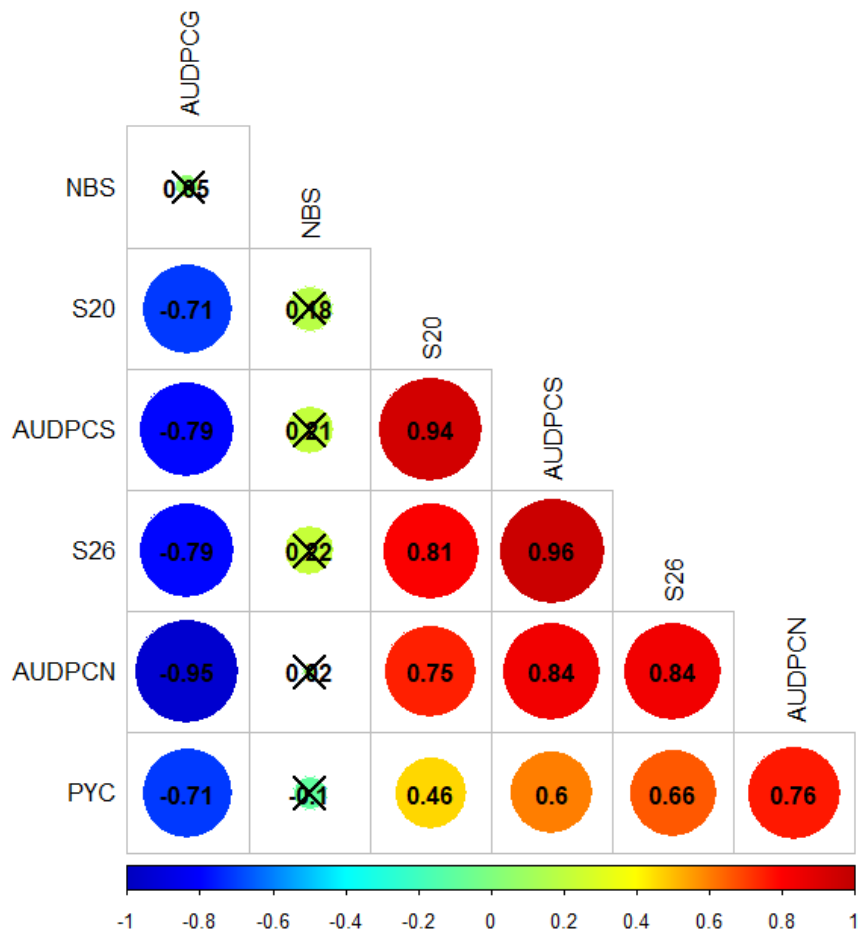


Figure 3. Bravais-Pearson correlogram for the phenotypic data; crossed out correlation values are not statistically significant (p -value < 0.05). PYC is the pycnidia density. NBS is the number of spores per pycnidiospore. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is the area under the disease progress curve for the sporulating leaf area. S20 and S26 are the sporulating leaf area at 20 and 26 days post-inoculation respectively.

Both ANOVAs (Table 2) showed that the genotype had a significant effect on phenotypes, though the significance was milder for PYC and NBS. They also showed that, overall, the replication had high statistical significance. This led to all subsequent analyses being carried out replication by replication. The traits that best performed in the statistical analyses were AUDPCG, AUDPCN and PYC with the ANOVA assumptions respected. For the other traits, the assumptions were not so well respected and heritability is not optimally estimated for these traits. Heritability ranged from 0.45 to 0.59 for S26, AUDPCG, AUDPCN and AUDPCS, values that are reasonably high. The values were lower for S20, PYC and NBS, ranging from 0.16 to 0.36.

Table 2. Statistical analysis of the phenotypic data

	Trait	Statistical significance of the genotype	Statistical significance of the replication	MSg	MS ϵ	Broad-sense heritability	Shapiro-Wilk normality test on residuals	Independence of residuals	Homoscedasticity Bartlett test of homogeneity of variances
No individuals with missing data	S20	***	***	114.9	55.7	0.26	< 2.2E-16	no	< 2.2E-16
	S26	***	***	765	160	0.56	6.23E-06	yes	6.893E-04
	AUDPCG	***	***	91740	23116	0.50	0.06	yes	0.4
	AUDPCN	***	***	74760	14626	0.58	4.24E-03	yes	0.11
	AUDPCS	***	***	20307	5800	0.45	< 2.2E-16	no	9.78E-12
	PYC	***	***	79898	45477	0.20	0.1417	yes	0.12
	NBS	**	***	1646264	1051014	0.16	< 2.2E-16	no	< 2.2E-16
only replicates 1 and 2	S20	***		4101	1951	0.36	< 2.2E-16	no	< 2.2E-16
	S26	***	**	456.7	117.7	0.59	2.21E-17	no	4.71E-03
	AUDPCG	***		71061	22560	0.52	0.56	yes	0.49
	AUDPCN	***	*	52337	12937	0.60	0.30	yes	0.20
	AUDPCS	***	*	8328	2356	0.56	2.37E-12	no	6.52E-07
	PYC	*	***	67311	47739	0.17	0.63	yes	0.90
	NBS	*	***	2481174	1748477	0.17	< 2.2E-16	no	3.24E-13

¹ Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 '.', 1

² MSg is the mean square value for the individual genotypes output by the ANOVA

³ MS ϵ is the mean square value for the residuals output by the ANOVA

105×107 linkage analyses reveal three pathogenicity QTL

The linkage analyses enabled us to detect three repeatable QTL: *Qzt-105-1*, *Qzt-105-6* and *Qzt-107-13*, on core chromosomes 1, 6 and 13, respectively (Table 3, Figure 4, Table S4). *Qzt-105-1* and *Qzt-105-6* were detected for all three replicates, while *Qzt-107-13* was detected only for replicates 2 and 3 (Table 3). *Qzt-105-1* is in a sub-telomeric region of chromosome 1. It covers a 4.96 cM long region on the genetic map spanning 138 kb based on the physical position of the flanking markers. The mean phenotypic variation explained by this QTL is 6.37% and the parental strain carrying the pathogenic allele for this QTL is I05. *Qzt-105-6* covers a 13.48 cM long region corresponding to a physical interval of 169 kb on chromosome 6. The mean phenotypic variation explained by this QTL is 24.91%, the highest among the three repeatable QTL detected, and the parental strain carrying the pathogenic allele for this QTL is I05. This QTL presents itself as being the most robust of the three. Finally, *Qzt-107-13* is found at a sub-telomeric region of chromosome 13. It covers an 8.64 cM long region corresponding to a physical interval of 186 kb. The mean phenotypic variation explained by this QTL is 6.93% and contrary to the other two QTL, the parental strain carrying the pathogenic allele is I07.

Table 3. QTL for pathogenicity detected with the phenotypic data obtained and the 105×107 genetic map

QTL	Number of detections	r ² max (%)	Mean r ² (%)	Parent carrying the pathogenic allele	Traits
<i>QZT-105-1</i>	8	7.43	6.37	I05	S26, AUDPCG, AUDPCN, AUDPCS
<i>QZT-105-6</i>	17	40.15	24.91	I05	S20, S26, AUDPCG, AUDPCN, AUDPCS, PYC, NBS
<i>QZT-107-13</i>	6	9.40	6.93	I07	S20, S26, AUDPCN, AUDPCS, NBS

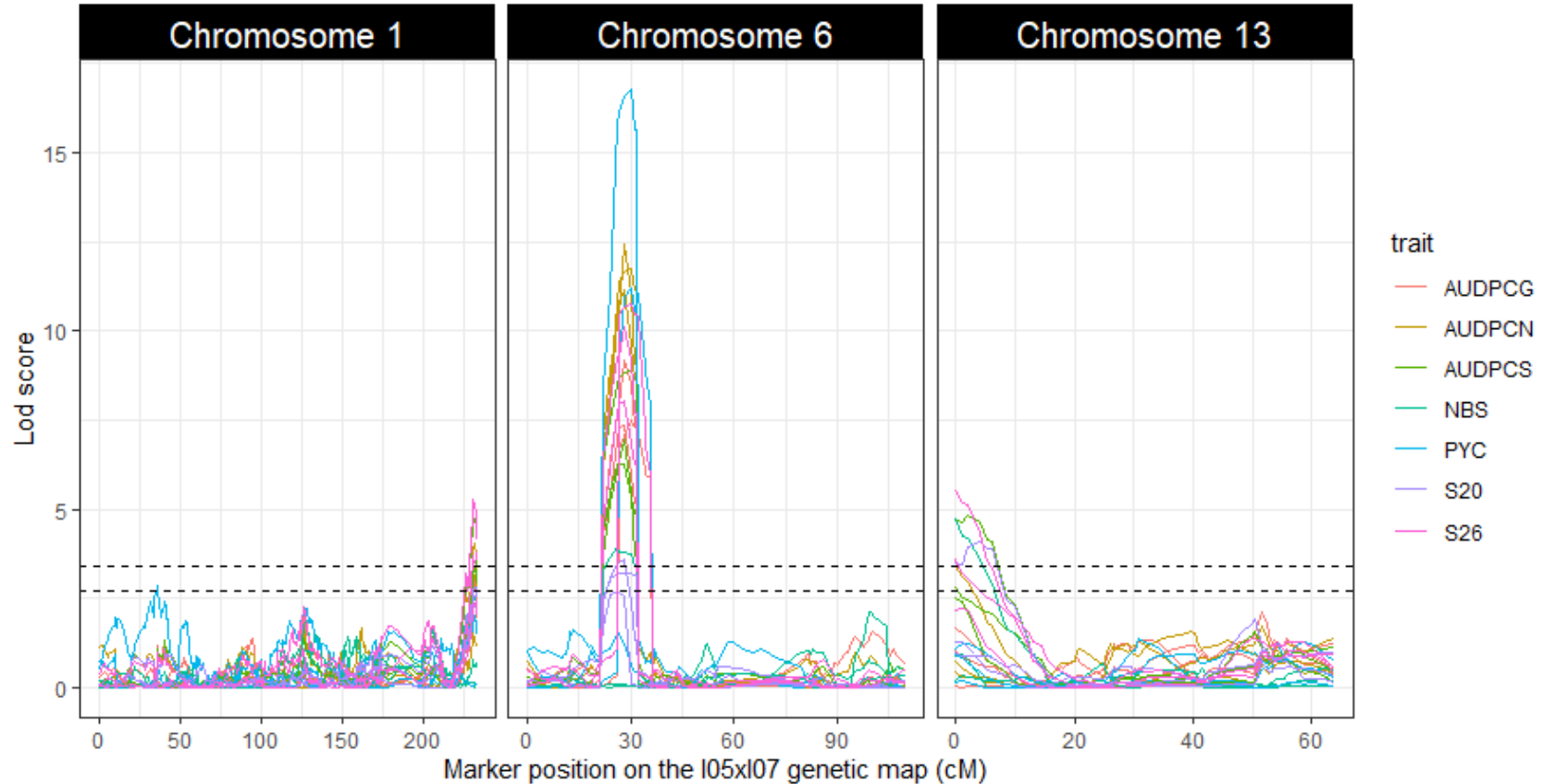


Figure 4. LOD score profiles for the linkage analyses. The X-axis represents the position of the markers on the genetic map in cM. The Y-axis represents the LOD score associated to the markers. In dotted lines are represented the minimal and maximal LOD threshold values obtained in the linkage analyses after 1000 permutations tests. Each column corresponds to a chromosome, chromosomes 1, 6 and 13 respectively. The colours in the graphs correspond to the studied traits. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. S20 and S26 are the sporulating areas (%) at 20 and 26 days post-inoculation respectively. Each trait was studied over three replicates, 1, 2 and 3.

Significant epistatic interactions were detected between *Qzt-105-1* and *Qzt-105-6* and between *Qzt-105-6* and *Qzt-107-13* (Table 4). *Qzt-105-6* and *Qzt-107-13* had the highest and most significant epistatic interaction explaining on average 7.12% of phenotypic variation. This epistatic interaction explains why strains carrying the pathogenic allele for these two QTL led to the highest S26 values among the strains combining the pathogenic allele for two QTL (Figure 5).

Table 4. Epistatic interactions detected between the identified QTL

Replication	Trait	Chromosomes on which QTL were detected	Epistatic effect r^2 (%)	Significance of the epistatic effect
2	S26	1, 13	0.68	
3	AUDPCN	1, 13	0.77	
3	AUDPCS	1, 13	1.3	
1	AUDPCS	1, 6	5.8	***
1	S26	1, 6	5.19	***
2	AUDPCG	1, 6	0.12	
2	AUDPCN	1, 6	0.48	
2	AUDPCS	1, 6	2.74	*
2	S26	1, 6	2.72	*
3	AUDPCN	1, 6	2.73	*
3	AUDPCS	1, 6	6.59	***
2	S26	6, 13	3.62	**
3	AUDPCN	6, 13	4.07	**
3	AUDPCS	6, 13	10.8	***
3	S20	6, 13	8.92	***
3	S26	6, 13	8.21	***
3	NBS	6, 13	1	

¹ Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1

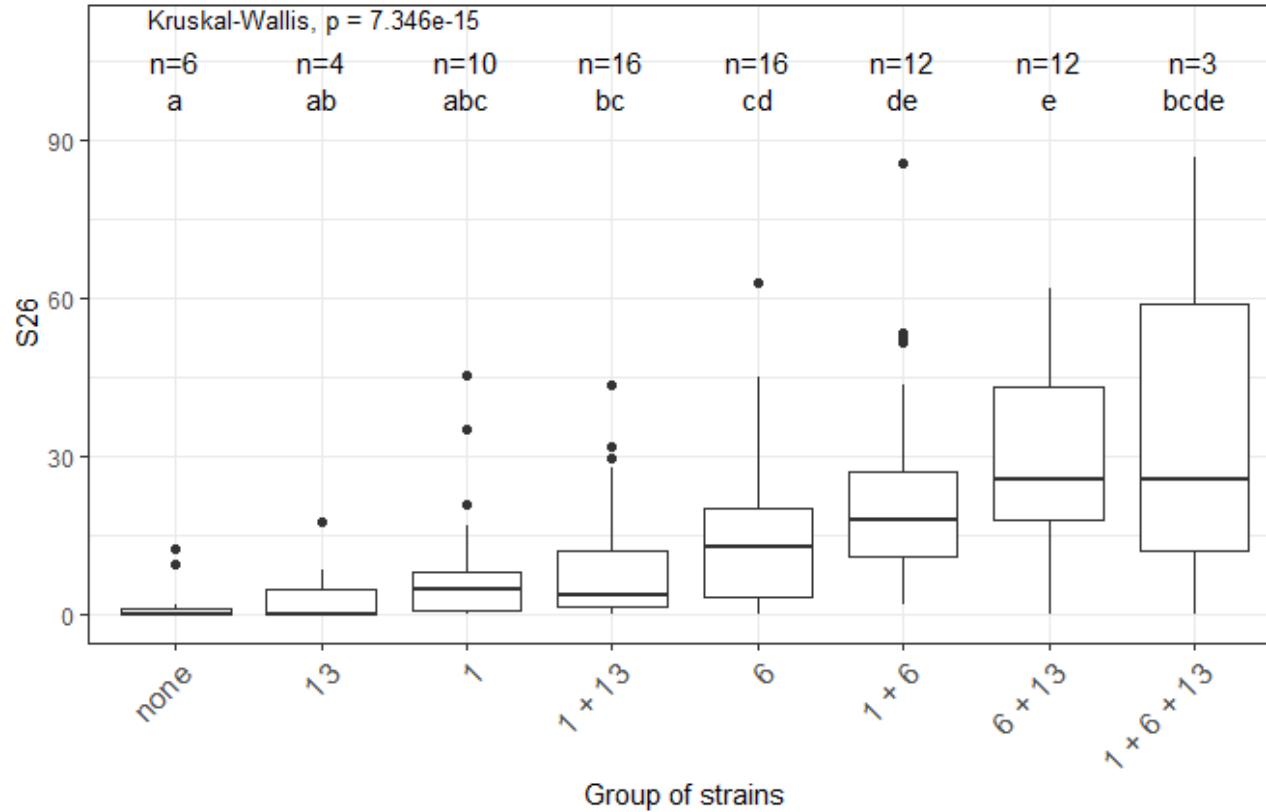


Figure 5. Impact of the different combinations of pathogenicity QTL on the performance of strains from the I05xI07 population on Renan, strains with recombination within the QTL intervals were excluded. The X-axis represents the different groups of strains concerning their pathogenic allele combination per QTL. For legibility, the QTL are referred to here as 1, 6 and 13. The n values correspond to the number of strains per group. The Y-axis represents the phenotypic values obtained. S26 is the sporulating area (%) at 26 days post-inoculation. The Kruskal-Wallis values indicate that the phenotypic value for at least one group of strains is significantly different to the others. Letters a, b, c, d and e indicate significant difference for a Wilcoxon pairwise comparison at alpha=0.05.

For further investigation, we chose to focus our efforts on *Qzt-105-6* and *Qzt-105-13* as these were the most robust QTL, with the most effect on phenotypic variation and were subjects to a highly significant epistatic interaction.

Qzt-105-6 harbours a previously unannotated effector-like gene in a dynamic region

The interval defined by *Qzt-105-6* holds 36 genes annotated by Grandaubert *et al.*, (2015) (Grandaubert *et al.*, 2015). There is a 60 Kb long TE-rich region in the middle of the QTL, with markers that came out as peak markers during the linkage analysis on either side. None of these 36 genes has any predicted functions, we therefore predicted functions with the InterPro database (<http://www.ebi.ac.uk/interpro/search/sequence/>) (Table S5). We searched for genes that have characteristics of known effectors, as these are often involved in fungal pathogenicity. Out of the 36 annotated genes, two had a signal peptide. The first named *Zt09_6_00095* in the Grandaubert *et al.* (2015) annotation comes out as being in the PTHR35523 family in the panther classification system (<http://www.pantherdb.org/>), this family regroups cell wall proteins. As a component of the cell structure, this gene does not seem to be a good candidate. The second gene, *Zt09_6_00123*, is predicted to encode a FAD binding domain, linking it to an oxidoreductase process. This gene is a little large for an effector with a length of 1272 bp and a corresponding protein of 423 amino acids (aa), the general cut-off being set at 300 aa (Sperschneider *et al.*, 2018), it has a predicted function and only one cysteine in its protein sequence. It does not seem to be a good candidate either.

The RNAseq data from Grandaubert *et al.* (2015) enabled us to notice some reads that mapped to a position that was not annotated, right next to the central TE region. The reads were only present for certain days post-infection, inferring a differential expression pattern. As pathogenicity genes can be found in regions such as this (Plissonneau *et al.*, 2018) we looked further into the corresponding position. Haueisen *et al.* (2019) produced RNAseq data which we used to make a bigwig file which was used in the integrative genomics viewer (IGV) software (Robinson *et al.*, 2011) as a means of annotating a previously unidentified gene. On chromosome 6 of IPO323, this gene is positioned at 470,027-470,324 bp. It has two exons, the first has a signal peptide when input to the InterPro database and there is no predicted function or family for the protein. It has an intron which is 61 bp long and has canonical splice site combination GT-AG (Kupfer *et al.*, 2004; Frey & Pucker, 2020). The gene is 237 bp long and encodes a 78 aa protein with 11 cysteines in its sequence (14% of the protein sequence). We identified four mutations between parental strains I05 and I07. A BLAST search against the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) did not yield

any significant results in other species, indicating that this gene is species-specific. In a recent, yet unreleased, annotation of the *Z. tritici* IPO323 genome sequence, specifically improved to detect genes encoding SSP, this gene was identified and named *G_07189* (unpublished data).

The study of the expression of this gene over the course of the infection revealed up-regulation at 12 dpi in both I05 and I07 strains (Figure 6), further consolidating its status as a very good effector candidate.

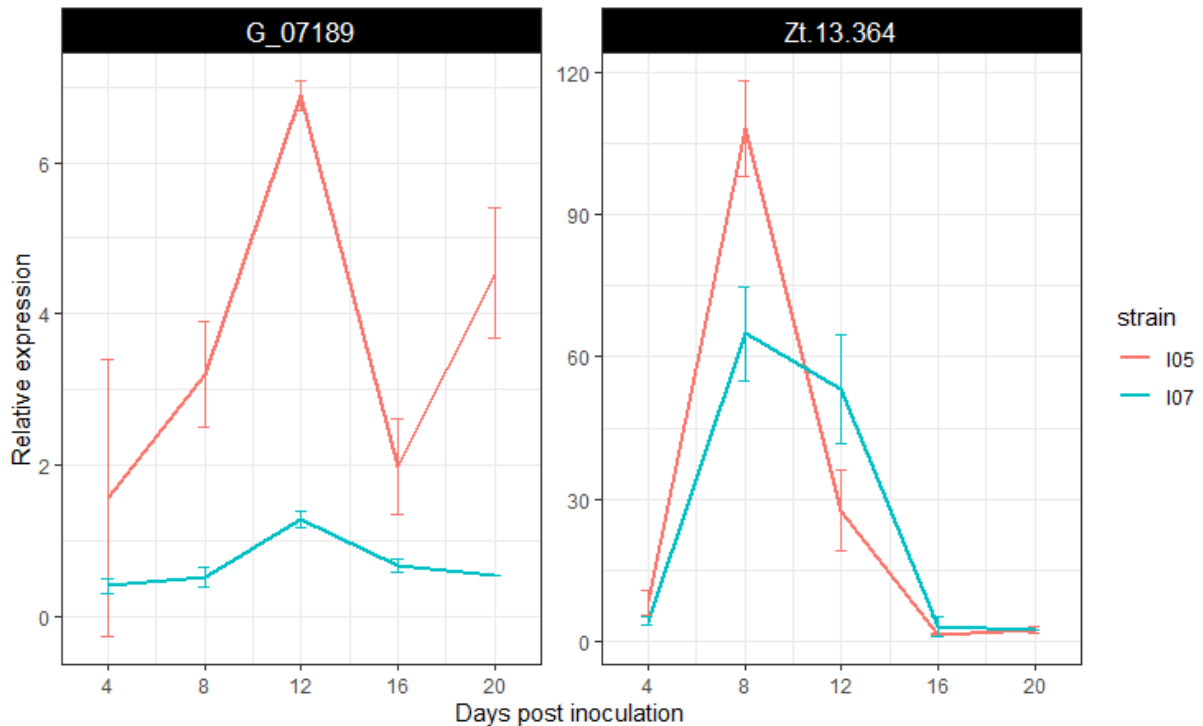


Figure 6. Expression profiles of *G_07189* and *Zt.13.364* in *Z. tritici* strains I05 (red) and I07 (blue) during infection on the *T. aestivum* cultivar Chinese Spring. The values shown are the relative expression levels for each gene with respect to the geometric mean obtained for the three housekeeping genes used *EF1 α* , *UBC* and *β -tubuline*, averaged over at least two biological replicates, except for I07 20 dpi, where all samples were degraded but one after RNA extraction and DNase treatment. Error bars represent the 95% confidence intervals of the averages.

Additionally, the comparison of I05 and I07 assembled contigs revealed that the newly annotated gene lies in a highly dynamic region, with several presence/absence polymorphisms of TE between both parental strains (Figure 7).

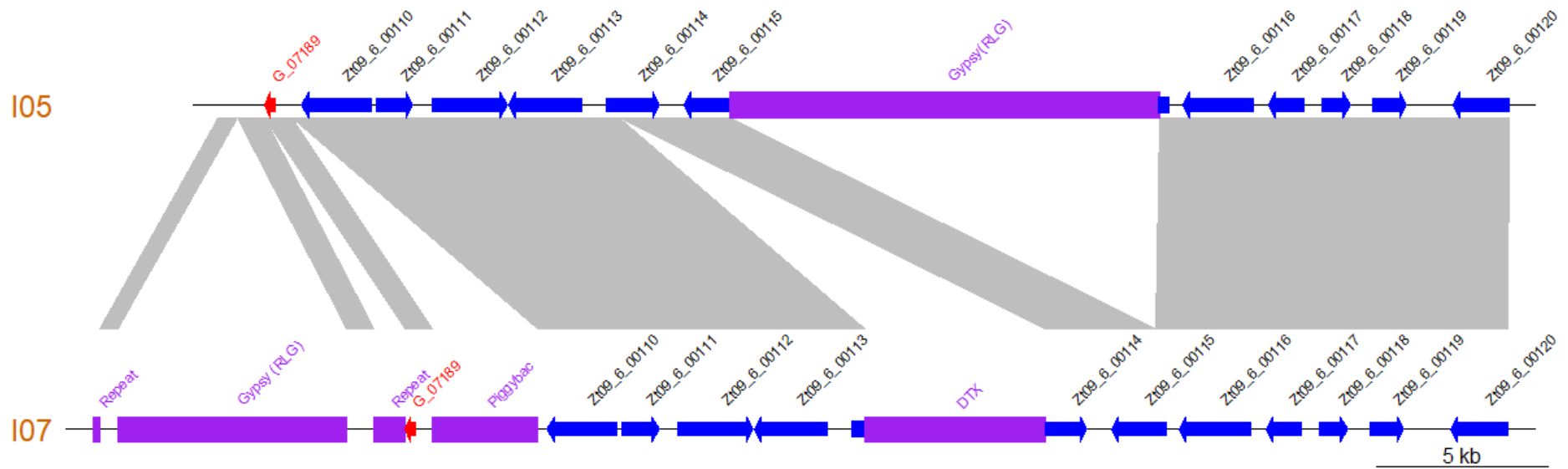


Figure 7. The region harbouring *G_07189* is highly polymorphic between strains I05 and I07 as illustrated by this synteny plot comparing the contigs of the I05 and I07 assemblies which carry *G_07189*. Previously annotated genes are represented as blue arrows according to their orientation. The newly annotated gene *G_07189* is represented by a red arrow according to its orientation. TE are represented by purple blocks. Collinear sequences between contigs are shown in grey.

Qzt-107-13 harbours several effector-like genes one of which is a strong candidate

The interval defined by *Qzt-107-13* holds 51 genes annotated by Grandaubert *et al.* (2015). Like the other two QTL, the interval also carries a smattering of TE regions. The peak was however well defined. No functions were previously predicted for these genes, so we compared them with the Inter Pro database (Table S6). Of the 51 genes, 8 carry a signal peptide, and among these, only 6 had no identifiable gene ontology (GO) terms and of the six, five had polymorphism for the protein sequence between I05 and I07. As the peak marker for the detection of *Qzt-107-13* was very stable between analyses, practically always the marker chr13_1114801, we decided to focus on *Zt09_13_00364* as it is the only one among the five aforementioned genes that is close to the peak marker. *Zt09_13_00364* has no functional pattern and has a signal peptide. The 205 aa corresponding protein has 12 cysteines in its sequence (5.8% of the protein sequence). We found a single non-synonymous SNP between the parental strains I05 and I07. Mirzadi Gohari *et al.* (2015) worked with *Zt09_13_00364*, naming it SSP118, and identified a differential expression over the infection period, with a peak at 8 dpi, in line with our qPCR results which identify this peak in both I05 and I07 (Figure 6). In the most recent annotation of the IPO323 genome sequence, this gene was named *G_12590* (unpublished data).

Genetic manipulation and pathology tests validate the involvement of G_07189 in pathogenicity

All knock-out, complementation and ectopic integration mutant strains were successfully generated following an ATMT protocol (Bowler *et al.*, 2010). Three randomly selected mutants per construction were able to successfully infect Chinese Spring and induce sporulation covering on average 99% of the inoculated area (Figures S2 and S3), with the exception of I05_Δ*Zt.13.364* + *Zt.13.364*_{I07}, where one of the three selected mutants was not able to infect Chinese Spring, or Renan, it was therefore excluded from subsequent analyses (Figure S3). In the I05 genetic background, deleting the gene *G_07189* has no effect on the phenotype while replacing the I05 allele (*G07189*_{I05}) by the I07 allele (*G07189*_{I07}) induces a suppression of sporulation (Figure 8). Indeed, I05 wild type induces a highly susceptible reaction on wheat cultivar Renan (>90% sporulating leaf area) while Renan becomes completely resistant when the I07 allele is introduced (Figure 8). In the I05 genetic background, the I07 allele of *G_07189* behaves as a classical avirulence gene inducing complete resistance in the host.

In the I07 genetic background, deleting the gene *G_07189* also has a strong and

significant effect on sporulation as I07 wild type induces 7% sporulating leaf area on average while the knock-out mutants I07 Δ G07189 induce 61% sporulating leaf area on average (Figure 8). In the I07 genetic background, the effect of the I07 allele of *G_07189* remains quantitative.

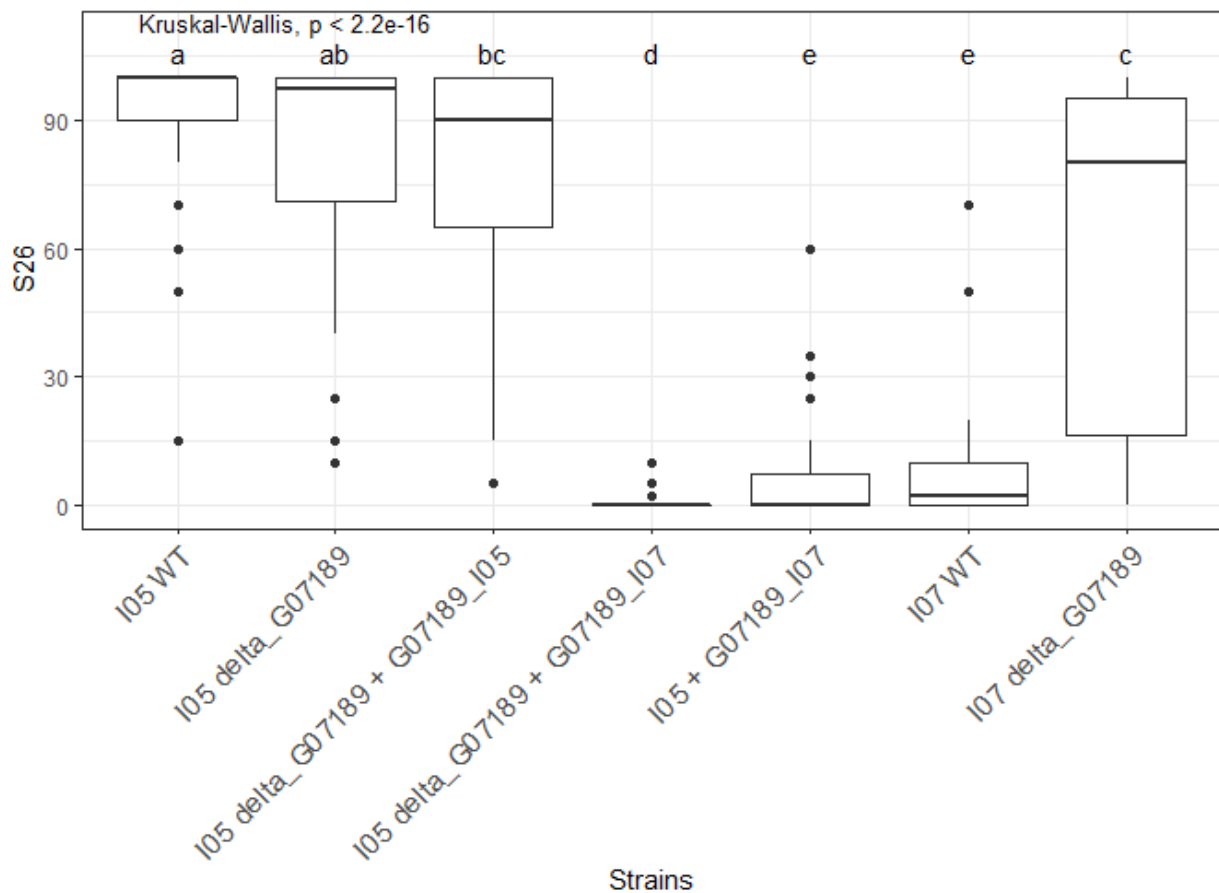


Figure 8. S26 values obtained for the different mutant strains obtained for *G_07189* inoculated on Renan. The Kruskal-Wallis value indicates that the phenotypic value for at least one type of strain is significantly different to the others. Letters a, b, c, d and e indicate significant difference for a Wilcoxon pairwise comparison at $\alpha=0.05$. The X-axis represents the different mutant strains tested. The Y-axis represents the phenotypic values obtained. The phenotypic data presented comprises all replicates for all individuals, the detail per individual is available in Supplementary Figure S2. S26 is the sporulating area (%) at 26 days post-inoculation.

In both genetic backgrounds of strains I05 and I07, deleting *Zt09_13_00364* or complementing with either allelic version of this gene has no significant effect on the phenotype (Figure 9). Therefore, we could not confirm a role of *Zt09_13_00364* in the capacity of *Z. tritici* to infect the wheat cultivar 'Renan'.

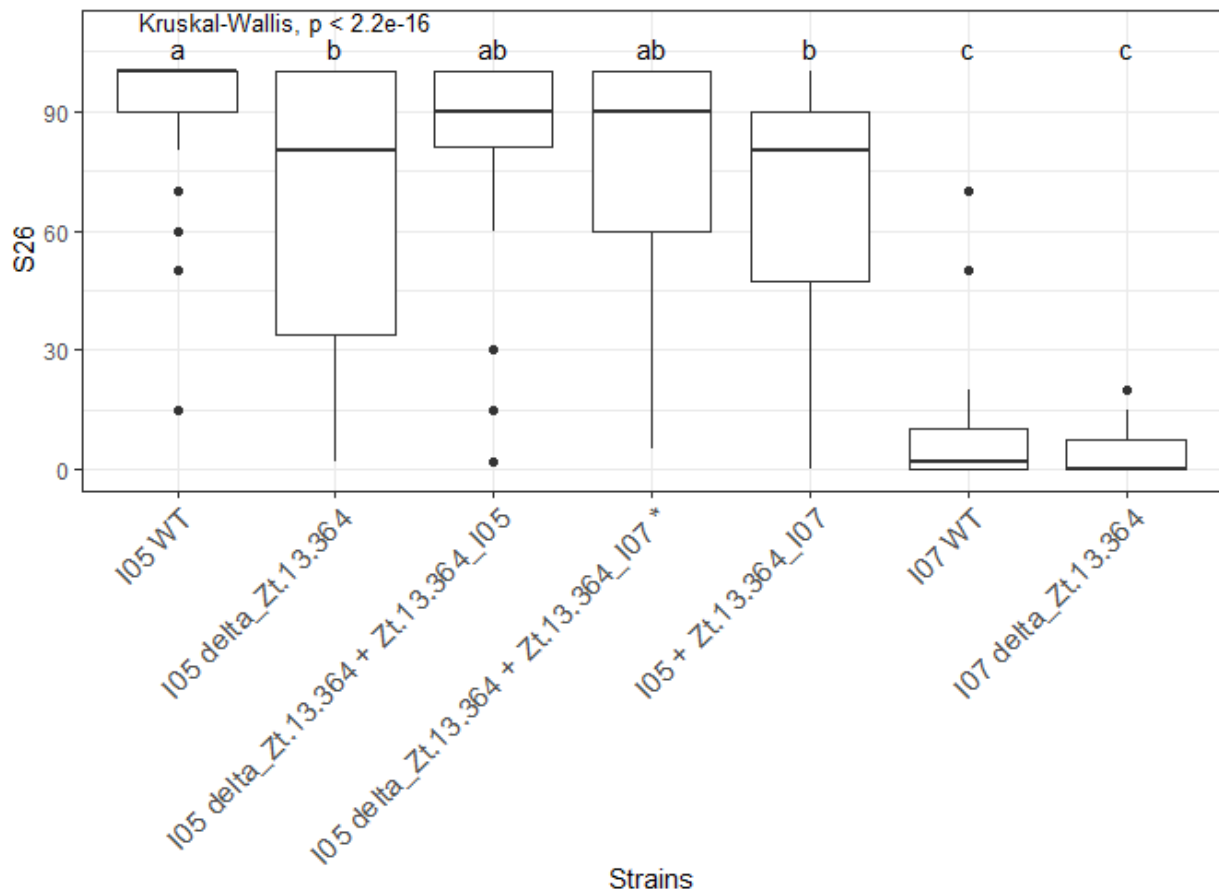


Figure 9. S26 values obtained for the different mutant strains obtained for *Zt09_13_00364* inoculated on Renan. The Kruskal-Wallis value indicates that the phenotypic value for at least one type of strain is significantly different to the others. Letters a, b, c, d and e indicate significant difference for a Wilcoxon pairwise comparison at $\alpha=0.05$. The X-axis represents the different mutant strains tested. The Y-axis represents the phenotypic values obtained. The phenotypic data presented comprises all replicates for all individuals, the detail per individual is available in Supplementary Figure S3. S26 is the sporulating area (%) at 26 days post-inoculation.

***G_07189* is highly conserved in French populations of *Z. tritici* but is under diversifying selection**

As the cloning experiments validated the effect of *G_07189*, we analysed its genetic diversity in the 229 French *Z. tritici* strains and searched for diversifying selection signatures. This analysis showed that *G_07189* is highly conserved among these strains, regardless of their being collected in 2009-2010 or in 2018-2019, with non-synonymous polymorphism for only 7 amino-acid residues out of 78 (Figure 10). With no collection period-related population structure, the strains carry genes encoding fifteen different isoforms of the *G_07189* protein with four isoforms representing 86% of all strains including I05 and I07 isoforms (Figure 10). All of the sequence diversity

observed for the gene is given by the second exon, with $\pi_{\text{CDS2}}=0.02254$ while π_{CDS1} and π_{intron} are around 0.0001 (Table 5). The ratio between non-synonymous and synonymous mutations ($\omega=dN/dS$) in the panel of strains was calculated at 2.118 for a one ratio model M0 (Table 6), a high value indicating diversifying selection, further validated by the selection M2 model having the highest lnL value (-487.686, $p<0.001$) among the three tested models (Table 6). According to the codon-based maximum likelihood approach, three residues are under significant diversifying selection ($p<0.01$) at positions 54, 71 and 72 in the protein sequence (Figure 10). We therefore have a highly conserved gene that is under diversifying selection.

French collections and the reference isolate IPO323. The avirulent (I07) and virulent parents (I05) are highlighted in green and red, respectively. (B) Top: Avr gene structure including UTRs (grey boxes) and the DNA coding sequence (CDS) of the mature protein (blue boxes). Bottom: Sliding window analyses of nucleotide diversity (π). (C) Top: The consensus sequence and sequence logos of Avr in the fungal population. Residues under significant diversifying selection ($p < 0.01$) are highlighted with red asterisks. Bottom: Conservation of Avr protein residues.

Table 5. Nucleotide diversity in the *G_07189* sequence

Sequence	Nucleotide diversity (π)
CDS 1	0.0001
Intron	0.00014
CDS 2	0.02254
Effector	0.01152

Table 6. Model test and parameter estimates of diversifying selection on *G_07189* coding sequence data.

Model	Parameter estimates			InL	PSS	LRT
M0: one ratio	$\omega = 2.118$			-498.543	none	-
M1: neutral	$p_0 = 0.517$	$p_1 = 0.482$		-497.661	NA	-
	$\omega_0 = 0$	$\omega_1 = 1$				
M2: selection	$p_0 = 0.903$	$p_1 = 0$	$p_2 = 0.096$	-487.686	3	M1 vs. M2 ($P < 0.001$)
	$\omega_0 = 0.772$	$\omega_1 = 1$	$\omega_2 = 21.711$			

L; likelihood estimate

PSS; number of positively selected sites with $p > 0.99$

ω ; omega dN/dS

LRT; likelihood ratio tests

Discussion

An ultra-dense genetic map of Z. tritici

In this study, we built an ultra-dense genetic map of *Z. tritici* based on RADseq data. It has 18,316 markers for 1,332 genetic positions, called bins. It covers 90% of the genome (chromosomes 14 and 18 discounted) over a genetic distance of 1,559 cM. Lendenmann *et al.* (2014) built two genetic maps using RADseq data. Their maps had 9,745 and 7,333 markers, about half the number in the map presented here. The map presented here therefore offers a far denser coverage than those two previous maps.

It does however not cover chromosomes 14 and 18. The previous linkage maps were also subject to presence/absence polymorphisms. Indeed, the 3D1x3D7 map lacked chromosomes 14, 15, 18 and 21, and the 1A5x1E4 map lacked chromosome 17. There are large gaps in the I05xI07 map, the two most problematic of these being those on chromosomes 1 and 6. These is not due to the type of sequencing and lack of *Pst*I restriction sites at certain positions as a closer look into the distribution of restriction sites along the genome debunks this theory, at least in the case of chromosome 1 (Figure 1). It could also be due to complex regions that are difficult to map or simply TE-rich regions, which were filtered out in the SNP calling pipeline. There is a large inversion on chromosome 13. Such inversions could result from a bad assembly of linkage groups, but have also been described as common in fungi and playing an important role in their genome plasticity (Plissonneau *et al.*, 2018). This map led to the identification of three QTL for quantitative pathogenicity with sufficient precision to target particular genes for subsequent work. RADseq is shown to be very well adapted for high-density genetic map building with full-genome coverage. Here from an F1 population we are able to build a map including the quasi-entirety of recombination events, illustrated by the saturation of the map, 18,316 mapped SNP for 1,332 genetic positions.

The interaction between Renan and I05xI07 is polygenic and quantitative

In this study we identified regions in the *Z. tritici* genome, which carry genes that contribute to quantitative pathogenicity towards *T. aestivum*. The cultivar used was Renan, known to carry at least three resistance QTL with different levels of resistance towards I05 and I07 on chromosomes 1D, 5D and 7B (Langlands-Perry *et al.*, 2022). Additionally, the resistance QTL on Renan show strain specificity between I05 and I07 and none of these QTL alone is able to confer total resistance to either I05 or I07, indicating that resistance is polygenic and quantitative in the Renan/I05-I07 interaction (Langlands-Perry *et al.*, 2022). Pathogenicity towards Renan is polygenic, with three QTL identified in this study, each contributing partially to the observed phenotypic variation. For two of these QTL, the parent carrying the pathogenic allele is I05, the parental strain known to be the most pathogenic on Renan. For the third, the parent carrying the pathogenic allele is I07, least pathogenic parental strain on Renan. We showed that these QTL have varying effects on the phenotype, *Qzt-I05-6* having the strongest effect, and are subject to epistatic interactions, notably in the case of the *Qzt-I05-6* and *Qzt-I07-13* interaction. None of the detected QTL colocalizes with an already known avirulence gene in *Z. tritici*. Indeed, *AvrStb6* is found on chromosome 5 (Zhong *et al.*, 2017), *AvrStb9* is on chromosome 1 but not in the region defined by *Qzt-I05-*

1(Amezrou *et al.* unpublished), and *Avr3D1* is on chromosome 7 (Meile *et al.*, 2018). Looking at progeny strains that did not have any recombination within the QTL intervals, we are able to separate the different strains according to the QTL allele combination that they carry in order to observe the impact of these allele combinations on the performance of the strains on Renan (Figure 5). We looked into the data for maximal sporulation S26 as this trait led to the detection of all three QTL. S26 was always higher for strains with the pathogenic allele for several QTL compared with strains carrying the pathogenic allele for only one QTL, though the difference was not always statistically significant, indicating incomplete additivity of the QTL effects. None of the non-pathogenic alleles of the QTL induced completely resistant phenotype such as that conferred by a major gene like *Stb6* in presence of *AvrStb6* (Zhong *et al.*, 2017). The only strains that were almost completely non-pathogenic were those that carried the non-pathogenic allele for all three QTL, demonstrating the quantitative and polygenic nature of pathogenicity and mirroring the quantitative and polygenic nature of resistance in Renan (Langlands-Perry *et al.*, 2022).

Fungal effectors involved in quantitative interactions

Two effector-like genes (i.e. coding for SSP) were identified as candidate genes to explain *Qzt-105-6* and *Qzt-107-13*. That on chromosome 6 had not been previously annotated but was supported by RNA sequences obtained from infected leaves (Grandaubert *et al.*, 2015; Haueisen *et al.*, 2019). Moreover, in a recent reannotation of the *Z. tritici* genome (unpublished data), which aimed at optimizing the detection of SSP, this gene was annotated and named *G_07189*. On chromosome 13, *Zt09_13_00364* had been previously identified as the best candidate gene to explain a pathogenicity QTL mapped on cultivars Taichung-29 and Gerek-79 (Gohari *et al.*, 2015). It encodes a 205 aa SSP comprising 12 cysteines with a differential expression during infection peaking at 8 dpi, primarily expressed during necrotrophy, fructification and sporulation. Additionally, qPCR performed on both candidate genes to evaluate their relative expression over the course of infection showed differential expression patterns, with an expression peak at 12 dpi for *G_07189* and at 8 dpi for *Zt09_13_00364*, further validating their status as good effector candidates. The results obtained after cloning these genes however led to different conclusions. In the case of *Zt09_13_00364* on chromosome 13, we found no significant difference in phenotypes between wild-types and their respective mutant strains, in both genetic backgrounds (Figure 9). We therefore could not confirm the role of this gene in the interaction with the cultivar Renan. It is possible that we simply worked with the wrong candidate. We identified other effector-like genes within the *Qzt-107-13* interval, with *Zt09_13_00355* standing

out with over 11% of cysteines in its protein sequence (Table S6), moreover chromosome 13 has been previously found to harbour a large number of putative effectors in comparison with the other chromosomes (Do Amaral *et al.*, 2012). It is also possible that *Zt09_13_00364* does contribute to pathogenicity with a quantitative effect, and that the very low effect of the *Qzt-107-13* on the phenotype ($\overline{r^2}=6.93\%$) is to blame for the difficulty to detect the potential effect of *Zt09_13_00364* in pathology assays.

We were however able to demonstrate the role of *G_07189* in the pathogenicity of I05 and I07 on the cultivar Renan. The knock-out of this gene from I05 (most pathogenic parental strain on Renan) which generated I05_Δ*G07189* strains, did not have a significant effect on the phenotype. The inclusion of the I07 (least pathogenic parental strain on Renan) allele for this gene, generating strains I05_Δ*G07189* + *G07189*_{I07} and I05 + *G07189*_{I07}, led to significantly lower S26 regardless of whether or not the I05 allele was present in the genetic background. The same phenomena were observed in the characterization of *Avr3D1* (Meile *et al.*, 2018), where the *Avr3D1* knock-out in the background of the most pathogenic parental strain of the pair in that study (3D7) was unaltered compared to the wild-type. The knock-out however led to increased disease symptoms in the background of the strain with lower pathogenicity used in that study (3D1) compared to the wild-type. This, corroborated with our results, suggests that *G_07189* encodes an avirulence factor. Additionally we showed that the region in which *G_07189* lies is TE-rich and presents with TE presence/absence polymorphisms (Figure 7), similar to what has been previously observed with *AvrStb6* and *Avr3D1* (Zhong *et al.*, 2017; Meile *et al.*, 2018). Moreover, while I07, which carries the avirulent allele for *G_07189*, is able to produce symptoms on Renan (*i.e.* S26 averaging at 10%), I05_Δ*G07189* + *G07189*_{I07} strains, which all carry this same allele, do not produce sporulation on Renan (*i.e.* S26 averaging at 0.8%). *G_07189* therefore leads to a quantitative phenotype in the I07 background, but to a qualitative, avirulent, phenotype in the I05 background. *G_07189* rather than acting like a classic avirulence gene in an *R/Avr* interaction has a spectrum of effects depending on the genetic background of the strain. This is illustrated in Figure 5, where the strains in the “none”, “13”, “1” and “1+13” categories, which all carry the avirulent allele for *G_07189*, have different effects, if not always significantly, on the phenotype. These different effects form a gradient depending on the QTL combinations.

Though we can only validate the effect of the *G_07189* with our work, we are able to present conclusive evidence that an *Avr* gene can have a quantitative or qualitative effect on the phenotypes depending on the fungal genetic background. Pathogenicity

is therefore not a strictly quantitative or qualitative variable, but rather fits somewhere on the continuum that they form.

On the plant side, it has been previously suggested that quantitative resistance genes in plants could be weaker forms of major resistance *R* genes (Poland *et al.*, 2009) and that most wheat resistance loci are matched by a specific effector (Plissonneau *et al.*, 2018). We therefore suppose that *G_07189* could interact with the wheat resistance QTL identified in Renan on chromosome 5D, designated as *Stb20q*, as this was the only QTL identified in Renan which had specific resistance to I07 (Langlands-Perry *et al.*, 2022). I07 carries the avirulent allele for *G_07189*, meaning that this gene could correspond to *AvrStb20q*.

Quantitative resistance durability despite gene-for-gene interactions

In a classic *R/Avr* gene-for-gene relationship, disease resistance is not considered durable due to the high probability of adaptation by the fungus with mutations in the *Avr* gene (Hartmann *et al.*, 2017; Meile *et al.*, 2018; Cowger & Brown, 2019; Niks *et al.*, 2019). In this case, we observe that the validated effector *G_07189* is highly conserved among French *Z. tritici* strains and is under diversifying selection. Moreover, no loss of *G_07189* was observed in the fungal populations or indeed any mutations in the effector-like features of the corresponding protein, i.e. the signal peptide and cysteine residues, inferring a potential fitness cost of such a loss. This is also what was observed for *Avr3D1*, the only other cloned effector in this pathosystem known to be involved in quantitative interactions (Meile *et al.*, 2018). In the latter case however, a high level of diversity was observed in fungal populations. *G_07189* has the capacity to evolve to adapt to wheat resistance, as demonstrated by the diversifying selection signatures in three residues. The low diversity and fact that the virulent, or most pathogenic, isoform (I05), is not the most abundant in recent strains may therefore seem paradoxical. Two potential explanations for this paradox are first that the corresponding resistance gene, which we hypothesize to be *Stb20q*, has not been largely deployed in the French cultivar landscape, meaning that no selection due to this resistance has been imposed on fungal populations. The cultivar on which *Stb20q* was identified is 'Renan' (Langlands-Perry *et al.*, 2022), mostly used in organic farming, and therefore not one of the majority cultivars in the landscape. The other explanation is that the selective pressure imposed on *G_07189* despite being present is low enough that adaptation is slowed down compared to what is observed with major *R/Avr* interactions. Indeed, the least pathogenic allele (I07) does not stop the fungus from completing its life cycle, as illustrated by the presence of pycnidia on the infected leaf surface. This second hypothesis fits in with the narrative that quantitative resistance is more durable than its

qualitative counterpart.

An example of a highly conserved effector under diversifying selection is APikL2 in *M. Oryzae* (Bentham *et al.*, 2021). Research has shown that a single amino-acid polymorphism is sufficient to evade host recognition, however, the evolutionary driver of this polymorphism was attributed to the expansion of the host target spectrum rather than immune receptor evasion (Bentham *et al.*, 2021). Moreover, similar to what we observe with *G_07189*, diversifying residues are centered at a specific part of the protein (Bentham *et al.*, 2021). This example opens a new avenue of possibilities as to the molecular interactions in which *G_07189* could be involved.

Conclusion

This study confirmed that pathogenicity in *Z. tritici* is complex and largely quantitative. We showed that several genes underlying QTL interact and contribute to the *T. aestivum* infection with varied impact on the phenotype. We demonstrated that genes underlying pathogenicity QTL can be effectors or *Avr* genes. These *Avr* genes can produce quantitative or qualitative phenotypes depending on the genetic background of the strains that carry them, advocating for a continuum between qualitative and quantitative notions of pathogenicity. We hypothesize the involvement of these effectors in minor gene-for-minor gene interactions, although this remains to be experimentally validated, in particular in the case of the putative *Stb20q/AvrStb20q* interaction. Furthermore, the low sequence diversity despite diversifying selection signatures observed for *G_07189* could advocate for the durability of quantitative resistance despite a potential gene-for-gene interaction context.

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Data Accessibility

Genome sequencing data for the 103 French *Z. tritici* isolates collected in 2009-2010 is available at NCBI BioProject PRJNA777581 (accessions SRR16762555 to SRR16762657; to be released), and for the 126 French *Z. tritici* isolates collected in 2018-2019 at NCBI BioProject (to be submitted). The sequence of G_07189, candidate gene for *AvrStb20q*, is available at NCBI Gene ID (to be submitted).

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SUPPLEMENTARY MATERIAL

Table S1. Primers used for qPCR

Number	Name	Sequence 5'-3'
99	qpcr 6 primer F 1	GTGCTGTCATCCATTTGTC
101	qpcr 6 R I05	GATCGTCCTTGTCCAAG
102	qpcr 6 R I07	TCCCGATCGCTACTGTC
105	qpcr 13 primer F 1	GCGAGATCCTTTCCAAG
107	qpcr 13 primer R 1	TGGTCGAGGTATTTGTGAA
109	EF1alpha F	AAGATTGGTGGTATCGGAACAG
110	EF1alpha R	GACTTGACTTCGGTGGTGAC
111	beta-tubuline F	AACGAGGCTCTCTACGACATCTG
112	beta-tubuline R	GGCGGAGACGAGGTGGTTG
113	UBC F	GTCTGCGGACCACAATACC
114	UBC R	CGACCTTCCTTGCTCTG

Supporting information 1. qPCR on our two top candidate genes

1. Biological material

To obtain *in planta* samples, a pathology assay was carried out according to the same protocol as previously presented. I05 and I07 were inoculated on Chinese Spring, 15 pots each containing three plants were inoculated per strain. After inoculation, three samples per strain, a sample here corresponds to all three inoculated leaves in a pot, were collected at 4, 8, 12, 16 and 20dpi respectively, following a similar kinetic as that used by Gohari et al. (2015). Upon collection, all samples were immediately flash-frozen in liquid nitrogen and conserved at -80°C.

In parallel, *in vitro* samples for I05 and I07 were obtained by growing both strains on a PDA (potato dextrose agar) culture medium. Both strains were grown over a week, then subcultured for 4 days before flash-freezing in liquid nitrogen and conservation at -80°C. Three replicates were generated for each strain.

2. RNA extraction

All samples were ground using sterile liquid nitrogen-cooled mortars and pestles. RNA was extracted from each obtained powder using the RNeasy® Mini Kit (Qiagen) according to the supplier's protocol. Following RNA extraction, a DNase treatment was performed to remove any contaminating genomic DNA.

3. Reverse transcription

cDNA was generated for each sample using ProtoScript® II First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA, USA) using 750ng RNA per reaction.

4. qPCR

Efficiency was determined for all qPCR primers using five-point standard curves generated with genomic DNA, each point as a triplicate.

Efficiency of qPCR primer pairs:

	E	R ²
111-112	97.3	0.998
109-110	99.1	0.998
113-114	97.1	0.998
99-101	94	0.996
99-102	86.6	0.997
105-107	91.7	0.92

qPCR mixes comprised 0.4µL of each primer at 10mM, 5µL of cDNA, 10µL of MESA GREEN qPCR MasterMix Plus for SYBR® Assay (Eurogentec) and 4.2µL of ultra-pure water. Amplification was performed with a 10min step of initial denaturation and enzyme activation at 95°C and 40 cycles of 95°C (10sec) and 60°C (1min). Each sample was run in technical duplicates.

As the primer efficiency was reasonably high (>85%) relative expression was calculated following the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001).

Table S2. Primers used for cloning

Number	Name	Sequence 5'-3'
1	pNOV_for	ATGACGCGGGACAAG
2	pNOV_rev	TAACACATTGCGGATACG
3	Hygro_for	TGATATTGAAGGAGCAT
4	Hygro_rev	TCTATTCCTTTGCCC
5	5' QTL6 for	CGGGGATCCTCTAGAGTCGACCATACGCACGATTGCTGTGTTGCTAC
6	5' QTL6 rev	CCAAAAAATGCTCCTTCAATATCAGGTTTTGGGTTGTGGAGATGCACG
7	3' QTL6 for	GTCCGAGGGCAAAGGAATAGATAAAACGAGACGTTCAAATG
8	3' QTL6 rev	TGCGGCCGCTCCGGATTCTGAATGCGGTAGCGGAGCTTCTTA
9	5' QTL6 for I07	gggatcctctagagtcgaccatTGTTAAATACAGCGGTAGTAGAC
10	3' QTL6 rev I07	CGGCCGCTCCGGATTCTGAATAGAGGCTGCCCTTACAACCTCT
11	5' QTL13 for I05	GGATCCTCTAGAGTCGACCATCAAAGATGGGCATCTCATCGG
12	5' QTL13 rev	CCAAAAAATGCTCCTTCAATATCAGGTTGGGTCGGTGTGCAAAAGAG
13	3' QTL13 for	cgtccgagggcaaaggaatagaGGGTGTTCAAACACTCTGGCG
14	3' QTL13 rev	GCGGCCGCTCCGGATTCTGAATATGGCACAAAACAAGCAACTGC
15	5' QTL13 for I07	ggggatcctctagagtcgaccatTTAAAACTAAAGGAGTTTCTAGCG
16	5' QTL13 for IPO323	gggatcctctagagtcgaccatCAGAGAGTCAGCTATTGGGTTG
17	5' QTL13 rev IPO323	CCAAAAAATGCTCCTTCAATATCAGGTTGGGTCGGTGTGCAAAAGAG
18	3' QTL13 for IPO323	gtccgagggcaaaggaatagaGGGTGGTCAAACACTCTGGCG
19	Hygro_rev2	GGGATCAGCAATCGC
20	Hygro_for2	CTGCCTGAAACCGAAC
21	Hygro_rev3	AGTTGCCTAAATGAACCATC
22	Hygro_for3	TCGATGATGCAGCTTG
23	pNOV_inv_for	ATTCGAATCCGGAGC
24	pNOV_inv_rev	ATGGTCGACTCTAGAGGATC

25	Zt-chr13-Univ107-05-F	TCCGTCCGATTCTCTCCATTCTC
26	Zt-chr13-Univ107-05-R	CTCGTGCCGCCTCGTTCTCTTG
27	Zt-chr6-I07-F1	ACCCGAATCTACTTTTTGTGACG
28	Zt-chr6-I07-R1	CCCTGCCGGTGAAGAAGACG
29	Zt-chr6-I05-F	TCGCATCCGTCATCGCTTCC
30	Zt-chr6-I05-R	AGGGTCCGTGCATGCTTTACA
31	pNOV_sulf_rev	CGCCTGGACGACTAAAC
32	QTL6 I05 for	CCGAATTCGAGCTCGGTACAGGAGGTCCTGATGGATCGA
33	QTL6 I05 rev	AATGCTCCTTCAATATCAAAGCTACCGACCCCGGCGG
34	QTL6 I07 for	AATTCGAGCTCGGTACTCTTGGGCAGGATTCGGATAA
35	QTL6 I07 rev	ATGCTCCTTCAATATCAACGTAAGGCGACGCTAC
36	QTL13 I05 for	CGAATTCGAGCTCGGTACATTCTCTCCATCCATCGTATC
37	QTL13 I05 rev	TGCTCCTTCAATATCAAATGGCACAAAACAAGCAACTGC
38	QTL13 I07 for	CGAATTCGAGCTCGGTACTCTGCAATCCTCGTTCTCTC
39	QTL13 I07 rev	AATGCTCCTTCAATATCAAGGTGGCATTTCGTGTAGAC
40	seq QTL6 I05 for	CTGTCATCCATTTGTCTGC
41	seq QTL6 I05 rev	CGTTTTATTACACGCATTCC
42	seq QTL6 I07 for	CTCTTTCTAATCGGCTAAAACG
43	seq QTL6 I07 rev	CTCTGCGTCCAAGTATGTG
44	seq QTL13 for	ACATGCGATCCCTTGC
45	seq QTL13 rev	AGTCGCGTTGGCATC
46	seq QTL 13 bis for	TACATCGCCAGGTCAC
47	seq QTL 13 bis rev	GGTTGGAAGGTATGG
48	Pnov_for BIS	CAGCGGCCATTTAAATC
49	Sulf_rev	ATGCTGCCAGTGACACG

Supporting information 2. Molecular cloning detail

1. Bacterial strains and DNA manipulation

I05 and I07 did not display any resistance to hygromycin and sulfonyleurea antibiotics. All primers used are referenced in Table S1. Original vectors pNOV2114 and pNOV_3Gate_SUL were used for the assemblies. Genomic DNA from I05, I07 and IPO323 used to obtain cloning fragment was extracted following the same protocol as for the DNA used for RADseq. For all PCR performed to obtain cloning fragments, the Taq polymerase Phusion (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used under adapted PCR conditions. For the plasmids used to generate knock-out mutants, pNOV2114 was linearized using the primers 23 and 24. A fragment carrying a hygromycin resistance gene and its promoter was obtained by performing a PCR on pNOV_3Gate_HYG using primers 3 and 4. Fragments of approximately 1000bp upstream and downstream of the start and stop of the candidate genes were generated by performing a PCR on genomic DNA of I05, I07 and IPO323 using primers 5 to 18. For the plasmids used to generate complementation mutants and ectopic integration mutants, pNOV_3Gate_SUL, which carries a sulfonyleurea resistance gene, was linearized by digestion with KpnI and HindIII following the supplier's recommendations (Thermo Fisher Scientific Inc., Waltham, MA, USA). Fragments containing the candidate genes and at least 499bp upstream of the start codon and at least 1kb downstream of the stop codon were generated by performing a PCR on genomic DNA of I05 and I07 using primers 32 to 39. The presence of transposable elements with polymorphism between I05 and I07 determined the size of the cloned fragments as we wished to exclude the potential effect of these transposable elements from the experiment. Indeed such regions have been shown to have an effect on pathogenicity (Mat Razali et al., 2019; Seidl and Thomma, 2017; Singh et al., 2021). Assembly of the fragments was performed by Gibson assembly (Gibson et al., 2009; Gibson et al., 2010) using the Gibson Assembly Cloning Kit (New England Biolabs, Ipswich, MA, USA). NEB 5-alpha Competent *E. coli* (High Efficiency) (New England Biolabs, Ipswich, MA, USA) were transformed by heat shock with the generated plasmids and used for their amplification. Successfully transformed strains were identified by PCR and mini-prepped plasmid constructs validated by Sanger sequencing (Eurofins, Luxembourg) using primers 1, 2, 19 to 22, 31, and 40 to 47. The *A. tumefaciens* strain AGL1 was then transformed by heat shock with each generated plasmid. Colonies were screened by PCR using the same primers as for the *E. coli* colonies.

2. *A. tumefaciens* mediated transformation of *Z. tritici*

The *Z. tritici* strains I05 and I07 were transformed by ATMT (Bowler et al., 2010) following the standard protocol to generate knock-out mutants and ectopic integration mutants. IPO323 was also transformed with the appropriate *A. tumefaciens* strains and used as a transformation control. This enabled us to obtain I05_ΔG07189, I05_ΔZt.13.364, I07_ΔG07189 and I07_ΔZt.13.364 mutants. I05_ΔG07189 and I05_ΔZt.13.364 mutants were transformed following the same protocol to generate complementation mutants I05_ΔG07189 + G07189_{I05}, I05_ΔG07189 + G07189_{I07}, I05_ΔZt.13.364 + Zt.13.364_{I05} and I05_ΔZt.13.364 + Zt.13.364_{I07} and ectopic integration mutants I05 + G07189_{I07} and I05 + Zt.13.364_{I07}.

Mutant strains were selected by hygromycin or sulfonyleurea screening depending on which resistance gene they were designed to carry. Obtained strains were verified by PCR using primers 25 to 30, 48 and 49 (Table S2) after genomic DNA extraction with DNeasy® Plant Mini Kit (Qiagen) according to the supplier's protocol.

Table S3. SNP identified in the *Z. tritici* genome using RADseq data

Chromosome	Number of SNP detected	Number of SNP/ Chromosome size in bp (‰)
1	11286	1.9
2	6544	1.7
3	6438	1.8
4	4405	1.5
5	5346	1.9
6	3279	1.2
7	5292	2
8	4898	2
9	4359	2
10	3519	2.1
11	3563	2.2
12	3221	2.2
13	2501	2.1
14	14	0
15	1738	2.7
16	1109	1.8
17	1837	3.1
18	14	0
19	1064	1.9
20	1385	2.9
21	1087	2.7
Total without chromosomes 14 and 18	72871	2.1

Table S4. Details on QTL for pathogenicity detected with the I05×I07 genetic map and phenotypic data

Replicate	Trait	5% threshold (1000 permutations)	Chromosome	LOD score	Flanking marker 1	Position flanking marker 1 (cM)	Peak marker	Peak marker position (cM)	Flanking marker 2	Position flanking marker 2 (cM)	r ² (%)	Parent carrying the pathogenic allele
1	AUDPCS	3.10	1	4.57	chr1_244317	228.56	chr1_217589	230.58	chr1_112362	232.87	7.43	I05
1	S26	3.14	1	5.06	chr1_244317	228.56	chr1_217589	230.58	chr1_112362	232.87	7.13	I05
2	AUDPCG	3.19	1	3.20	chr1_250666	227.91	chr1_217589	230.58	chr1_112362	232.87	5.09	I05
2	AUDPCN	3.16	1	3.21	chr1_244317	228.56	chr1_217589	230.58	chr1_112362	232.87	4.40	I05
2	AUDPCS	3.05	1	3.82	chr1_244317	228.56	chr1_143600	232.54	chr1_112362	232.87	6.79	I05
2	S26	3.09	1	4.04	chr1_244317	228.56	chr1_143600	232.54	chr1_112362	232.87	6.71	I05
3	AUDPCN	3.22	1	4.01	chr1_244317	228.56	chr1_181231	231.90	chr1_112362	232.87	6.64	I05
3	AUDPCS	3.07	1	3.32	chr1_244317	228.56	chr1_158919	232.22	chr1_112362	232.87	6.74	I05
1	AUDPCG	3.17	6	7.43	chr6_366855	24.18	c6.loc30	30.00	chr6_532314	34.19	20.52	I05
1	AUDPCN	3.13	6	11.65	chr6_366855	24.18	c6.loc28	28.00	chr6_532314	34.19	30.18	I05
1	AUDPCS	3.10	6	8.85	chr6_366855	24.18	c6.loc28	28.00	chr6_532314	34.19	23.95	I05
1	PYC	3.33	6	16.77	chr6_366855	24.18	c6.loc28	28.00	chr6_506156	31.13	40.15	I05
1	S20	2.80	6	3.22	chr6_363606	20.71	chr6_370491	26.54	chr6_532314	34.19	9.43	I05
1	S26	3.14	6	10.77	chr6_366855	24.18	c6.loc28	28.00	chr6_532314	34.19	28.28	I05
2	AUDPCG	3.19	6	7.29	chr6_366855	24.18	chr6_370491	26.54	chr6_506156	31.13	20.04	I05
2	AUDPCN	3.16	6	10.84	chr6_366855	24.18	c6.loc28	28.00	chr6_506156	31.13	28.46	I05
2	AUDPCS	3.05	6	6.24	chr6_366855	24.18	chr6_370491	26.54	chr6_506156	31.13	17.38	I05
2	S26	3.09	6	7.93	chr6_366855	24.18	chr6_370491	26.54	chr6_506156	31.13	21.54	I05
3	AUDPCG	3.24	6	8.58	chr6_370491	26.54	c6.loc28	28.00	chr6_506156	31.13	29.85	I05
3	AUDPCN	3.22	6	11.44	chr6_370491	26.54	c6.loc28	28.00	chr6_506156	31.13	37.70	I05
3	AUDPCS	3.07	6	6.49	chr6_366855	24.18	c6.loc28	28.00	chr6_506156	31.13	23.62	I05
3	NBS	3.39	6	3.95	chr6_363606	20.71	c6.loc26	26.00	chr6_532314	34.19	13.64	I05
3	PYC	3.22	6	11.14	chr6_370491	26.54	c6.loc32	32.00	chr6_532314	34.19	33.77	I05
3	S20	2.72	6	3.48	chr6_363606	20.71	chr6_370491	26.54	chr6_506156	31.13	12.57	I05

3	S26	3.32	6	9.57	chr6_366855	24.18	c6.loc28	28.00	chr6_506156	31.13	32.39	I05
2	S26	3.09	13	3.47	chr13_1114801	0.00	chr13_1114801	0.00	chr13_978875	5.19	4.96	I07
3	AUDPCN	3.22	13	3.40	chr13_1114801	0.00	chr13_1114801	0.00	chr13_978875	5.19	3.22	I07
3	AUDPCS	3.07	13	4.64	chr13_1114801	0.00	chr13_1114801	0.00	chr13_928663	8.64	7.85	I07
3	NBS	3.39	13	4.15	chr13_1114801	0.00	chr13_1114801	0.00	chr13_978875	5.19	9.40	I07
3	S20	2.72	13	3.92	chr13_1114801	0.00	c13.loc4	4.00	chr13_928663	8.64	8.85	I07
3	S26	3.32	13	5.33	chr13_1114801	0.00	chr13_1114801	0.00	chr13_978875	5.19	7.31	I07

Table S5. Characteristics of annotated genes within the *Qzt-105-6* confidence interval

START (bp)	STOP (bp)	Gene name	Length of coding sequence (bp)	Number of exons	Protein length (aa)	Number of cysteines	Percentage of cysteines	Associated GO terms	Signal peptide
366114	365404	Zt09_6_00095	621	2	206	3	1.46	0031505;0005199	Yes
370562	372546	Zt09_6_00096	1677	4	558	7	1.25	0003824	No
373148	372672	Zt09_6_00097	477	1	158	3	1.90	0055114;0051537;0016491;0008942	No
373314	375494	Zt09_6_00098	2028	3	675	7	1.04	0006351;0008270;0003677;0005634	No
376101	377583	Zt09_6_00099	1338	3	445	5	1.12	0055085	No
378502	377794	Zt09_6_00100	654	2	217	7	3.23	0016407	No
379073	379997	Zt09_6_00101	759	4	252	3	1.19		No
384231	380136	Zt09_6_00102	4047	2	1348	2	0.15	0032065;0005515;0005543;0005938	No
386490	387778	Zt09_6_00103	1206	2	401	5	1.25	0005515;0005543	No
389764	387944	Zt09_6_00104	1449	2	482	10	2.07	0016491	No
390263	392435	Zt09_6_00105	1725	3	574	6	1.05		No
395620	392810	Zt09_6_00106	2811	1	936	13	1.39	0006298;0005524;0030983;0003677;0032300	No
397507	396649	Zt09_6_00107	795	2	264	5	1.89		No
399277	397937	Zt09_6_00108	1341	1	446	2	0.45	0003725	No
401008	399674	Zt09_6_00109	1098	4	365	4	1.10	0016020	No
473175	471117	Zt09_6_00110	1908	3	635	3	0.47		No
473336	474422	Zt09_6_00111	900	2	299	0	0.00		No
474997	477222	Zt09_6_00112	2169	2	722	8	1.11	0005515	No
479459	477267	Zt09_6_00113	1872	5	623	9	1.44	0055114;0016614;0050660	No
480182	481778	Zt09_6_00114	1485	3	494	5	1.01		No
484136	482498	Zt09_6_00115	1581	2	526	7	1.33		No
484521	486674	Zt09_6_00116	2154	1	717	3	0.42	0007094	No
488156	487125	Zt09_6_00117	792	3	263	9	3.42		No
488690	489541	Zt09_6_00118	852	1	283	2	0.71		No
490213	491217	Zt09_6_00119	1005	1	334	6	1.80		No

494292	492608	Zt09_6_00120	1575	3	524	5	0.95	0055114;0004470;0004471;0051287	No
496252	498486	Zt09_6_00121	2235	1	744	2	0.27	0008289	No
499583	499158	Zt09_6_00122	369	2	122	4	3.28		No
501728	500457	Zt09_6_00123	1272	1	423	4	0.95	0071946	Yes
503651	502914	Zt09_6_00124	738	1	245	1	0.41	0005515	No
509490	511448	Zt09_6_00125	1755	4	584	7	1.20	0006470;1902751;0004725	No
520560	520022	Zt09_6_00126	399	2	132	1	0.76		No
522235	523014	Zt09_6_00127	720	2	239	0	0.00		No
525247	523882	Zt09_6_00128	1245	3	414	2	0.48		No
530080	531954	Zt09_6_00129	1596	5	531	6	1.13		No
532291	535332	Zt09_6_00130	3042	1	1013	9	0.89	0006355;0008134;0003677;0005730	No

Table S6. Characteristics of annotated genes within the *Qzt-107-13* confidence interval

START (bp)	STOP (bp)	Gene name	Length of coding sequence (bp)	Number of exons	Protein length (aa)	Number of cysteines	Percentage of cysteines	Associated GO terms	Signal peptide
948898	949212	Zt09_13_00321	315	1	104	3	2.88		no
949257	950254	Zt09_13_00322	891	3	296	7	2.36		no
952272	954036	Zt09_13_00323	1629	3	542	17	3.14	0055085;0022857;0016020	no
955534	956591	Zt09_13_00324	933	3	310	2	0.65	0016491	no
957159	958217	Zt09_13_00325	1059	1	352	3	0.85	0032979;0005743	yes
958662	959299	Zt09_13_00326	351	3	116	1	0.86	0006413;0003743	no
960690	961190	Zt09_13_00327	501	1	166	4	2.41		yes
961604	962281	Zt09_13_00328	678	1	225	3	1.33		no
963149	963615	Zt09_13_00329	390	2	129	10	7.75		yes
964130	965996	Zt09_13_00330	1641	5	546	16	2.93	0046872	no
966549	967064	Zt09_13_00331	516	1	171	1	0.58	0008608;0042729;0005876	no
967386	968603	Zt09_13_00332	1218	1	405	1	0.25	0003676	no
976414	977174	Zt09_13_00333	672	2	223	0	0.00	0006777;0030366;0005829;0019008	no
979112	980155	Zt09_13_00334	1044	1	347	5	1.44		no
981376	982617	Zt09_13_00335	1242	1	413	7	1.69	0009107;0051539;0016992;	no
982840	985542	Zt09_13_00336	1869	9	622	10	1.61	0007264;0005525;0003924	no
986283	986816	Zt09_13_00337	471	2	156	4	2.56		no
987345	990272	Zt09_13_00338	1368	8	455	9	1.98	0006749;0005515	no
992073	992671	Zt09_13_00339	351	5	116	0	0.00	0045048;0044183;0030176	no
993985	998469	Zt09_13_00340	4485	1	1494	23	1.54	0006357;0003712;0016592	no
998966	1000969	Zt09_13_00341	2004	1	667	5	0.75		no
1003086	1003394	Zt09_13_00342	309	1	102	8	7.84		yes
1004049	1005251	Zt09_13_00343	1203	1	400	6	1.50		yes

1018593	1019831	Zt09_13_00344	1239	1	412	4	0.97		no
1020265	1022410	Zt09_13_00345	1896	3	631	9	1.43		no
1023001	1024444	Zt09_13_00346	1389	2	462	5	1.08		no
1025350	1025959	Zt09_13_00347	561	2	186	4	2.15		no
1026491	1027867	Zt09_13_00348	1377	1	458	7	1.53		no
1053154	1056491	Zt09_13_00349	2997	5	998	15	1.50	0000160;0007165;0016310;	no
1056842	1058535	Zt09_13_00350	1479	5	492	16	3.25		no
1058812	1060564	Zt09_13_00351	1689	2	562	13	2.31	0055085;0022857;0016021	no
1061163	1062904	Zt09_13_00352	1686	2	561	13	2.32		no
1064242	1065225	Zt09_13_00353	774	5	257	6	2.33	0005975;0016810;0003824	yes
1066460	1068243	Zt09_13_00354	1728	2	575	3	0.52		no
1086334	1086612	Zt09_13_00355	162	3	53	6	11.32		yes
1089167	1090449	Zt09_13_00356	1230	2	409	4	0.98	0016787	no
1090731	1092547	Zt09_13_00357	1560	5	519	8	1.54	0055085;0022857	no
1093146	1094577	Zt09_13_00358	1317	3	438	8	1.83		no
1095853	1097659	Zt09_13_00359	1473	7	490	11	2.24	0016491	no
1097892	1099692	Zt09_13_00360	1473	6	490	11	2.24	0055085;0022857	no
1108416	1110419	Zt09_13_00361	1629	6	542	10	1.85		no
1110704	1112251	Zt09_13_00362	1227	6	408	12	2.94	0055085;0022857	no
1114143	1114912	Zt09_13_00363	627	3	208	2	0.96	0003824	no
1116277	1116950	Zt09_13_00364	618	2	205	12	5.85		yes
1118475	1120592	Zt09_13_00365	2118	1	705	8	1.13	0016702	no
1126071	1128010	Zt09_13_00366	1863	2	620	6	0.97	0009058;0004311;0016872;0016765	no
1128251	1130164	Zt09_13_00367	1914	1	637	6	0.94	0016117;0016491	no
1132439	1133774	Zt09_13_00368	1101	4	366	5	1.37	0006811;0005216;0016020	no
1134425	1136228	Zt09_13_00369	1698	3	565	5	0.88		no
1138015	1139802	Zt09_13_00370	1788	1	595	18	3.03	0005515	no
1149603	1150793	Zt09_13_00371	1191	1	396	5	1.26		no

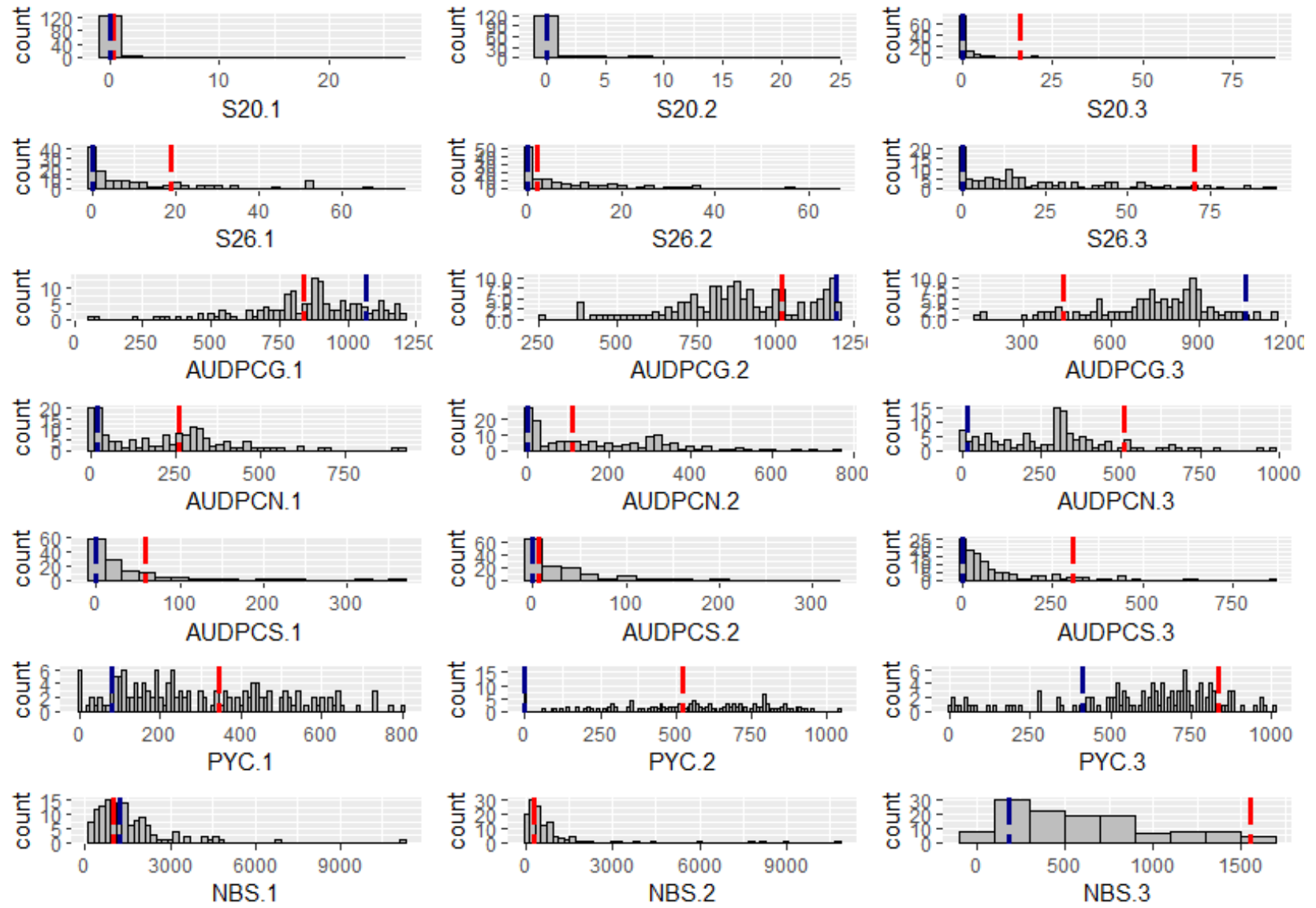


Figure S15. Histograms representing the phenotypic data distribution. For all of the histograms, the X-axis represents the trait values and the Y-axis represents the number of individuals from the $10^5 \times 10^7$ population, which have the corresponding value for the phenotypic trait. Each line corresponds to a phenotypic trait. S20 and S26 are the sporulating areas (%) at 20 and 26 days respectively and are expressed in percentages. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. PYC is the pycnidia density. NBS is the number of spores per pycnidiospore. Each trait was studied over three replicates: 1, 2 and 3. Each column corresponds to a replicate. The blue dotted line corresponds to the trait value for I07, the red dotted line corresponds to the trait value for I05.

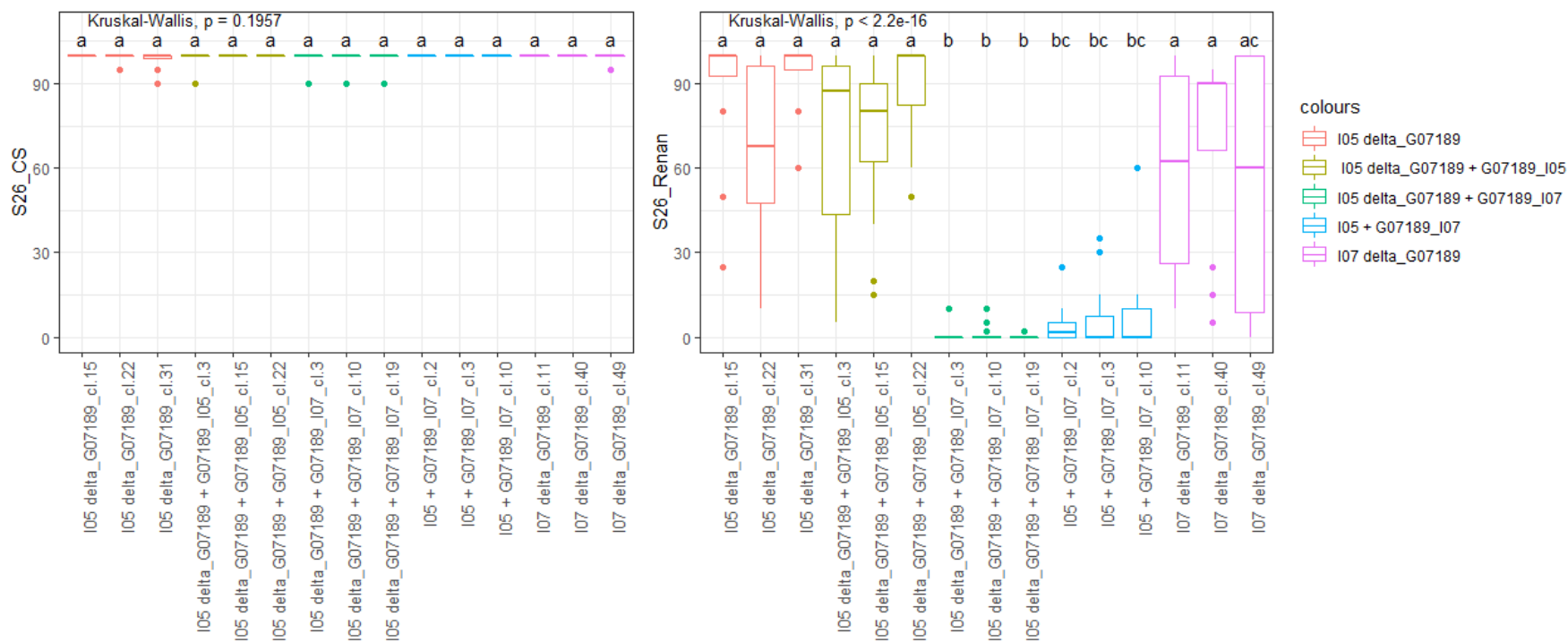


Figure S2. Boxplots representing the S26 in % (sporulation a 26 days post inoculation) for all mutant strains generated for *G_07189*. On the left are the results for the inoculation on cultivar Chinese Spring, on the right for the inoculation on cultivar Renan. The X-axis represents the trait values for each mutant strain. Colours represent mutant strain types. The Kruskal-Wallis value indicates that the phenotypic value for at least one type of strain is significantly different to the others if $p < 0.05$. Letters a, b and c indicate significant difference for a Wilcoxon pairwise comparison at $\alpha=0.05$.

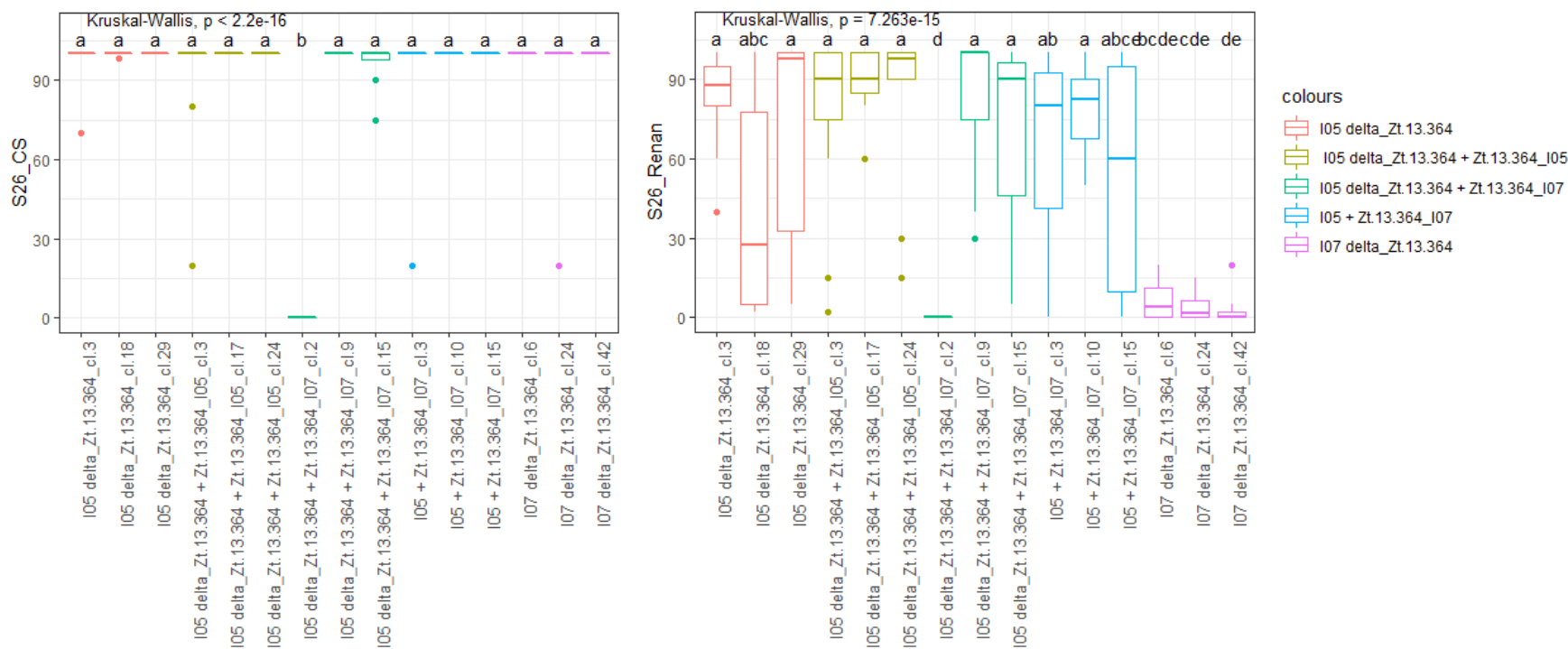


Figure S3. Boxplots representing the S26 in % (sporulation a 26 days post inoculation) for all mutant strains generated for *Zt09_13_00364*. On the left are the results for the inoculation on cultivar Chinese Spring, on the right for the inoculation on cultivar Renan. The X-axis represents the trait values for each mutant strain. Colours represent mutant strain types. The Kruskal-Wallis value indicates that the phenotypic value for at least one type of strain is significantly different to the others if $p < 0.05$. Letters a, b and c indicate significant difference for a Wilcoxon pairwise comparison at $\alpha=0.05$.

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CHAPTER 4: GENE-FOR-GENE INTERACTIONS BETWEEN HOST AND PATHOGEN BLUR THE LINES BETWEEN QUALITATIVE AND QUANTITATIVE DEFINITIONS OF RESISTANCE

Gene-for-gene interactions between host and pathogen blur the lines between qualitative and quantitative definitions of resistance

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Abstract

- In phytopathology, quantitative and qualitative forms of resistance have been described as two separate systems with their own distinct mechanisms for the last 60 years, although the mechanisms underlying quantitative resistance have rarely been described. Working with the *Zymoseptoria tritici*-wheat pathosystem, we aimed to demonstrate that many overlaps exist between what is deemed quantitative and qualitative, emphasizing a need for these terms to be re-defined.

- Using two bi-parental populations carrying known pathogenicity (fungus-side) and resistance (wheat-side) QTL, we demonstrated QTL-for-QTL interactions by linkage analysis and demonstrated a gene-for-gene interaction involving a previously cloned *Z. tritici* effector gene. We also looked into the haplotype diversity in the region harbouring the cloned effector in a panel of diverse strains and its impact on phenotypes by combining genetic analyses and pathology tests.
- Two QTL-for-QTL interactions were identified, one of these explained by an *R/Avr* interaction involving *Stb20q* and *AvrStb20q* underlying quantitative phenotypes.
- We demonstrated that gene-for-gene interactions can underlie quantitative phenotypes, highlighting a major issue with the generally accepted dichotomy between quantitative and qualitative definitions of disease resistance. We suggest that rather than compartmentalizing qualitative and quantitative forms of disease resistance, they should be seen as a continuum, with qualitative interactions being the extreme cases of overall quantitative systems.

Keywords

Quantitative interaction, Quantitative trait loci, Plant-pathogen interactions, Septoria tritici blotch, *AvrStb20q*

Introduction

Pathogen-borne disease is a major driver of yield losses in cultivated crops. In order to fight disease, an arsenal of prophylactic, disease-limiting and curative measures is available. When a disease is not avoidable, it is necessary to attempt to control it. This can be done effectively by chemical means, however this is associated with high economic and environmental costs. Turning to plant immunity then seems a reasonable path to follow. In a two-tiered model of plant immunity, the first level acts on the exterior of the host cell, where pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) are recognized by pattern recognition receptors (PRRs). PRR stimulation leads to PAMP-triggered immunity (PTI). This first defence system is effective against a broad range of pathogens (Dodds and Rathjen, 2010; Yu et al., 2017). Adapted pathogens can produce effectors, generally found to be cysteine-rich small secreted proteins, which undetected by PTI, are able to penetrate host cells (Oliva et al., 2010), causing effector-triggered susceptibility (ETS).

The second layer of plant immunity then concerns intracellular receptors that are capable of recognizing these effectors, thus inducing effector-triggered immunity (ETI) (Dodds and Rathjen, 2010). ETI is associated with highly specific gene-for-gene interactions for which the groundwork was carried out by Flor who worked with the flax/flax rust pathosystem (Flor, 1956). Since this work, the notions surrounding gene-for-gene interactions have been studied in a plethora of pathosystems involving fungi, oomycetes, bacteria and even insects (Gabriel et al., 1986; de Wit, 1995; Bonas and Van den Ackerveken, 1999; Jia et al., 2000; Gout et al., 2006; Stukenbrock and McDonald, 2009; Hall et al., 2009; Stuart, 2015). The gene-for-gene interaction is between an *R* major resistance gene which recognizes the product of an *Avr* avirulence gene, often an effector, thus inducing a hypersensitive response (HR) (Niks et al., 2019). This type of resistance can also be called qualitative resistance. This genetic interaction between the plant and the pathogen is associated with constant coevolution, which causes a veritable arms race, illustrated by the Zig-Zag model proposed by Jones and Dangl with endless cycles of ETI/ETS that correspond to the adaptation of the host and pathogen to each other (Jones and Dangl, 2006). This arms race is representative of why durability of major resistance genes is recurrently put into question. Indeed, the higher the selection pressure induced by *R* genes, the more likely it is for these *R* genes to be overcome (Mundt, 2018; Nelson et al., 2018; Witek et al., 2021). As will be discussed later on, using strict dichotomy is a hazardous way of defining biological events, some even describe the dichotomy between PTI and ETI as ambiguous (Thomma et al., 2011). Here, the plant immunity model separates PTI on the exterior of the cell and ETI on the interior. However, certain pathogens do not act on an intracellular level, *Zymoseptoria tritici* for example is a strictly apoplastic fungal pathogen (Kettles et al., 2017; Hill and Solomon, 2020). Some *R* genes have similar products to PRRs but interact with extracellular effectors and can encode receptor-like proteins (RLPs) such as receptor-like kinases (RLKs) or wall associated kinases (WAKs), leading to the term effector-triggered defence (ETD) which covers *R* gene mediated host resistance against apoplastic effectors (Stotz et al., 2014). Qualitative resistance is often opposed to quantitative resistance, which is a weaker or partial form of disease resistance exerting a lower selection pressure on pathogens (Niks et al., 2015). These quantitative resistances are generally polygenic and are mapped to QTL, which each contribute to the overall resistance of the host. They are collectively called quantitative resistance (Niks et al., 2015). In a segregating population, quantitative resistance is associated to a continuous distribution of phenotypes (Corwin and Kliebenstein, 2017; Niks et al., 2015). If it is of ever growing interest, quantitative resistance is still relatively poorly understood (St Clair, 2010), though the underlying mechanisms have been hypothesized (Poland et al., 2009). One particular hypothesis, points towards the genes

involved in quantitative resistance simply being weaker forms or indeed alleles of *R* genes (Poland et al., 2009), which do not completely stop disease progression, but rather limit symptoms. If quantitative resistance is conferred by weak *R* genes, these should have corresponding *Avr* genes. Quantitative gene-for-gene, or indeed minor gene-for-minor gene interactions, have been suggested in several pathosystems including barley/barley leaf rust (González et al., 2012; Parlevliet, 1977; Qi et al., 1999), barley/barley leaf stripe (Arru et al., 2003), potato/*Phytophthora infestans* (Leonards-Schippers et al., 1994), pepper/potyvirus (Caranta et al., 1997), wheat/*Z. tritici* (Meile et al., 2018) and rapeseed/*Leptosphaeria maculans* (Jiquel et al., 2021). For the last two mentioned pathosystems, the publications go beyond suggestion. Meile et al. (2018) worked with *Avr3D1*, an effector gene responsible for differences in quantitative virulence between *Z. tritici* strains 3D1 and 3D7. Interaction of this effector with known major resistance genes *Stb7* and/or *Stb12* is postulated, though not formally demonstrated. The use of the term “quantitative” relates to the incomplete resistance to the avirulent allele of the effector gene (Meile et al., 2018). Jiquel et al. (2021) worked with *LmSTEE98*, an effector gene involved in stem colonization, which occurs late in the *L. maculans* infection cycle when plants are grown up and resistance is considered mostly quantitative. Once again, “quantitative” relates to the incomplete or partial resistance observed in the phenotype. Interestingly, when expressed at the cotyledon stage, this gene induced HR on the genotype bearing the corresponding *R* gene *RlmSTEE98*, a dominant gene that has been fine-mapped in rapeseed (Gervais, 2017; Jiquel et al., 2021). Another example of what can be considered quantitative gene-for-gene interaction is observable in the wheat/*P. nodorum* interaction. In this case, it is not the classic gene-for-gene which is observed, but what has been dubbed an “inverse gene-for-gene” interaction wherein necrotrophic effectors (NE) target host sensitivity genes (*Snn*) (Friesen et al., 2007). In this pathosystem, if a strain carrying two NE infects a cultivar carrying the matching *Snn* genes, the infection can be more severe than with only one of the NE (Abeysekara et al., 2012; Chu et al., 2010; Phan et al., 2021). The particularity here, as opposed to HR induced by a single *R* gene, lies in the polygenic potential of the infection, blurring the limits between what can be considered as either qualitative or quantitative. On one side of the argument, Cowger et al. (2020) clearly separate *Snn*-NE interactions from quantitative resistance because of the nature of the genes involved. Indeed the sensitivity genes targeted by NE are thought to be *R*-genes that normally elicit resistance upon pathogen recognition, such as NLRs (nucleotide leucine-rich receptors) or ETI associated RLPs or RLKs, but that have been hijacked by necrotrophic pathogens (Cowger et al., 2020; Lin et al., 2020). On the other side however, the authors state that the apparent durability of disease resistance observed in field trial data is incompatible with specific interactions like *R/Avr* or indeed *Snn/NE*.

In this case, the phenotypes observed fall into what could be considered quantitative, but the underlying molecular mechanisms fall into the qualitative category. With a growing number of examples of potential, and indeed demonstrated, gene-for-gene interactions underlying quantitative interactions, it would seem that such interactions are after all not limited to what is deemed qualitative, suggesting that generally accepted definitions may be in need of some revision.

Here we propose to look into the mechanisms underlying quantitative resistance in a system that is polygenic, where phenotypes have a continuous distribution and where the different genes or QTL involved each contribute to the overall phenotype. To this effect, we worked with two bi-parental populations. The first is derived from a cross between two *Z. tritici* strains I05 and I07, which led to the mapping of three pathogenicity QTL towards wheat cultivar 'Renan' (Chapter 3; this thesis). I05 carries the pathogenic allele for *Qzt-I05-1* and *Qzt-I05-6* that were respectively identified on chromosomes 1 and 6, and I07 carries the pathogenic allele for *Qzt-I07-13*, identified on chromosome 13. *Qzt-I05-6* was cloned and corresponds to a previously unannotated cysteine-rich small secreted protein (Chapter 3; this thesis). The second bi-parental population is composed of recombinant inbred lines (RILs) obtained from a cross between cultivars 'Renan', mostly resistant to septoria leaf blotch (STB), and 'Chinese spring', susceptible to the disease. Linkage analyses led to the detection of three resistance QTL on chromosomes 1D, 5D and 7B with the resistance allele carried by 'Renan' (Langlands-Perry et al., 2022). These QTL showed strain specificity between I05 and I07. *Qstb-Renan-1D* provides resistance to I07, *Qstb-Renan-5D* provides resistance to I05, finally *Qstb-Renan-7B* provides resistance to both strains. In this study we aim to identify gene-for-gene interactions underlying quantitative phenotypes by a combination of approaches including linkage analyses and a genome-wide association study (GWAS) using plant and fungal material selected specifically for the resistance and pathogenicity QTL that they carry.

Materials and methods

Plant and fungal materials

The wheat and fungal populations used in this study were previously used to identify STB resistance QTL in wheat (Langlands-Perry et al., 2022) and pathogenicity QTL in *Z. tritici* strains (Chapter 3; this thesis). The wheat population referred to as R×CS is derived from a cross between cultivars Renan, mostly resistant to STB, and Chinese Spring, susceptible to the disease. It comprises 148 recombinant inbred lines (RILs; F6

generation) chosen randomly from a larger population. Are also included in R×CS, Renan and Chinese Spring, the parental cultivars.

The fungal population referred to as I05×I07 results from a cross between strains “I05” (INRA09-FS0813, Mat1-1), pathogenic on Renan, and I07 (INRA09-FS0732, Mat1-2), which has a lower level of pathogenicity on Renan, sampled in 2010 from STB lesions on wheat cv. Soissons in Grignon, France (48510 N, 1580 E) as described in Chapter 3 (this thesis).

Three different progeny were selected from the I05×I07 population. These progeny carry different allele combinations for the three known pathogenicity QTL based on available single nucleotide polymorphism (SNP) marker data. Strain 7558 has the two pathogenic alleles *Qzt-I05-6* and *Qzt-I07-13*. Strain 7510 has only one pathogenic allele, *Qzt-I05-6*. Strain 6096 has the non-pathogenic alleles for the three QTL. A knock-out mutant for the cloned gene underlying *Qzt-I05-6* in an I07 background, *I07_ΔG07189_cl.40*, was previously obtained by *A. tumefaciens* mediated transformation (Chapter 3; this thesis).

The recombinant inbred line (RIL) ‘2019’ was selected from the R×CS population, it carries the non-resistant alleles for *Qstb-Renan-1D* and *Qstb-Renan-7B* and the resistant allele for *Qstb-Renan-5D*.

A diverse panel of 126 *Z. tritici* strains was collected in 2018-2019, from two locations in France (*i.e.* Bignan in Brittany and Rots in Normandy). The pathogenicity of 120 of these strains was evaluated on the RIL ‘2019’. A *de novo* genome assembly of these strains was realized with previously available PE-150 sequences obtained on an Illumina NovaSeq-6000 sequencing system with a 30x mean genome coverage (to be submitted). Illumina paired-end reads were assembled using a combination of VELVET (Zerbino and Birney, 2008), SOAPDENOV0 and SOAPGAPCLOSER (Luo et al., 2012), as previously described for the assembly of the *Botrytis cinerea* genome (Mercier et al., 2021). These strains carry a diversity of different haplotypes for the cloned *Qzt-I05-6* gene *G_07189* as was shown by the generation of a protein sequence phylogenetic tree (Chapter 3; this thesis) using the RaxML algorithm with the GAMMA JTT model and 100 bootstrap replicates (Stamatakis, 2014). The isoforms of *G_07189* are named I1, I2, I3, I4 and I5, which correspond to the most common isoforms, carried by 6 strains or more in the panel of 120 strains (represent 111 of the 120).

Experimental set-up and procedure for pathology assays

For all experiments, inoculations were performed on 16 day-old seedlings grown in a

growth chamber with a 16h photoperiod, hygrometry fixed at 70% and temperatures at 16 °C night and 20 °C day. Post-inoculation, plants were placed in a climate chamber with a 16h photoperiod, hygrometry fixed at 90% by day, 80% by night and temperatures of 22 °C during daytime and 18 °C during night-time. In the climate chambers, light conditions were maintained at 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with eight neon tubes (Osram Lumilux L58W/830) placed 40 cm above the trays (OSRAM GmbH, Munich, Germany).

Inocula were prepared for each strain using blastospores generated after a 6 day preculture period in a YPD liquid culture medium (1% yeast, 2% bacto-peptone, 2% glucose) followed by a 4 day culture on a PDA (potato dextrose agar) solid culture medium inoculated with 50 to 80 μL of inoculum. Inocula were prepared to have a concentration of $10^6 \pm 1.10^5$ spores. mL^{-1} suspended in water. Before inoculation, a drop of Tween 20[®] was added per 15mL of inoculum to insure adherence of the inoculum to the leaf. For the pathology assays involving 7558, 7510, 6096 and I07_ΔG07189_cl.40 inoculated on R×CS, inoculations were done using a square-tipped flat paintbrush as described by Langlands-Perry et al. (2022), while for the RIL 2019 inoculated with the panel of 120 strains, inoculations were done using cotton swabs as described in Chapter 3 (this thesis).

For all experiments, two replications were performed.

Evaluated traits

The inoculated leaf area was visually evaluated at 14, 20 and 26 dpi. Were evaluated the percentage of the surface which was green, necrotic and sporulating. The sporulating area is defined here as the area which presents pycnidia, regardless of density, colour or size. The chlorotic area was not evaluated because it is deductible from the green and necrotic areas. These notations were used to calculate different values. Firstly, S20 and S26 were evaluated, these correspond to the percentage of the inoculated area presenting with pycnidia at 20 and 26 dpi respectively. Were also evaluated AUDPCs (Area Under the Disease Progress Curve) for the green, necrotic and sporulating areas (AUDPCG, AUDPCN and AUDPCS respectively) as previously described (Langlands-Perry et al., 2022).

Statistical analysis of phenotypic data

The obtained data for strains 7558, 7510, 6096 and I07_ΔG07189_cl.40 were analysed with the R software (R Core Team, 2019), for each trait an analysis of variance (ANOVA) was performed with the following model:

$$Y_{ij} = \mu + I_i + r_j + Ir_{ij} + \varepsilon_{ij}$$

With Y_{ij} the trait which is being studied, μ the mean value for this trait, I_i the individual genotype, r_j the repetition, Ir_{ij} the interaction and ε_{ij} the residual. For the following analyses, Ir_{ij} was included in the residual.

The following hypotheses were verified after the variance analyses.

$$\varepsilon \sim N(0, \sigma^2) \quad \text{cov}(\varepsilon, \varepsilon') = 0 \quad \text{Homoscedasticity (homogeneity of } \text{var}(\varepsilon))$$

Broad sense heritability is defined by the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

With H^2 the heritability, σ_g^2 genotypic variance and σ_e^2 residual variance.

Correlation between traits was studied using the Bravais-Pearson correlation.

For the data generated from the inoculation of the panel of 120 strains on RIL '2019', a Kruskal-Wallis test was carried out along with a Wilcoxon pairwise comparison, which were performed with R on all values from both replicates (R Core Team, 2019).

Linkage and association analyses

The genetic map used for the linkage analyses was the linkage map for R×CS generated by Langlands-Perry et al. (2022). A linkage analysis was carried out using the R/qtl software (Broman et al., 2003) version 1.42-8. This analysis included for each trait an initial Simple Interval Mapping (SIM), followed by a Composite Interval Mapping (CIM). Analyses were performed repetition by repetition. For SIM, 1000 genome-wide permutations were used to calculate the significant logarithm of odds (LOD) threshold. Were considered significant only QTL that showed P-values <0.05. The CIM was carried out with the peak marker of the QTL with the highest LOD used as a covariate. QTL intervals were evaluated with the LOD support interval with a drop in LOD of 1 and the "expandtomarkers" argument as true. QTL effects were calculated with the "effectplot" and "effectscan" functions. Possible epistatic interactions between QTL were looked into using the "addint" function.

Variant calling in the 126 strain panel

We used Trimmomatic v.0.32 (Bolger et al., 2014) and then mapped to the reference genome IPO323 (Goodwin et al., 2011) using the mem algorithm from BWA v0.7.7 (Li

and Durbin, 2009) with default options. Samtools v0.1.19 and Picard tools v1.106 were used to filter reads (Li et al. (2009); Picard tools <<http://broadinstitute.github.io/picard/>>) to remove optic duplicates, secondary alignments, reads with a mapping quality below 30 and to keep only pairs in which both reads met quality checks. SNP and short indels calling was done with Genome Analysis Toolkit (GATK) v.4.0.1.2 (McKenna et al., 2010). We used GATK HaplotypeCaller on each isolate with the command `--min-base-quality-score 18 --minimum-mapping-quality 30 -ERC "GVCF" -ploidy 1` (*Z. tritici* is haploid). All positions detected as low complexity regions or transposable elements (TE) were excluded. Joint variant calls were performed using GenotypeGVCFs on a merged gvcf variant file with the option `-maxAltAlleles 2`. To filter out low quality variants and calls from ambiguous mapped reads we used GATK VariantFiltration following GATK recommendations for SNPs and indels separately and GATK SelectVariants to select variants that passed quality control checks. We obtained a final dataset by filtering variant with a genotyping call rate of $\geq 80\%$ and minor allele frequency (MAF) $> 5\%$ using VCFtools (Danecek et al., 2011) which resulted in 1,003,343 biallelic SNPs and indels across all 21 *Z. tritici* chromosomes.

GWAS of 120 strains inoculated on RIL 2019

We used the mixed linear model implemented in the R package GAPIT for association analysis (Lipka et al., 2012). To reduce spurious associations due to population structure and genetic relatedness, we added the first three principal components computed from genome-wide variant data and a kinship matrix calculated using the VanRaden method as covariates in the statistical model. We used a Bonferroni threshold of $P < 4.98e-8$ ($\alpha=0.05$) to declare significant variants associated with AUDPCN and AUDPCS variations. Linkage Disequilibrium (LD) measures (r^2) for the lead SNP were obtained with PLINK v.1.9 using the `--r2` command in PLINK SNPs with an $r^2 \geq 0.8$ were considered in strong LD.

PCR on a TE insert 26 bp upstream of G_07189

A PCR was performed on the 120 panel strains to identify their genotype for a TE insertion previously identified in the three prime untranslated region (3' UTR) of the cloned gene only 26 bp from the STOP codon, this insert is referred to as Insert C (Figure 1) (Chapter 3; this thesis). PCRs using a forward primer (5'-3': GTCATTTGATGTGTGAGTGGG) and a reverse primer (5'-3': CCGGGACGGAGAGCATTAGGA) were carried out using the GoTaq® polymerase (Promega, Madison Wisconsin USA) under PCR conditions adapted for the detection

of insertions up to 1,137 bp ($T_m = 60\text{ }^\circ\text{C}$, elongation time: 1min 8 sec) as this was the size of the amplicon for strain I07 in which the insert had been previously identified (Chapter 3; this thesis). Haplotypes were evaluated based on amplicon sizes.

Additionally, sequence assemblies of the strains were used to gain further insight into the nature of the 3'UTR inserts. The insert sequences, when the region was sufficiently well assembled, were obtained through an "*in silico* PCR" performed by blasting conserved regions either side of the insert corresponding to "*in silico* primers" and extracting the "*in silico* amplicon" sequence for each assembly. These "amplicon" sequences were then annotated for TE using RepeatMasker (Smit et al., 2013) and a previously generated TE library for this organism (Grandaubert et al., 2015) according to the TE nomenclature defined by (Wicker et al., 2007).

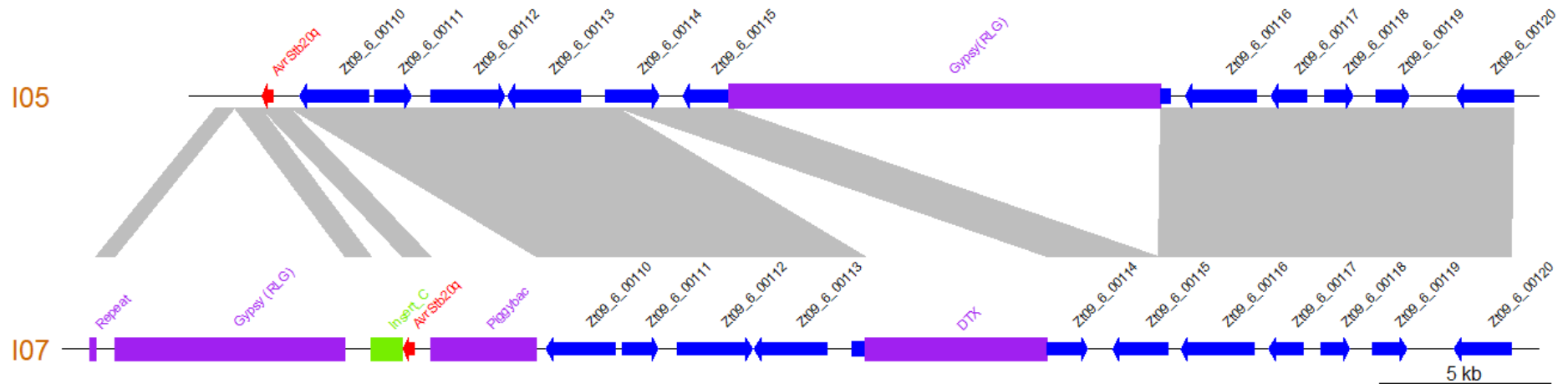


Figure 1. The region harbouring *AvrStb20q* is highly polymorphic between strains I05 and I07 as illustrated by this synteny plot comparing the contigs of the I05 and I07 assemblies which carry the gene. Other annotated genes are represented as blue arrows according to their orientation. *AvrStb20q* is represented by a red arrow according to its orientation. TE are represented by purple blocks. The repeat TE insert C is represented by a green block. Collinear sequences between contigs are shown in grey. Modified from Chapter 3 (this thesis).

Results

Quantitative phenotypes obtained in the R×CS mapping population

For all of the studied strains in all replicates, Renan had a healthier phenotype than Chinese Spring (Figures S1-S4). The strains which led to the least sporulation were 6096 and 7510. 7558 yielded intermediary phenotypes for sporulation. Finally, I07_ΔG07189_cl.40 led to the most sporulation. Despite these differences in overall infection levels between strains, they all led to phenotypic data with a continuous distribution throughout the R×CS. Transgressive individuals appear in all experiments. Correlation coefficients were calculated from the mean values between replicates and showed that all the studied traits were highly correlated, with the traits S20, S26 and AUDPCS being particularly highly correlated (Figures S1-S4). The lower values for correlations with AUDPCG and AUDPCN can be explained by the sensitivity of these traits to external factors, which are not avoidable despite a controlled environment. These external factors can be for example leaves being submerged after watering due to their length, which leads to leaf damage which cannot be differentiated from disease induced necrosis. High correlations between traits suggest a similar genetic base.

The ANOVA results (Table 1) show that the individual genotype systematically has a large effect on phenotypes, with a slightly lower significance rate for AUDPCN strain 7510. The replication had an overall very strong effect on phenotypes leading to subsequent linkage analyses being carried out replicate by replicate. On average between traits and per strain, the highest heritability values were obtained with 6096 ($\overline{H^2} = 0.49$), followed by 7558 ($\overline{H^2} = 0.42$), then 7510 ($\overline{H^2} = 0.33$) and finally I07_ΔG07189_cl.40 led to the lowest heritability on average ($\overline{H^2} = 0.29$). In the subsequent linkage analyses, lower trait heritability does not seem to affect QTL detection or indeed the consistency thereof.

Table 1. Statistical analysis of the phenotypic data obtained from the inoculations of 6096, 7510, 7558 and I07_ΔG07189_cl.40 on R×CS

Isolate	Trait	Statistical significance of the genotype	Statistical significance of the replication	MSg	Mse	Broad-sense heritability	Shapiro-Wilk normality test on residuals	Independence of residuals	Homoscedasticity Bartlett test of homogeneity of variances
6096	S20	***	***	411,7	149,9	0,47	0,00	no	0,00
	S26	***	***	964,6	210,3	0,64	0,00	no	0,00
	AUDPCG	***	***	115045,0	55077,0	0,35	0,30	yes	0,37
	AUDPCN	***		96084,0	38897,0	0,42	0,01	yes	0,00
	AUDPCS	***	***	43803,0	11976,0	0,57	0,00	no	0,00
7510	S20	***	***	384,0	227,0	0,26	0,00	no	0,08
	S26	***		1004,2	372,0	0,46	0,12	yes	0,05
	AUDPCG	***	***	59829,0	30032,0	0,33	0,91	yes	0,90
	AUDPCN	**	***	56400,0	36470,0	0,21	0,22	yes	0,99
	AUDPCS	***	**	41925,0	17697,0	0,41	0,00	yes	0,25
7558	S20	***		1190,3	423,1	0,48	0,49	yes	0,82
	S26	***	***	1043,0	404,0	0,44	0,05	yes	0,57
	AUDPCG	***	***	52485,0	25060,0	0,35	0,00	no	0,03
	AUDPCN	***	.	60534,0	31284,0	0,32	0,83	yes	0,09
	AUDPCS	***	***	93138,0	30221,0	0,51	0,36	yes	0,79
I07_ΔG07189_cl.40	S20	***	***	1491,0	839,0	0,28	0,01	yes	0,04
	S26	***	***	1020,0	545,0	0,30	0,11	yes	0,83
	AUDPCG	***	***	67615,0	38284,0	0,28	0,16	yes	0,79

	AUDPCN	***	***	78121,0	44390,0	0,28	0,04	yes	0,40	
	AUDPCS	***	***	105193,0	53854,0	0,32	0,17	yes	0,57	
	1 Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '!', 0.1 ' ';									
	2 MSg is the mean square value for the individual genotypes output by the ANOVA;									
	3 Mse is the mean square value for the residuals put out by the ANOVA									

Resistance QTL were detected depending on the alleles of the fungal pathogenicity QTL

The analyses of the data led to the detection of the three same QTL as previously detected with strains I05 and I07 (Langlands-Perry et al., 2022) in different combinations depending on the strain inoculated on R×CS (Figure 2). *Qstb-Renan-1D* was detected 5 times overall, spanning a 23.8cM region and explaining on average 11.8% of phenotypic variation. This QTL was detected with strains 6096 and 7510. *Qstb-Renan-5D* was detected 10 times overall, only with strain 6096, spanning 15.5cM and explaining on average 18.3% of phenotypic variation. *Qstb-Renan-7B* was detected 31 times overall, with all four tested strains. It spans 47.9cM and explains 16.7% of phenotypic variation on average. No highly significant epistatic interactions were detected between QTL. Low effect interactions were identified with sporulation related traits S26 and AUDPCS for the *Qstb-Renan-1D/Qstb-Renan-7B* and *Qstb-Renan-5D/Qstb-Renan-7B* pairs, explaining on average 2.4% and 2.9% of phenotypic variation respectively. Moreover, unlike with I07 in previous analyses (Langlands-Perry et al., 2022), I07_ΔG07189_cl.40 did not lead to the detection of a resistance QTL on chromosome 5D.

Correlations can be made between the presence of certain fungal pathogenicity QTL in used strains and the detection of resistance QTL in the wheat population. *Qstb-Renan-1D* was detected only in the presence of the non-pathogenic allele for *Qzt-I07-13* (Figure 2). *Qstb-Renan-5D* was detected only in the presence of the non-pathogenic allele for *Qzt-I05-6* or when the underlying gene was knocked-out (Figure 2). *Qstb-Renan-7B* was detected with all strains and it could not be correlated with the presence/absence of any of the known fungal pathogenicity QTL (Figure 2). Table S1 presents the detail of all QTL detected in this study.

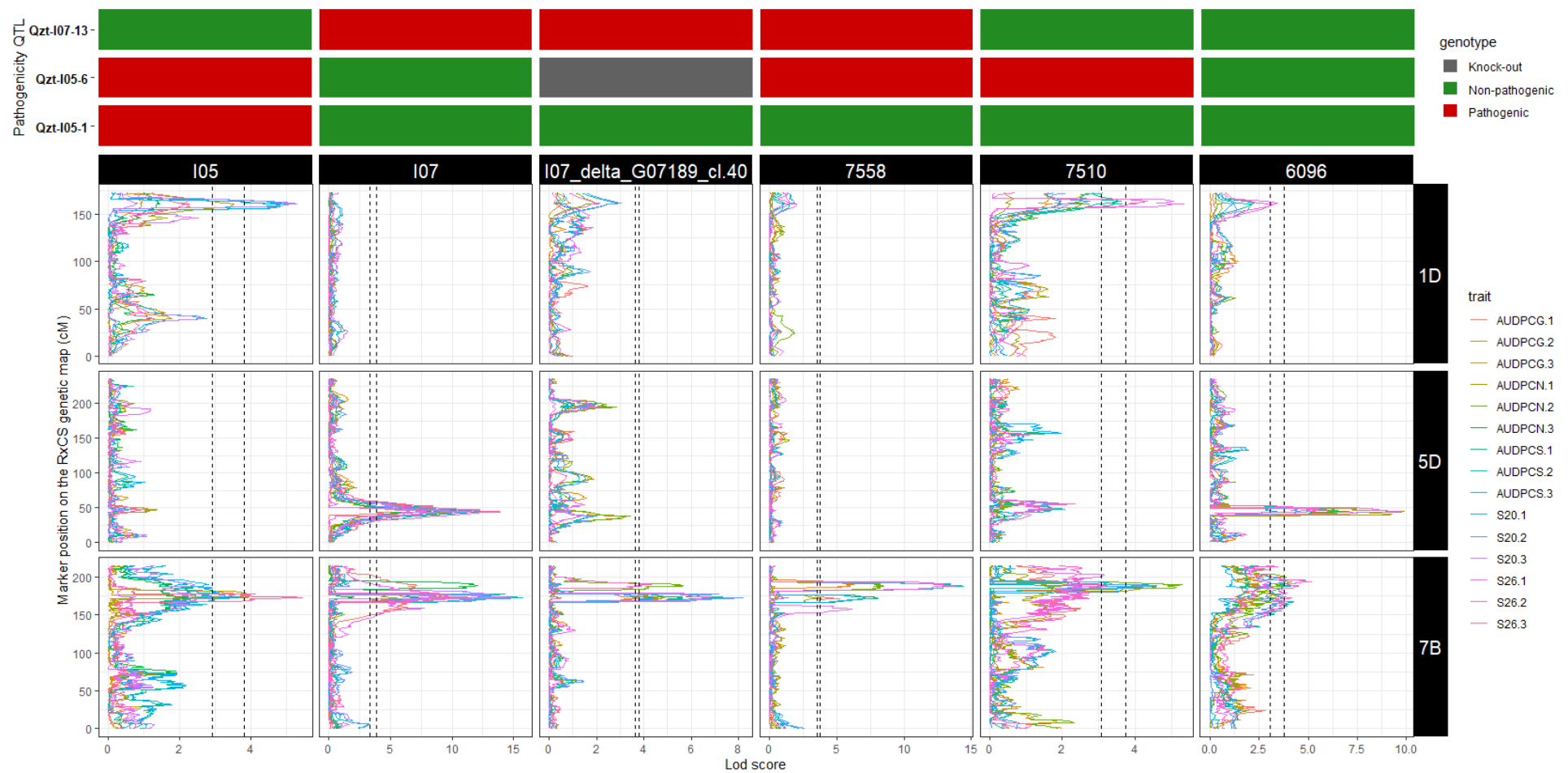


Figure 2. Lod Score values obtained after linkage analyses of the phenotypic data. The red, green and grey boxes represent the genotype of the strains for the pathogenicity QTLs *Qzt-107-13*, *Qzt-105-6* and *Qzt-105-6*, with red as pathogenic, green as non-pathogenic, and grey as knocked-out for the causal gene underlying the QTL. The graphs are organized in lines by wheat

chromosomes, are represented chromosomes 1D, 5D and 7B, and in columns by inoculated strain, these are I05, I07, I07_ΔG07189_cl.40, 7558, 7510 and 6096. The Y-axis represents the position of the markers on the R×CS genetic map in cM. The X-axis represents the LOD score associated to the markers. In dotted lines are represented the minimal and maximal LOD threshold values obtained in the linkage analyses after 1000 permutations tests. The colours in the graphs correspond to the studied traits. AUDPCs are the Area Under the Disease Progress Curve for the green, necrotic and sporulating areas (AUDPCG, AUDPCN and AUDPCS respectively). S20 and S26 are the sporulating area at 20 and 26 days post-inoculation respectively. The data presented for I05 and I07 was generated by Langlands-Perry et al. (2022).

Detection of *G_07189* by GWAS

GWAS was performed for pathogenicity of the 120 strains on RIL 2019 carrying only the resistance allele at *Qstb-Renan-5D* (i.e. *Stb20q*). The phenotypic data presented with a continuous distribution for all studied traits and consistent with previous studies (Chapter 3; this thesis), I05 was systematically associated with higher pathogenicity than I07 (Figure 3).

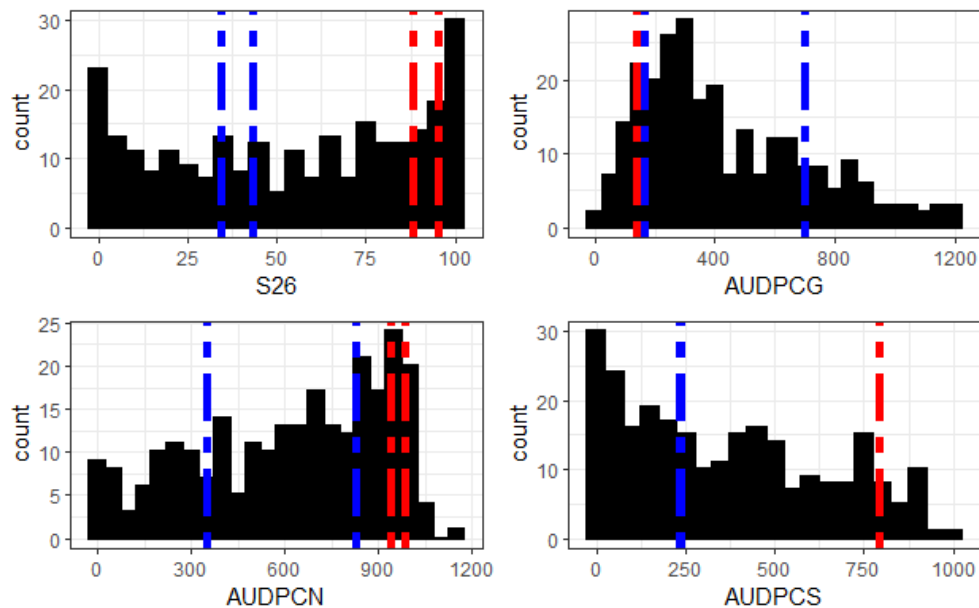
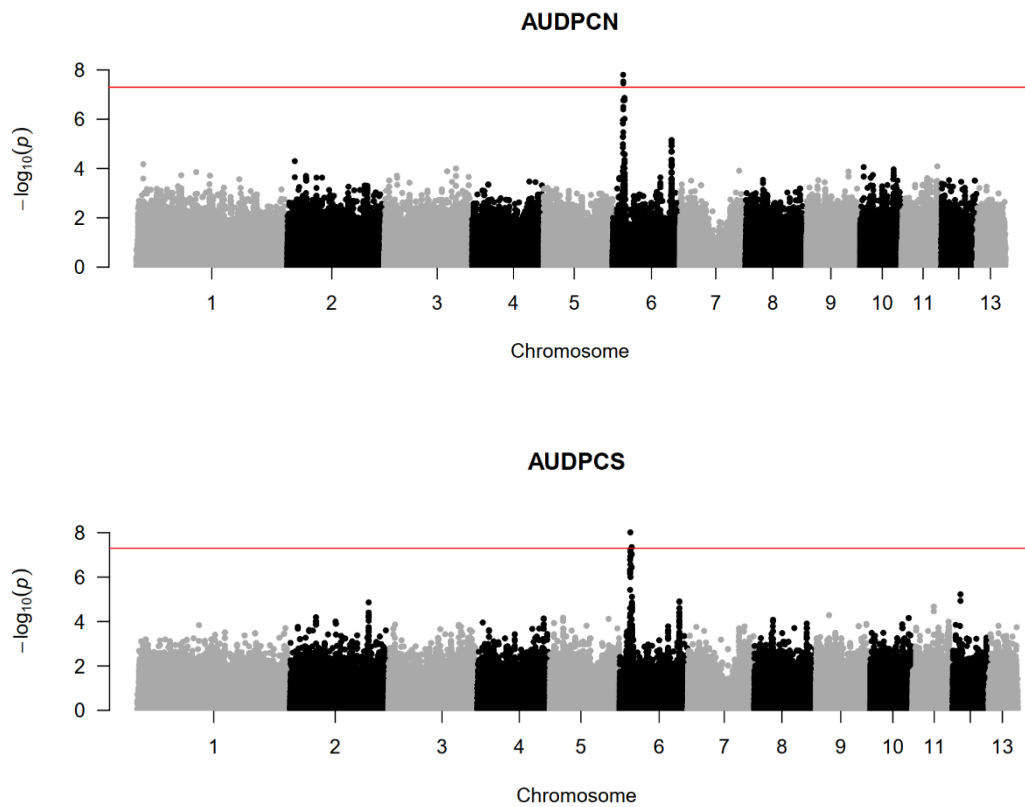


Figure 3. Distribution of the phenotypic data obtained from the inoculation of a diverse panel of strains on RIL '2019', which carries the resistance allele only for *Qstb-Renan-5D*. The data obtained from both replicates is represented. Blue dashed lined correspond to the phenotypic values obtained for strain I07 and red dashed lines represent the phenotypic values obtained for strain I05. AUDPCs are the Area Under the Disease Progress Curve for the green, necrotic and sporulating areas (AUDPCG, AUDPCN and AUDPCS respectively). S26 is the sporulating area at 26 days post-inoculation.

We used a linear mixed model to associate genetic polymorphisms with quantitative pathogenicity on RIL 2019 and identified 3 and 2 SNPs on chromosome 6, significantly associated with AUDPCN and AUDPCS, respectively, at the Bonferroni threshold (Figure 4. a). The highly significant SNPs mapped to the TE-rich region of chromosome 6 flanking *G_07189* (Figure 4. b). The lead SNP was in strong LD with variants in an interval of 406,297 bp to 469,816 bp, located 103 bp upstream of *G_07189* (Figure 4.b). No other genomic region was significantly associated with quantitative pathogenicity traits, indicating that *G_07189* is likely the only avirulence factor interacting with *Stb20q*.

a.



b.



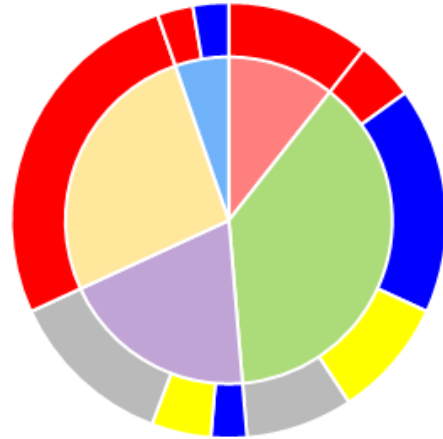
Figure 4. GWAS identifies a single avirulence factor, *AvrStb20q*, associated with quantitative pathogenicity on RIL_2019. **a.** Manhattan plot representing the strong association of 3 and 2 SNPs with the Area Under the Disease Progress Curve for the necrotic area AUDPCN (top panel) and for the sporulating area AUDPCS (bottom panel), respectively. The X and Y axes indicate *Z. tritici* core chromosomes and $-\log_{10}(P\text{-value})$ for marker-trait associations, while the red line represents the Bonferroni threshold ($\alpha=0.05$). **b.** LD pattern in the genomic region of *AvrStb20q* (bottom panel) and an annotation bar of the corresponding region (top panel). The black arrow is *AvrStb20q*, grey boxes represent repetitive elements while red bars are the GWAS SNPs associated with phenotypic variation.

Strains with different haplotypes at the Qzt-I05-6 locus lead to a variety of phenotypes on RIL '2019', which carries Stb20q

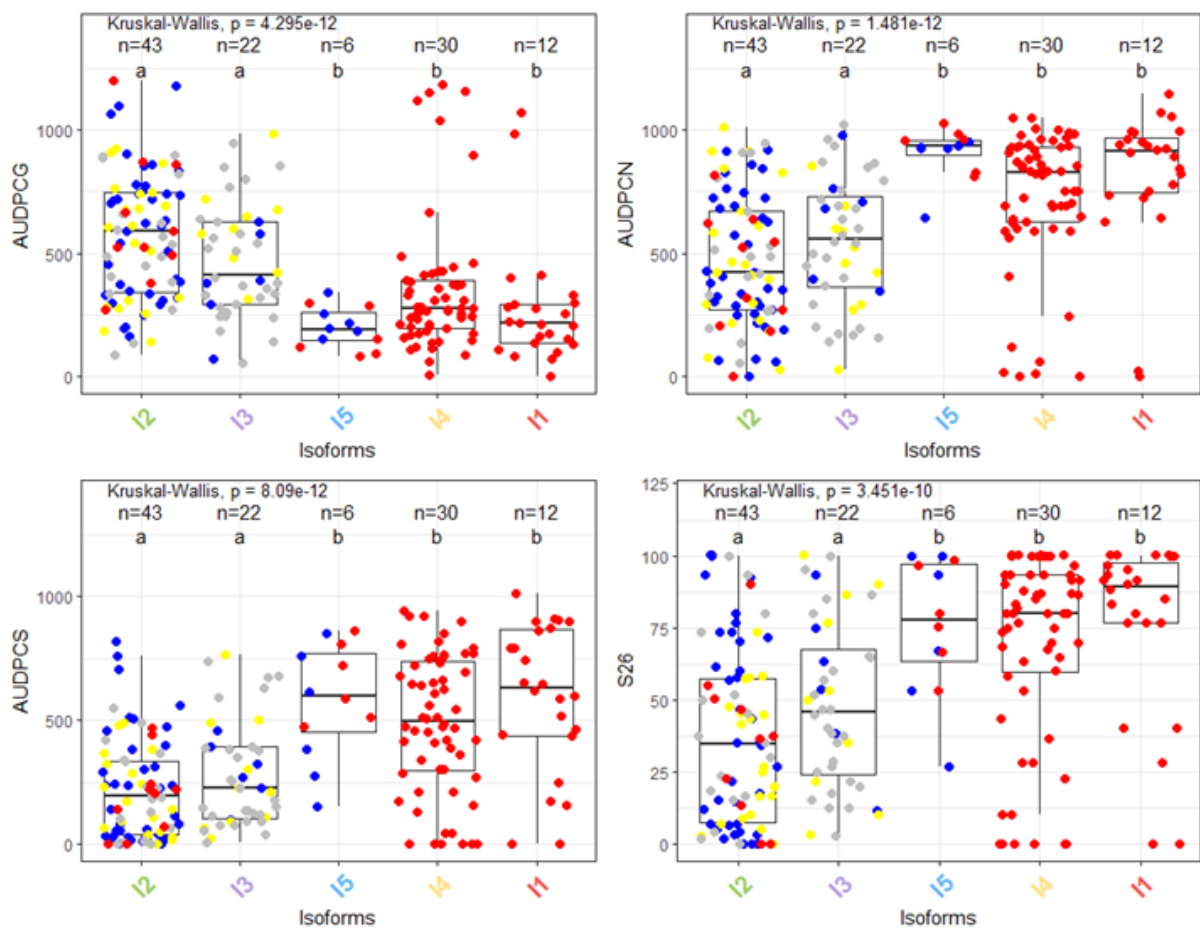
The data from both replications was analysed together, as despite a significant effect of the replication (ANOVA p -value <0.05), both replications were carried out in controlled conditions by the same experimenter. Boxplots generated from phenotypic data obtained for different strains grouped according to their G_07189 isoform show responses that fit into two categories. The strains with the I2 and I3 isoforms, including I07, are the least pathogenic of our sample, while the strains with the I5, I4 and I1 isoforms, including I05, are the most pathogenic (Figure 5. b). The difference between the most pathogenic and least pathogenic strains grouped according to their G_07189 isoform was statistically significant as shown by a Wilcoxon pairwise comparison (Figure 5. b). No obvious link appears between isoform sequence polymorphisms and strain pathogenicity (Figure 5. c).

a. Strains grouped by isoform

■ I1	n=12
■ I2	n=43
■ I3	n=22
■ I4	n=30
■ I5	n=6
Insert haplotypes	
■ No insertion	n=50
■ 750-3,300 bp inserts	n=25
■ 3,800-14,300 bp inserts	n=15
■ No amplification	n=23



b. Phenotypes



c. Isoform sequences

I1	MKLF ICAMLAASVVARAPY SPCTHGGWEAAC PYWRCCHPFVCADDKHCHLMCEWGGQGCPPGLSCQVAPWNKDDRECV
I2	MKLF ICAMLAASVVARAPY SPCTHGGWEAAC PYWRCCHPFVCADDKHCHLMCEWGGQGCPPGFSCQVAPWDSSDRECV
I3	MKLF ICAMLAASVVARAPY SPCTHGGWEAAC PYWRCCHPFVCADDKHCHLMCEWGGQGCPPGFSCQVAPWDKTDRECV
I4	MKLF ICAMLAASVVARAPY SPCTHGGWEAAC PYWRCCHPFVCADDKHCHLMCELGQGQCPPGFSCQVAPWDKTNRECV
I5	MKLF ICAMLAASVVARAPY SPCTHGGWEAAC PYWRCCHPFVCADDKHCHLMCEWGGQGCPPGFSCQVAPWDKGNRECV

Figure 5. *AvrStb20q* (*G_07189*) presents with a diversity of possible isoforms in combination with a diversity of 3' UTR haplotypes. The most pathogenic isoforms are associated with the absence of TE insert haplotype of the 3' UTR. The letter n represents

the number of strains in a given groups. **a.** Different isoforms are associated with different Insert C haplotypes. **b.** Boxplots representing the phenotypic values obtained for strains and scatter plot representing the values obtained for strains coloured by insert haplotype. A Kruskal-Wallis test was performed, followed by a Wilcoxon pairwise comparison, letters a and b indicate the statistical groups at $\alpha=0.05$. AUDPCs are the Area Under the Disease Progress Curve for the green, necrotic and sporulating areas (AUDPCG, AUDPCN and AUDPCS respectively). S26 is the sporulating area at 26 days post-inoculation. **c.** Isoform sequences of AvrStb20q for groups I1, I2, I3, I4 and I5. In green is the signal peptide sequence, in bold are the conserved cysteine residues, letters coloured in blue, red, purple and pink correspond to the polymorphic residues. The red asterisk marks residues under significant diversifying selection. For all parts of the figure, colours are consistent for strain groups by isoform and by insert C haplotype.

Genotyping insert C by PCR identified different cases of no TE insertion (e.g. I05), inserts of different sizes estimated on the resulting gels at 500 bp, 1000 bp (e.g. I07), 1500 bp and up to 4000 bp, and no amplification at all in 33 strains. Cases of no amplification can be linked to the primers not matching the sequence in the strains or the insert in these strains being too large to be amplified under standard PCR conditions. Furthermore, whenever possible, the sequence at insert C was retrieved from the genome assemblies of the strains in the panel. The sequence was successfully assembled and retrieved for 85 of the 120 strains. The assemblies confirmed the insert sizes that we had already been able to identify by PCR, and also enabled us to explain 9 of the cases of lack of amplification. Indeed, for these strains, insert sizes according to assembly data ranged from 7,300 bp to 14,300 bp, sizes which are too large for amplification using the GoTaq® polymerase (Promega, Madison Wisconsin USA). Assembly analyses therefore enabled us to identify even more polymorphism for this insert. Additionally, TE annotation in the inserts showed that while shorter inserts consisted mostly of class II DNA transposons, including mariners and helitrons, the longer inserts were the only ones in which class I retrotransposons were annotated, consisting mostly of Gypsy elements with some RLX-TRIM and a LINE RIX. Uncategorized elements were identified in shorter and longer inserts (data not shown).

Almost all the 'most pathogenic strains' with G_07189 isoforms I1, I4 and I5, do not have any insertion at Insert C; only 3 strains with isoform I5 have an insert of 800 bp on average (estimated at 750 bp for two of the strains, and at 900 for the other based on assembly data), annotated as containing non-categorized elements and repeats. The majority of the 'least pathogenic strains' with AvrStb20q isoforms I2 and I3, have

inserts larger than 3,300 bp at Insert C or no amplification, as in no PCR amplification and no assembly derived “*in silico* PCR” result. The lack of assembly data for the region and lack of PCR amplification could indicate a large TE insertion, as was shown for some other strains with insertions larger than 7,000 bp. Indeed TE-rich regions are difficult to assemble, particularly with short read-based sequencing, due to the high number of repeats that they contain, which can induce errors in assemblies and reduce their quality. Nevertheless, some strains with the I2 isoform have no insertion at Insert C, indicating that the absence of an insertion in the 3’UTR does not strictly mean lower pathogenicity. This was further shown by the association study. Taking the most associated SNP obtained in the GWAS, and crossing the genotype at that SNP with the TE haplotype previously identified by PCR and assembly analysis, we show that bar ten strains out of the panel of 99, all absence of data at the SNP strains discounted, an absence of insert haplotype in the *G_07189* 3’UTR is associated with a “virulent” allele, while the presence of a TE insertion is associated with an “avirulent” allele regardless of isoforms (Figure 6).

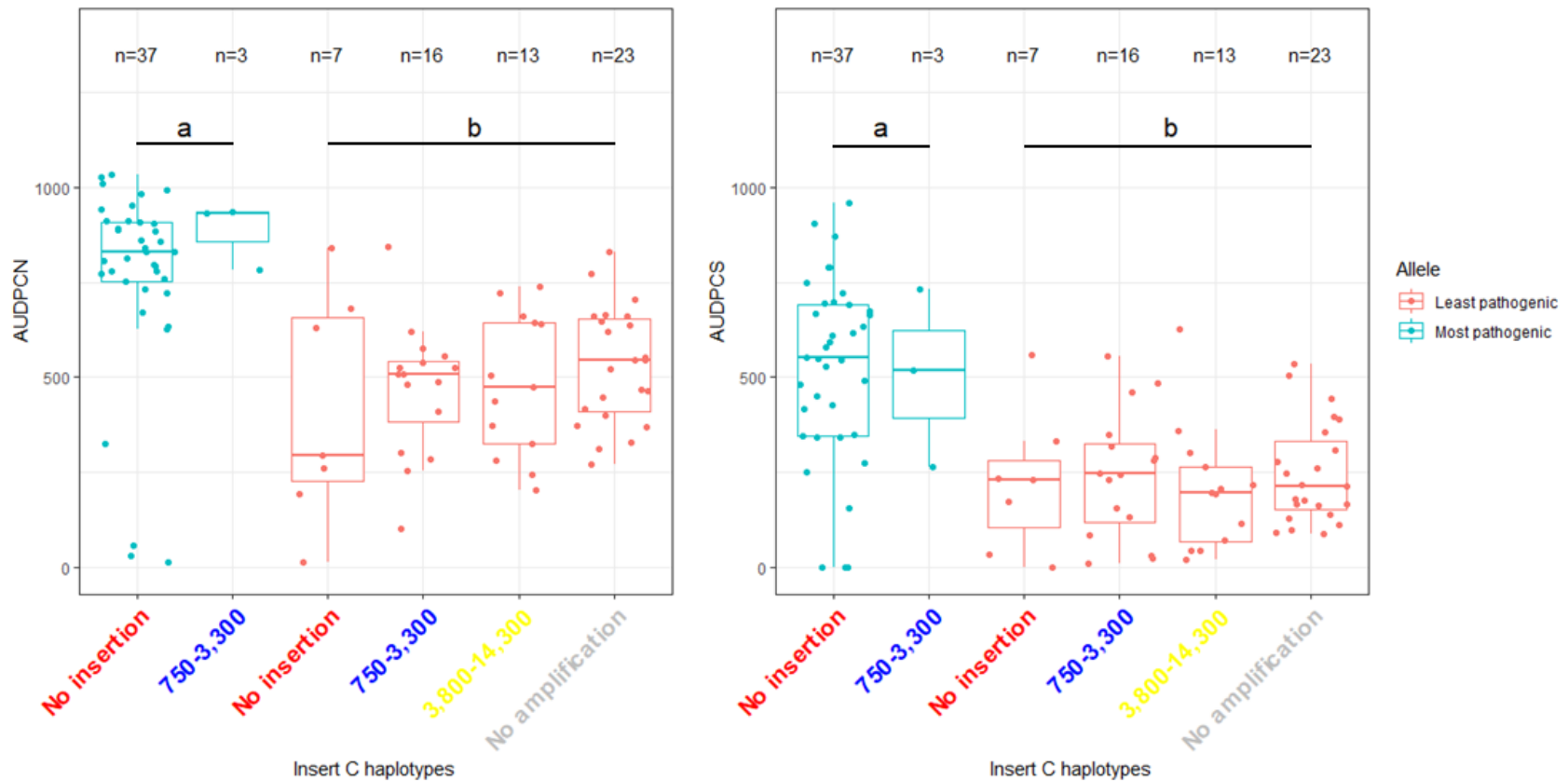


Figure 6. Boxplots representing the phenotypic values obtained for strains coloured according to the allele they have at the most associated SNP identified by GWAS. A Wilcoxon pairwise comparison was performed, letters a and b indicate the statistical groups at $\alpha=0.05$. AUDPCN (left panel) is the area under the disease progress curve for the necrotic leaf area. AUDPCS (right panel) is area under the disease progress curve for the sporulating leaf area. The y-axis presents the different haplotypes at insert C.

Discussion

Gene-for-gene interactions underlying an overall quantitative phenotype

Qstb-Renan-1D had previously been detected for resistance to *Z. tritici* strain I05 (Langlands-Perry et al., 2022), and was identified in this study with progeny strains 7510 and 6096 (Figure 2). With the contribution of this QTL to the overall phenotype varying between 8% and 15%, this locus is clearly quantitative and contributes to partial resistance. With regards to pathogenicity QTL carried by these three strains, the only common factor is the *Qzt-I07-13* allele (Figure 2). We can therefore safely state that the detection of *Qstb-renan-1D* is dependent on the presence of the non-pathogenic allele for *Qzt-I07-13* in the fungal genome. Thus, we have identified a QTL-for-QTL interaction between *Qstb-renan-1D* and *Qzt-I07-13*. Moreover, *Qstb-renan-1D* colocalizes with major resistance gene *Stb19* (Yang et al., 2018), and it has been suggested that this QTL could be a weak allele of this resistance gene (Langlands-Perry et al., 2022). It is therefore probable that *QTL-I07-13* harbours *AvrStb19*. This strongly suggests that qualitative resistance genes can be involved in quantitative interactions, thus blurring the delimitations between what is considered quantitative or qualitative.

Similarly to what we have observed for *Qstb-Renan-1D*, *Qstb-Renan-5D* showed strain specificity, being detected only with progeny strain 6096 (Figure 2). This QTL had been previously identified for resistance to I07 (Langlands-Perry et al., 2022). Detection of this QTL, which explained 18% of phenotypic variation on average, was found to be highly repeatable for all studied traits. I07 and 6096 have a common non-pathogenic allele for both *Qzt-I05-1* and *Qzt-I05-6* (Figure 2). This QTL was however not detected with mutant strain I07_ΔG07189_cl.40 (Figure 2), from which the candidate effector gene for *Qzt-I05-6* had been knocked-out and its role in pathogenicity validated (Chapter 3; this thesis). With these results, not only can we conclude that there is a QTL-for-QTL interaction between *Qstb-Renan-5D* and *Qzt-I05-6*, but also that the previously cloned effector gene in *Qzt-I05-6* is the interacting gene. Moreover, GWAS on the RIL '2019' carrying only *Qstb-Renan-5D*, identified an SNP upstream of the gene, which further corroborates these results and rules out any other possible candidate region for the interaction. *Qstb-Renan-5D* has previously been designated *Stb20q* (Langlands-Perry et al., 2022), we therefore propose to designate the cloned effector gene *AvrStb20q* in accordance with common nomenclature.

Qstb-Renan-7B had been previously identified with I05 and I07 (Langlands-Perry et al., 2022). This QTL was consistently detected with the different parental, progenies and mutant strains, and it explains more than 10% of phenotypic variation. Thus it can be

considered a major QTL (Kou and Wang, 2010). Nevertheless, position of the peak marker varied between analyses in an interval of 36.6 cM, independently of the isolate or the trait considered. This suggests that the region covered by *Qstb-Renan-7B* is complex with potentially several underlying causal genes which complicate the dissection of the genomic area in linkage analyses. This QTL could contribute to broad resistance to STB with no strain specificities. It could on the other hand be strain-specific, or indeed some of the causal genes could show specificities to other isolates than I05 and I07. It has been suggested that what is called “non-specific resistance, is that resistance which hasn’t yet been shown to be specific” (Ellingboe, 1975), this could well be the case for *Qstb-Renan-7B*, especially seeing as this QTL colocalizes with known major resistance gene *Stb8* (Adhikari et al., 2003). Further testing of the *Qstb-Renan-7B* resistance locus with other *Z. tritici* strains could verify these hypotheses.

We therefore have a complex system involving several QTL plant-side and fungus-side all quantitatively contributing to an overall phenotype with underlying QTL-for-QTL interactions. This clearly goes against long upheld definitions of quantitative resistance as a non-specific and broad-spectrum type of disease resistance (Cowger and Brown, 2019; Nelson et al., 2018; Van Der Plank, 1966). Additionally, the resistance QTL involved in these interactions can colocalize with known major resistance genes. This suggests that major resistance genes can lead to quantitative phenotypes. This had been previously implied with the *Avr3D1* interaction with *Stb7* or *Stb12*, where the phenotypes observed with the “avirulent” strain were not free of disease, but rather had reduced symptoms compared to other “virulent” strains (Meile et al., 2018). That the same genes should underlie qualitative and quantitative interactions indicates that these two forms of disease resistance should not be differentiated in terms of underlying mechanisms, as they can overlap, but should rather only be differentiated in terms of phenotypes.

Two hypotheses underlying the interaction between AvrStb20q and Stb20q

Based on classic *R/Avr* interactions, we can hypothesize that the mechanisms underlying the gene-for-gene interaction of *AvrStb20q* and *Stb20q* is a sort of on/off mechanism wherein if *AvrStb20q* is recognized, there is resistance induced by *Stb20q*, and if there is no recognition, there is no resistance induced. The differences observed in strains with the different isoforms of *AvrStb20q* inoculated on RIL 2019 support this hypothesis as phenotypes are distributed into two statistically different classes seemingly dependant on isoforms which are less pathogenic (I2 and I3) and more pathogenic (I1, I4 and I5) (Figure 5. b). In a study on the *P. infestans* effector AVR2 different haplotypes were shown to segregate into two categories in a similar way, with

haplotypes being either virulent or avirulent (Yang et al., 2020). The sole difference between the *AvrStb20q/Stb20q* interaction and a classic *R/Avr* is that the resistant phenotype leads to partial resistance rather than total resistance. The cause for the virulence or avirulence would then be linked to the avirulence protein. In the case of *AvrStb6*, one single amino-acid mutation has been hypothesized to explain the switch between virulent and avirulent at position 41 in the *AvrStb6* protein sequence (Stephens et al., 2021). However, the protein sequences of the different *AvrStb20q* do not have any mutations that are clearly associated with either phenotype. Indeed, no mutation in common to the least pathogenic isoforms I2 and I3 is strictly absent in the other isoforms, and no mutation common to the more pathogenic isoforms is strictly absent in I2 and I3 (Figure 5. b and c).

A second hypothesis is that pathogenicity phenotypes could follow a gradient from “not pathogenic” to “highly pathogenic” with all intermediates in between depending on isoforms, though we did not identify such a gradient in our study. This was previously suggested as a possibility in the case of *Avr3D1* where the authors state that other isoforms could potentially lead to a stronger resistance response than those they studied (Meile et al., 2018). Additionally, it has been previously reported in the *Puccinia striiformis* f. sp. *tritici-Triticum dicoccoides* pathosystem that cultivars with different haplotypes of the *Yr36 (WKS1)* resistance gene inoculated with the same fungal strain led to qualitative extreme phenotypes and variable quantitative intermediary phenotypes, although in this case it was the *R*-gene diversity which was in play (Huang et al., 2016). However, resistance genes and effectors can be considered as two sides of the same coin. A study on haplotype diversity of *AvrStb6* identified nine isoforms of *AvrStb6* in more than one *Z. tritici* isolate of their sample collection (Stephens et al., 2021). Only one of these isoforms (I01) led to an avirulent phenotype, avirulent here meaning no disease symptoms. Another isoform (I13) however, was also classified as “avirulent” despite some sporulation observed on the cultivar Cadenza, which has *Stb6*, after inoculation of a strain with the I13 encoding allele. This classification as “avirulent” was based on significantly reduced sporulation of that strain on Cadenza compared with that on *CadenzaΔStb6*, a CRISPR/Cas9-induced deletion mutant. The I13 isoform is therefore recognized by *Stb6*, but the resistance is partial. I01 is then the extreme case in a spectrum of possibilities.

We identified TE insertions in the 3' UTR of *AvrStb20q* with a variety of haplotypes. In the GWAS, we showed that though there are some exceptions, an absence of TE insertion in the 3'UTR is associated with a “most pathogenic” allele, while an absence of insertion is associated with a “least pathogenic” allele at the most associated SNP

(Figure 6). Additionally, the strains with the isoforms I2 and I3 were the only strains to carry annotated class I retrotransposon TE in the insertions, consisting of Gypsy, RLX-TRIM and LINE RIX elements. 3' UTRs are known to regulate mRNA localization, stability and translation and repeat TE inserts have been shown to have an effect on their function (Mayr, 2019; Niu et al., 2019). They are also known to regulate protein-protein interactions (Mayr, 2017). In wheat for example, it has been shown that a miniature inverted-repeat TE (MITE) insertion in the 3' UTR of *TaHSP16.9* enhances gene transcription (Li et al., 2014). Influence of MITEs in 3' UTR on translation regulation has also been shown in rice (Shen et al., 2017). Moreover, 3'UTR length, influenced by the presence of repeat TEs in the UTR has been associated with the presence RNA binding sites and miRNA targets in certain types of fish, this is thought to be linked to an important post-transcriptional regulatory mechanism (Xiong et al., 2018). In *Magnaporthe oryzae*, shorter 3'UTRs were linked to more stable mRNA, for some pathogenicity genes however, a long 3'UTR was required for infection (Rodríguez-Romero et al., 2019). In *Z. tritici* a TE insertion in the promoter 5' UTR of *MFS1* was found to induce overexpression of the gene leading to a multi-drug resistance phenotype (Omrane et al., 2017), insertions in 3' UTR however have not to our knowledge been previously linked to pathogenicity in this fungus, although 3' UTR polymorphism has been previously used as a criterion for candidate gene screening in relation to oxidative stress tolerance (Zhong et al., 2020). With the effect of 3'UTR on mRNA stability and expression, we can suppose that the TE insertions in our study may modulate the expression of *AvrStb20q*. Though we do not have expression data for all of the panel strains, we do know that in strains I05 and I07, which encode *AvrStb20q* isoforms I1 and I2 respectively, there is overexpression of the effector at 12 dpi in both strains but with a lower level of expression in I07 (Chapter 3; this thesis). However, if TE size and nature modulate expression and pathogenicity, we would expect to see a gradient of phenotypes such as that proposed as a second hypothesis. In reality however, we observe a distinction of two classes of phenotypes (Figures 5 and 6). Moreover, the study on haplotype diversity of *AvrStb6* suggests that it is the isoforms themselves which are causal in resistance breakdown as no suppression of expression could be correlated to phenotypes (Stephens et al., 2021).

Finally, with the emergence of research on effector families defined by protein structures rather than by sequence (Franceschetti et al., 2017; Guillen et al., 2015; Lazar et al., 2022; Mukhi et al., 2020), it is possible that TE insertions have a role to play in structure-related virulence/avirulence mechanisms.

An update in definitions is needed for quantitative and qualitative notions of disease resistance

Disease resistance is and has been subject to a strict dichotomy classically separating “basal”/“horizontal”/“partial”/“broad-spectrum” resistance from “vertical”/“total”/“specific” resistance. Van Der Plank (1966) provided the definitions of the now relatively archaic terms. He described horizontal resistance as a polygenic, non-specific, minor-gene resistance akin to field resistance or tolerance. Vertical resistance on the other hand was described as monogenic or oligogenic (governed by a small number of genes), major gene resistance, *R*-gene resistance, racial resistance (meaning race or strain-specific) or HR inducing resistance (Van Der Plank, 1966). Nowadays, quantitative is used for phenotypes following a continuous distribution, a gradient, shades of grey, and qualitative for a discrete distribution into two categories, resistant or non-resistant, black or white (Niks et al., 2015; Poland et al., 2009; St Clair, 2010). A resistant phenotype being generally associated with no symptoms or HR. In general, qualitative resistance is used in reference to *R* genes while quantitative resistance is used in reference to smaller effect quantitative trait loci (QTL). Gene-for-gene interaction is associated with qualitative or major-gene resistance, the mechanisms underlying quantitative resistance remaining relatively poorly understood but usually considered to be involved in broad-range defence and to be linked to pathogen fitness (Cowger and Brown, 2019). In the present study we demonstrate several gene-for-gene interactions involving at least one genuine *Avr* gene *AvrStb20q*, underlying an overall quantitative phenotype. This goes along with a previous hypothesis that genes underlying quantitative resistance could be weaker forms of *R* genes (Poland et al., 2009). These results show that gene-for-gene interactions are not exclusive to qualitative resistance and concur with previous studies (Jiquel et al., 2021; Meile et al., 2018), thus calling for a much needed update of the concepts surrounding disease resistance in plants.

Conclusion

In this study we identified QTL-for-QTL interactions underlying quantitative phenotypes. We also validated an *R/Avr* interaction between STB resistance locus *Stb20q* and the cloned effector gene *AvrStb20q*. The findings highlight an issue with the generally accepted dichotomy between quantitative and qualitative definitions of disease resistance as gene-for-gene interactions and *R/Avr* type interactions have long been thought to be specific to qualitative systems. We suggest that rather than

compartmentalizing qualitative and quantitative forms of disease resistance, they should be seen as a continuum, with qualitative interactions being the extreme cases of overall quantitative systems.

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Data Accessibility

Genome sequencing data for the 120 French *Z. tritici* isolates used in this study will be made available at NCBI BioProject (To be submitted). The sequence of *AvrStb20q* is available at NCBI Gene ID (To be submitted).

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SUPPLEMENTARY FIGURES

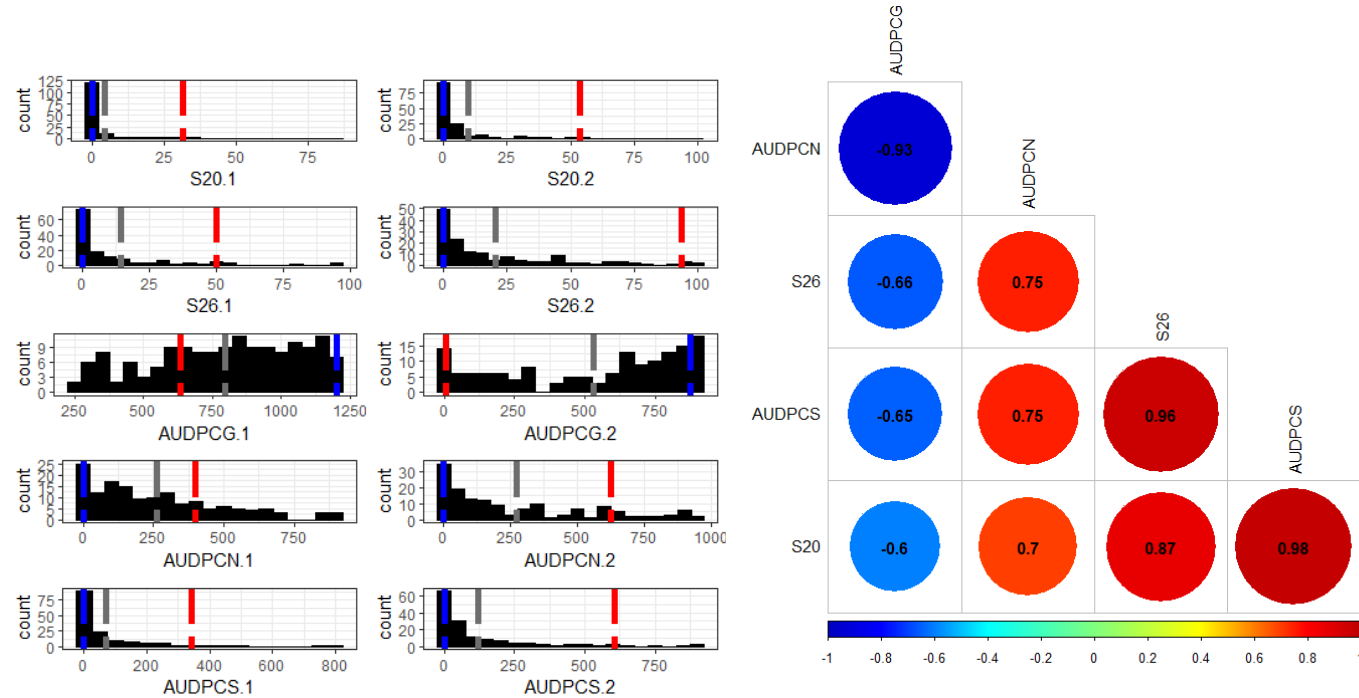


Figure S1. Phenotypic data obtained from the inoculation of strain 6096 on R×CS. On the left are histograms representing the distribution of data for R×CS for each trait and replicate. The blue dashed lines represents the values obtained for Renan, the red dashed lines represent the values obtained for Chinese Spring and the grey dashed lines represent the mean value for R×CS overall. On the right is a correlogram obtained from the values for both replications for all traits. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. S20 and S26 are the sporulating area at 20 and 26 days post-inoculation respectively.

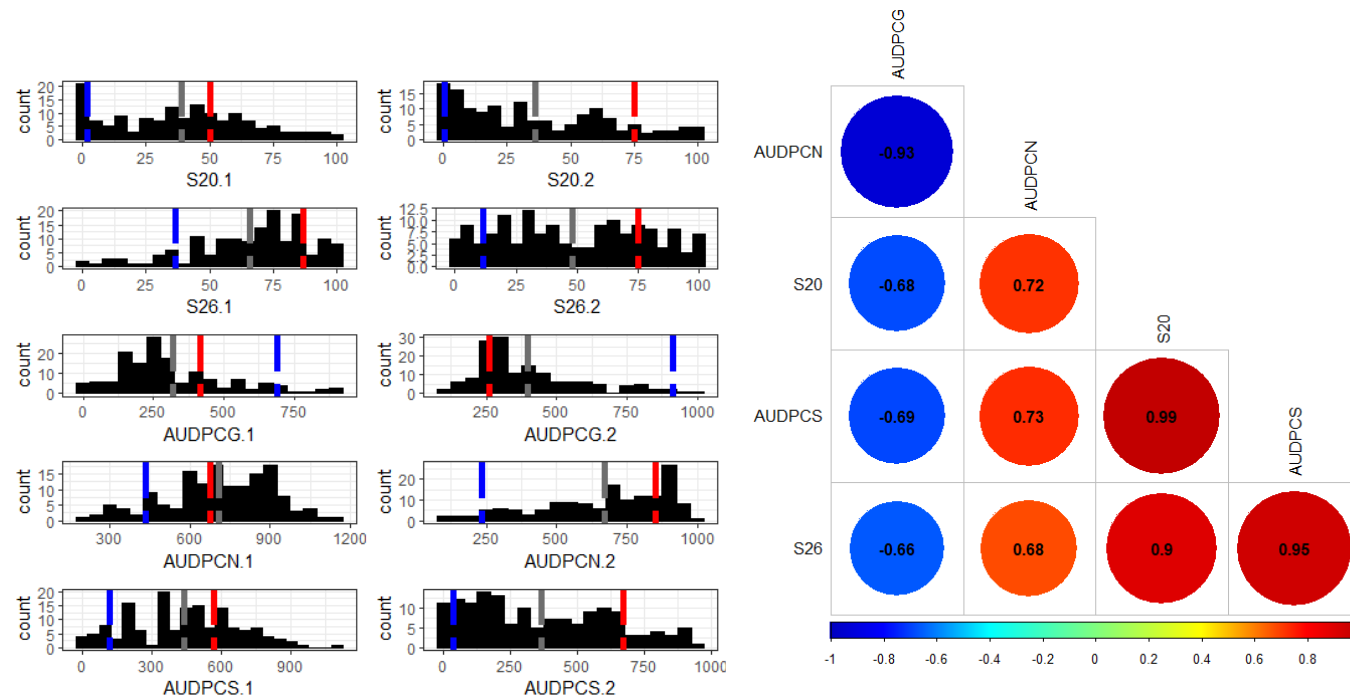


Figure S2. Phenotypic data obtained from the inoculation of strain 7558 on R×CS. On the left are histograms representing the distribution of data for R×CS for each trait and replicate. The blue dashed lines represents the values obtained for Renan, the red dashed lines represent the values obtained for Chinese Spring and the grey dashed lines represent the mean value for R×CS overall. On the right is a correlogram obtained from the values from both replications for all traits. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. S20 and S26 are the sporulating area at 20 and 26 days post-inoculation respectively.

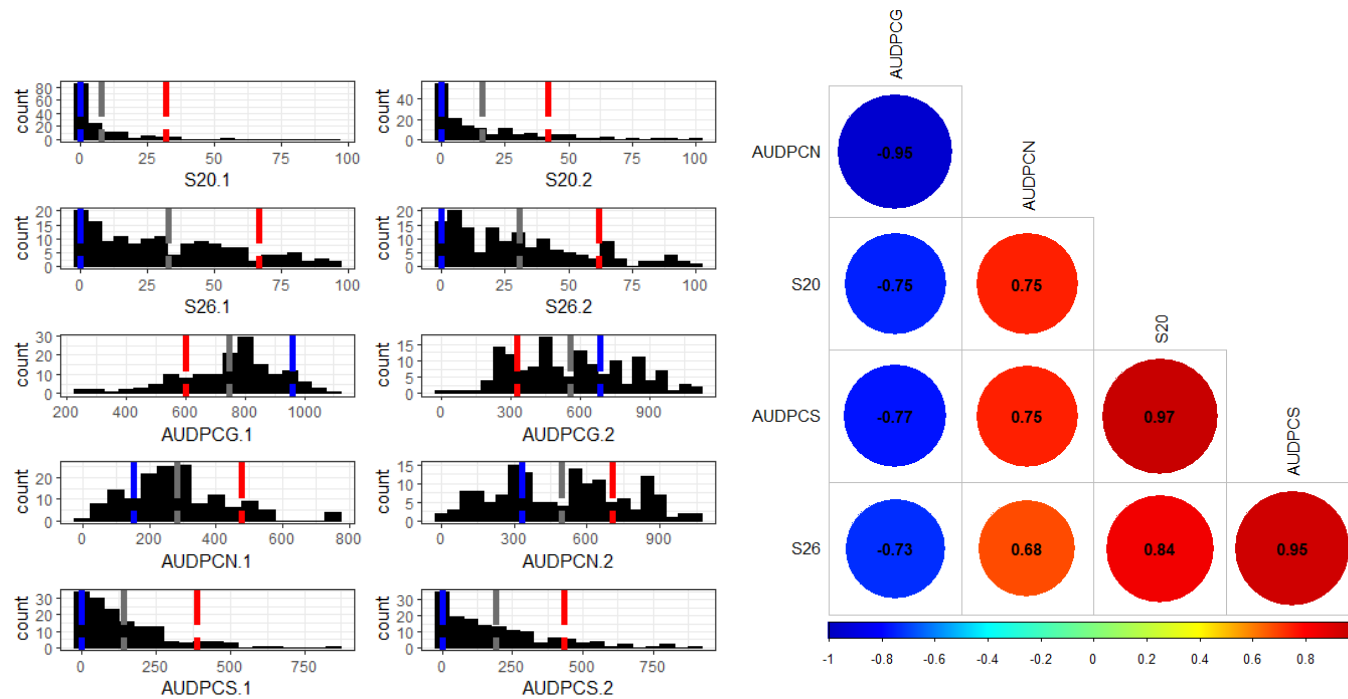


Figure S3. Phenotypic data obtained from the inoculation of strain 7510 on R×CS. On the left are histograms representing the distribution of data for R×CS for each trait and replicate. The blue dashed lines represents the values obtained for Renan, the red dashed lines represent the values obtained for Chinese Spring and the grey dashed lines represent the mean value for R×CS overall. On the right is a correlogram obtained from the values from both replications for all traits. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. S20 and S26 are the sporulating area at 20 and 26 days post-inoculation respectively.

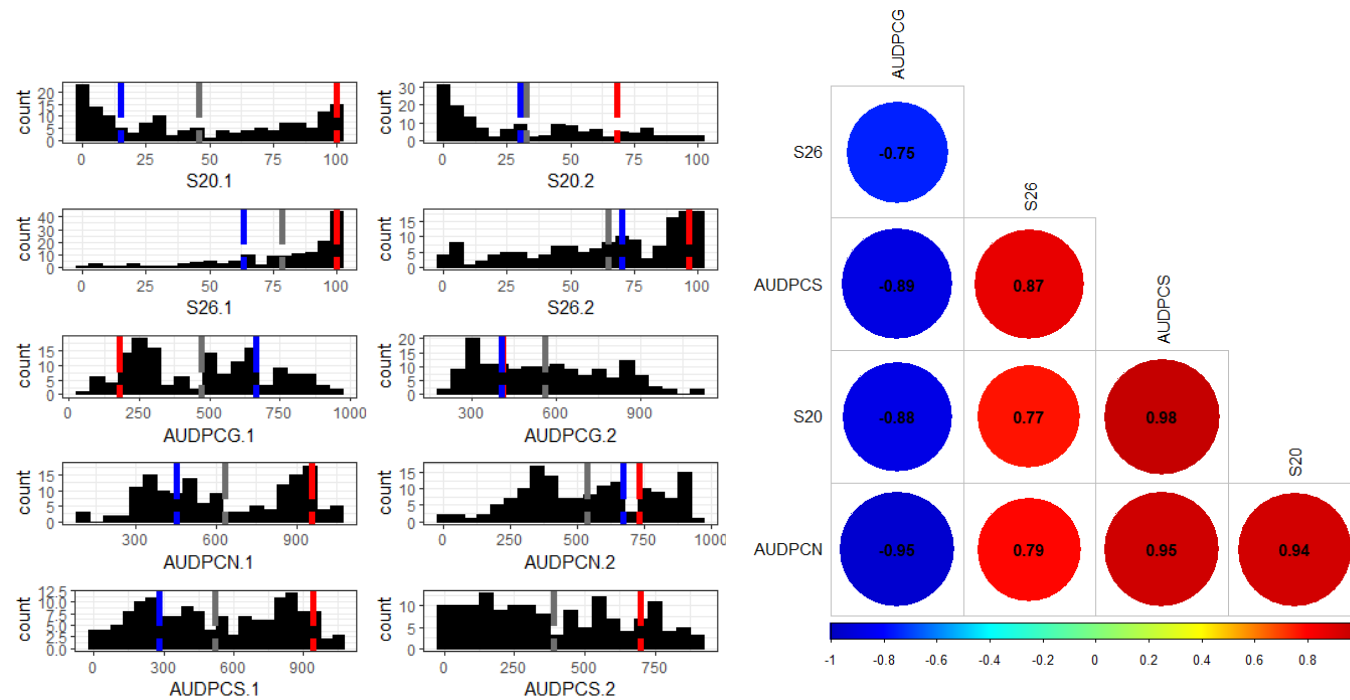


Figure S4. Phenotypic data obtained from the inoculation of strain I07_ΔG07189_cl.40 on R×CS. On the left are histograms representing the distribution of data for R×CS for each trait and replicate. The blue dashed lines represents the values obtained for Renan, the red dashed lines represent the values obtained for Chinese Spring and the grey dashed lines represent the mean value for R×CS overall. On the right is a Bravais-Pearson correlogram obtained from the values from both replications for all traits. AUDPCG is the area under the disease progress curve for the green leaf area. AUDPCN is the area under the disease progress curve for the necrotic leaf area. AUDPCS is area under the disease progress curve for the sporulating leaf area. S20 and S26 are the sporulating area at 20 and 26 days post-inoculation respectively.

CHAPTER 5: DISCUSSION AND PERSPECTIVES

The objective of this project was to better understand the mechanisms underlying quantitative resistance which for years has been described as non-specific broad-range resistance despite mounting exceptions to the rule. In understanding these mechanisms, the goal was then to comment on their potential impact on resistance durability.

Throughout the different chapters and corresponding experiments carried out over the last three or so years I was able to identify novel players in the *Z. tritici*-*T. aestivum* interaction both on the fungus and plant sides. In the second chapter of this document, I was able to identify quantitative resistance loci towards fungal strains I05 and I07, on chromosomes 1D, 5D and 7B in the wheat cultivar 'Renan', the QTL on chromosome 5D was a new resistance locus and was designated *Stb20q*. In the third chapter, I identified pathogenicity QTL in strains I05 and I07 and subsequently cloned and validated a gene encoding a cysteine-rich small-secreted protein identified in the interval of a QTL on *Z. tritici* chromosome 6. This cloned gene was shown to have an avirulence role in wheat infection but leading to qualitative or quantitative phenotypes depending on the fungal genetic background. Finally in the fourth chapter, I was able to identify two QTL-for-QTL interactions underlying the quantitative phenotypes observed in the R×CS and I05×I07 populations. Was also identified a gene-for-gene interaction between the previously cloned fungal gene and *Stb20q*, prompting the designation *AvrStb20q* for the cloned gene. With 22 previously known *Stb* genes and over 100 mapped resistance QTL, there was already a relatively large repertoire of resistance sources available in wheat. On the fungus side however, only two avirulence genes had previously been cloned, with the interacting gene pair known only in the case of *AvrStb6-Stb6*. The cloning of a newly identified effector and its involvement in a gene-for-gene interaction with a known plant locus therefore contributes to a large part of what we now know of the genetic interactions in this pathosystem.

I. GENE-FOR-GENE INTERACTIONS DO OCCUR IN QUANTITATIVE PLANT-PATHOGEN RELATIONSHIPS

A. DIFFERENTIAL INTERACTIONS ARE A HALLMARK OF GENE-FOR-GENE INTERACTIONS

That partial resistance can be specific to the isolate is indisputable as shown in many pathosystems (Arru et al., 2003; Caranta et al., 1997; Jiquel et al., 2021; Leonards-Schippers et al., 1994; Marcel et al., 2008; Meile et al., 2018; Qi et al., 1999). However, in order to demonstrate a gene-for-gene interaction underlying specificity, a

differential interaction must be observed between strains and cultivars. A differential interaction as explained by Niks et al. (2019) can be illustrated with the example of two host plant loci for hypersensitivity resistance and two pathogen loci for avirulence. In this context, the resistance allele of a given host resistance locus will trigger resistance mechanisms upon recognition of the avirulent allele of the corresponding pathogen locus but will not recognize the virulent allele. On the fungus side, the pathogen will be successful in infection if there is no resistance from the host, meaning that no resistance gene is triggered. The differential interaction is observed if the avirulence allele of a given fungal gene triggers resistance for a given resistance gene, but not for another. In the example in Table 1, Avr1 triggers resistance from R₁ but not from R₂ while Avr2 triggers resistance from R₂ but not R₁.

Table 1: A differential interaction is the signature of a gene-for-gene interaction, as proposed by Niks et al. (2019). Upper case letters represent the resistant allele plant-side and the avirulent allele fungus side, while lower case letters represent the susceptible and virulent alleles respectively.

		Pathogen			
		Avr1 Avr2	avr1 Avr2	Avr1 avr2	avr1 avr2
Host	r ₁ r ₁ r ₂ r ₂	S	S	S	S
	R ₁ R ₁ r ₂ r ₂	R	S	R	S
	r ₁ r ₁ R ₂ R ₂	R	R	S	S
	R ₁ R ₁ R ₂ R ₂	R	R	R	S

In the wheat-*Z. tritici* pathosystem, *Stb* resistance genes are known to be involved in gene-for-gene interactions, this was illustrated particularly in the case of the *Stb6/AvrStb6* interaction (Brading et al., 2002; Zhong et al., 2017). In this project, I was able to demonstrate that gene-for-gene interactions can also underlie quantitative resistance. In studying quantitative resistance from the cultivar Renan and the genetic architecture of pathogenicity in fungal strains I05 and I07, a differential interaction is observed between two recombinant inbred lines (RIL) carrying a different resistance QTL and the two parental strains carrying different pathogenicity QTL (Figure 1). The non-pathogenic allele of *Qzt-107-13* triggers resistance from *Qstb-Renan-1D* and the non-pathogenic allele of *Qzt-105-6* triggers resistance from *Stb20q* on chromosome 5D. As the phenotypes observed with non-pathogenic alleles were partial, rather than complete resistance as in classic gene-for-gene interactions, the system described here is quantitative, and the on/off motif observed in resistance QTL depending on the alleles of the pathogenicity QTL corresponds to a differential interaction, the hallmark

of gene-for-gene interactions.

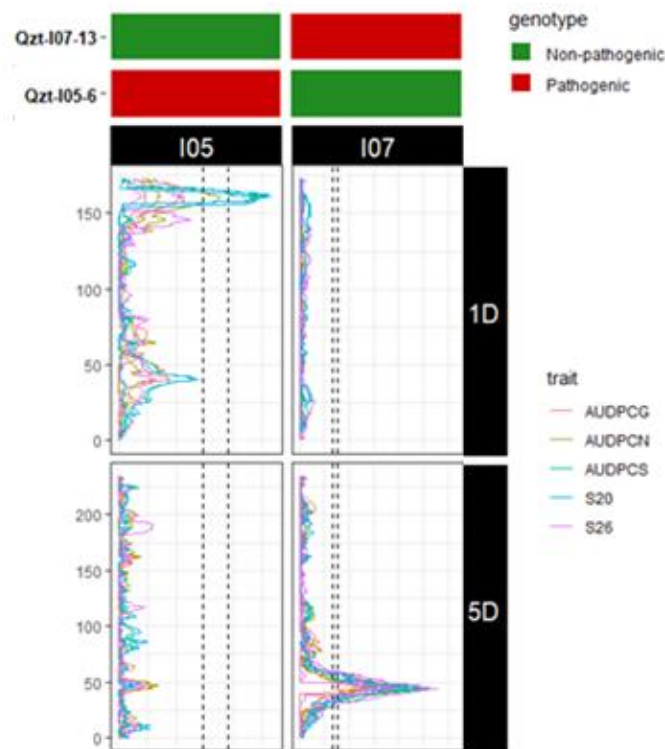


Figure 16: Differential interaction between two *Zymoseptoria tritici* isolates carrying different QTL for pathogenicity, *Qzt-105-6* (*AvrStb20q*) and *Qzt-107-13*, and two RIL carrying the corresponding QTL for resistance, *Qstb-Renan-5D* (*Stb20q*) and *Qstb-Renan-1D* respectively.

For this model to work however, it is necessary to have two loci involved in interactions, which is not often the case in publications. In general, only one interaction is demonstrated. Instances of gene-for-gene interactions leading to partial phenotypes do however exist in literature.

B. *STB* RESISTANCE GENES UNDERLYING RESISTANCE QTL IN THE T. AESTIVUM/Z. TRITICI PATHOSYSTEM

In the second chapter of this document, I identified three resistance QTL to septoria leaf blotch (STB) on chromosomes 1D, 5D and 7B. While that on chromosome 5D was a novel locus, those on chromosomes 1D and 7B colocalized with known resistance genes, *Stb19* (Yang et al., 2018) and *Stb8* (Adhikari et al., 2003) respectively. Moreover, at least two of these QTL are involved in a gene-for-gene relationship with the fungus as previously presented in Figure 1, strongly suggesting that the underlying mechanism is that of an *R/Avr* interaction but leading to quantitative phenotypes. *Qstb-Renan-1D*,

which has all of the candidate genes for *Stb19* proposed by Yang et al. (2018) in its confidence interval therefore presents as a probable “weaker form” of *Stb19*.

In this pathosystem, the identification of colocalized resistance genes with different effect magnitudes on the phenotype is not entirely new. An example of an *Stb* gene with an eventful story is *Stb2*. This gene had originally been mapped to the short arm of chromosome 3B (Adhikari et al., 2004), this position was however later corrected to the short arm of chromosome 1B (Liu et al., 2013). This would not have been a problem, had *Stb11* not been mapped to the short arm of chromosome 1B in the meantime. This novel gene explained 50% of phenotypic variation for resistance to the strain used in the study, which the authors link to partial resistance (Chartrain et al., 2005b). Both *Stb2* and *Stb11* have been linked to microsatellite marker *Xbarc008* (Chartrain et al., 2005b; Liu et al., 2013). As of 2018, no allelism test has been carried out to verify whether or not these two genes are simply variations of each other (Vagndorf et al., 2018) and I was not able to find reference to one in any more recent literature. Moreover, this is not the only occurrence in published works of a potential confusion of two *Stb* genes. Indeed, two other coupled *Stb* genes have been identified in very close proximity to each other. *Stb4* was identified in 1996 (Somasco et al., 1996) and mapped to the short arm of chromosome 7D (Adhikari et al., 2004). *Stb5* was already mapped in the same area (Arraiano et al., 2001), which has been referred to as a supercluster of resistance genes (Adhikari et al., 2004). Both genes have been mapped using *Xgwm44* (Vagndorf et al., 2018). As of 2015, no allelism test has been carried out for these two genes either (Brown et al., 2015) and I was not able to find any indication of one in more recent literature. The other couple is that of *Stb7* and *Stb12* on the long arm of chromosome 4A, these however are genuinely two different genes, this is based on separate isolate specificities and different microsatellites for each gene although *Xwmc219* has links to both, but this is due to their being clustered (Brown et al., 2015; Chartrain et al., 2005a; Louriki et al., 2021; McCartney et al., 2003). For these last genes, gene-for-gene interaction has been hypothesized but not formerly demonstrated with cloned avirulence gene *Avr3D1*, an interaction that results in partial resistance phenotypes (Meile et al., 2018). This interaction means that these *R* genes lead to partial phenotypes much as has been observed with the *Stb20q/AvrStb20q* interaction.

To verify these claims, it would be interesting to perform the allelism tests required, but also it would be interesting to have a better idea of the nature of the genes underlying these interaction mechanisms. The different known components of resistance in wheat to *Z. tritici* were thoroughly reviewed in 2015 (Brown et al., 2015) but the exact genes involved remain for the most part unknown. That resistance QTL

identified in this study could be weaker alleles of *R* genes suggests that they would encode proteins with structures similar to known *R* genes such as receptor-like proteins (RLPs), receptor-like kinases (RLKs) and potentially nucleotide-binding site leucine-rich repeat immunoreceptors (NLRs), though the third possibility seems least likely for this pathosystem as it involves a strictly apoplastic fungus. And indeed, within the resistance QTL intervals identified, only one, on chromosome 1D, carried annotated NLR or pseudo-NLR genes, while WAKs and other kinase domain carrying protein encoding genes were found in all three QTL intervals. The possibility does remain that the genes underlying resistance QTL identified in this study could also be of other types as previously presented (General introduction; this thesis), bearing in mind that so far, only two major resistance genes against STB have been cloned: *Stb6* which encodes a wall-associated kinase (Saintenac et al., 2018) and *Stb16q* which encodes a cysteine-rich receptor-like kinase (Saintenac et al., 2021).

On the fungus side, three avirulence genes have been cloned, which are *AvrStb6*, *Avr3D1* and *AvrStb20q* all encoding cysteine-rich small secreted proteins (SSP) (Meile et al., 2018; Zhong et al., 2017), classic of effectors involved in *R/Avr* interactions, none of them however have any known patterns comparable to other proteins. There are only three cloned effectors for this pathosystem despite there being over 20 known *R* genes, making any conclusions difficult to make, however, based on these three, one can state that cysteine-rich SSP represent one of the fungal components of gene-for-gene interactions in this pathosystem, regardless of the quantitative or qualitative nature of the interaction.

C. WEAKER *R* GENES MAY INDEED EXPLAIN QUANTITATIVE RESISTANCE

In their review on the world of quantitative disease resistance, Poland et al. (2009) suggested that genes underlying quantitative resistance could be weaker forms of *R* genes. And indeed, some cloned genes shown to be involved in quantitative resistance, partial resistance, encode genes that are similar to known *R* genes including NB-LRR domain carrying proteins and kinase domain carrying proteins (Dmochowska-Boguta et al., 2020; Gadaleta et al., 2019; Pilet-Nayel et al., 2017; Wang et al., 2019) and an NBS-ARC gene (Jiang et al., 2020). There are also examples of *R* genes that lead to partial phenotypes. An example is found in the apple-*Venturia inaequalis* pathosystem. *V. inaequalis* is a hemibiotrophic ascomycete that develops in the subcuticular space of apple leaves and fruit without penetrating host cells causing apple scab; also known as black spot. Two known resistance genes in apple to this disease *Rvi6* and *Rvi12* are particular in that unlike other *R* genes in this pathosystem, they do not induce a hypersensitive response (HR) or necrosis. Instead, they induce chlorosis upon

recognition of the corresponding *Avr* gene. This chlorosis is often accompanied by limited sporulation, hence they provide only partial resistance (Bowen et al., 2011; Khajuria et al., 2018). *Rvi6* encodes a Leucine-rich repeat RLP (Bus et al., 2011; Chizzali et al., 2016; Khajuria et al., 2018). The corresponding avirulence gene *AvrRvi6* has been identified and cloned, but the data has yet to be published (EcoFun research team, n.d.). A strain specific QTL named QTL T1 colocalizes with *Rvi6* but no link could be found between strain aggressiveness with regards to the QTL and their virulence/avirulence towards *Rvi6* (Caffier et al., 2016). Another resistance gene in this pathosystem that confers partial resistance leading to chlorosis and none to a little sporulation is *Rvi12*. This gene has been fine-mapped, but not cloned (Padmarasu et al., 2014), and no breakdown has been reported of yet (Khajuria et al., 2018). Other examples are found in rusts which are haustoria-forming biotrophic plant pathogens. Race-specificity of partial resistance to rusts has been identified suggesting gene-for-gene interactions, with some stating that an intermediate response, *i.e.* quantitative phenotypes, in the greenhouse or field could be due to a weak recognition between an effector and resistance elicitor (Herrera-Foessel et al., 2015). *Lr13* is one of the most widely distributed resistance genes against leaf rust, caused by *Puccinia triticina*, and is also widely ineffective. It was identified as an APR gene with race-specificity (McIntosh et al., 1995). Despite being an APR gene, *Lr13* was found to encode an NLR (Hewitt et al., 2021), this gene-type had been previously hypothesized by previous authors due to *Lr13* race-specificity (Qiu et al., 2020). Another rust resistance gene called *Yr27* confers partial but specific resistance to yellow rust caused by the fungus *Puccinia striiformis* f. sp. *tritici* (McDonald et al., 2004). It was recently shown that *Yr27* is allelic to *Lr13* (Athiyannan et al., 2022). These are therefore two cases of NLRs potentially involved in gene-for-gene interactions and which lead to partial phenotypes.

D. OTHER GENES INVOLVED IN QUANTITATIVE GENE-FOR-GENE INTERACTIONS

In rice, partial resistance of the cultivar Chubu 32 to *Magnaporthe grisea* (also known as *Pyricularia grisea*) was found to be explained by a single locus showing specificity (Zenbayashi et al., 2002). The corresponding locus named *Pi34* was later mapped to a region carrying transposon proteins, but no genes with commonly found *R* domains such as NLRs or Kinase domains (Zenbayashi-Sawata et al., 2007). A gene-for-gene interaction had however been demonstrated between *Pi34* and *AvrPi34* leading to a partial resistance phenotype (Ballini et al., 2008; Zenbayashi-Sawata et al., 2005), interestingly the *Avr* denomination was used in the article for convenience despite

being a term normally associated with qualitative or full resistance (Zenbayashi-Sawata et al., 2005). *AvrPi34* is not among the cloned avirulence genes in *M. oryzae* (Lopez et al., 2019). In rusts, a study highlighted strong indications for minor gene-for-minor gene interactions, corresponding to quantitative gene-for-gene interactions, making a point of stating that there was no evidence supporting that these minor genes belonged to the NLR class (González et al., 2012), and a few years later, after fine-mapping, the strongest candidate gene identified for *Rphq11*, one of these partial resistance QTL involved in a gene-for-gene interaction, encodes a phospholipid hydroperoxide glutathione peroxidase (Yeo et al., 2017).

Mechanisms underlying quantitative gene-for-gene interactions could therefore call upon similar genes to those involved in gene-for-gene interactions resulting in qualitative phenotypes. However other mechanisms could also underlie such interactions, leading to a very high diversity of molecular mechanisms in quantitative disease interactions. In the wheat-*Z. tritici* pathosystem, only two *R* genes have been cloned, both of which encode RLKs (Saintenac et al., 2018; Saintenac et al., 2021). With at least 20 other *Stb* genes and a plethora of resistance QTL with the underlying genes and precise mechanisms still unknown, there is no way to conclude for now, on the precise nature of the genes involved in gene-for-gene interactions in this pathosystem, quantitative or qualitative alike. However, although classically, it is RLPs, RLKs and NLRs which are considered to be the best candidates, other genes, for example those encoding proteins with enzymatic activity or transporters, should not systematically be overlooked.

E. GENE-FOR-GENE INTERACTIONS EXIST IN BOTH FORMS OF RESISTANCE, WHY ARE THEY SO DIFFICULT TO EVIDENCE?

Gene-for-gene interactions may have been overlooked in the context of quantitative phenotypes, due to the previously presented definitions of quantitative resistance which excluded the possibility of their existence despite many contradictory examples.

Strong effect loci are easier to study because phenotypes are clear-cut, scientists are likely to choose them over quantitative effect loci for further studies. Strong effect loci are more immediately effective, this could explain why they might be preferentially used by breeders (Cowger and Brown, 2019). In this thesis for example, the difficulty in studying smaller effect loci is illustrated by the unclear result obtained in cloning the candidate gene underlying *Qzt-107-13* despite the gene being a strong candidate. The phenotypes obtained with mutant strains were not clear-cut and could not be interpreted, then again, the QTL involved on the plant and fungus-sides explained only

small percentages of the phenotypic variation. It would take a larger and more time and energy consuming experiment to validate this gene, or as the case may be, to exclude it as a candidate. However, despite the small effect of QTLs *Qzt-107-13* and *Qstb-Renan-1D*, chapter 4 showed that the interaction is detectable and though the gene itself is not validated, the interaction is validated at a QTL level.

One factor highlighted by Cowger and Brown (2019), is that there is a publication bias affecting quantitative resistance. I feel that a prime example of this was found in the study of the allelic variants of *AvrStb6* which identified two avirulent alleles, one of which led to a classic qualitative phenotype, while the other led to a partial but resistance phenotype upon recognition by *Stb6* (Stephens et al., 2021). The authors do not highlight the particularity of this allele despite *Stb6* being widely known as a major resistance locus.

Another reason could be relatively limited knowledge of pathogen genomes up until relatively recently, which hindered the study of the pathogen genes involved in these interactions. In the case of *AvrStb20q* for example, before this work, the gene was not annotated. Additionally, implementing forward genetics methods, *i.e.* using a without *a priori* approach, led to the identification of this particular gene, while reverse genetics, *a priori* methods based on pre-existing annotations, would not have been sufficient to identify the gene.

Another factor may be that though two organisms are involved in the interaction, studies often seem to focus on one side or the other, *i.e.* on the host side or on the pathogen side rather than treating the pathosystem as a whole, which renders the identification of differential interactions near impossible. While a focus on the host side makes sense from a breeding point of view, the pathogen side cannot be discounted as the factors determining pathogenicity and adaptation are key determinants of resistance durability, as previously seen with durability prediction models.

Finally, in the context of the wheat-*Z. tritici* interaction, although gene-for-gene interactions are detectable through differential interactions, the components host and plant-side remain difficult to identify as the pathosystem is highly polygenic and involves quantitative effect genes (Kema et al., 1996). It is therefore relatively lacking in clear-cut phenotypes which require less precision work compared to what was done for this project. Complex systems such as plant pathogen interactions require a combination of complementary methods for their study.

II. TRANSPOSABLE ELEMENTS COULD HAVE AN IMPACT ON PATHOGENICITY

In the fourth chapter of this thesis, I obtained results which strongly suggest that TE could have an impact on strain pathogenicity in *Z. tritici* beyond evolutionary capacity.

TE play a significant role in genome evolution as they can induce gene inactivation, impact gene expression, induce illegitimate recombination (i.e. recombination between non-homologous DNA strands) and are heavily involved in insertion/deletion polymorphisms (Muñoz-López and García-Pérez, 2010). They have been classified into two classes, class I corresponds to retrotransposons, which move and spread by a copy-and-paste mechanism, and class II corresponds to DNA transposons which move by a cut-and-paste mechanism (Goodier and Kazazian, 2008; Muñoz-López and García-Pérez, 2010). The presence of TE has been shown to promote variability and adaptability in fungi (Mat Razali et al., 2019) and they are to be located significantly closer to orphan genes than to nonorphan genes (Grandaubert et al., 2015), and are physically associated with pathogenicity-related genes in several plant-pathogenic fungi (Lorrain et al., 2021).

In *Z. tritici*, TE represent around 18% of the 39.7 Mb genome with the majority of these belonging to class I (Grandaubert et al., 2015). It has been previously shown that TE insertions in *Z. tritici* upstream of the promoter of *Zmr1* can impact its expression. This gene encodes a transcription factor controlling the expression of genes involved in the biosynthesis of melanin (Krishnan et al., 2018). Similarly, a TE insertion in the promoter of *MFS1* induces overexpression of the gene, leading to a multidrug resistance phenotype (Omrane et al., 2017). Both of these observations demonstrate that TE can shape the adaptation of *Z. tritici* to stresses. The two previously cloned avirulence gene in *Z. tritici*, *AvrStb6* and *Avr3D1*, were both identified in TE-rich regions which were shown to be highly polymorphic between strains (Meile et al., 2018; Zhong et al., 2017). Another putative avirulence gene *Zt_8_609*, was also identified in a highly polymorphic region subject to TE-induced plasticity which in this case was even linked to partial deletions of the gene (Hartmann et al., 2017). None of these studies however linked the presence of the TE directly to strains pathogenicity, although the link has been suggested by others since (Badet et al., 2020; Oggenfuss et al., 2021). Additionally, TE mobility has been shown to be still active in *Z. tritici* (Lorrain et al., 2021).

Like previously cloned avirulence genes in *Z. tritici*, *AvrStb20q* was identified in a dynamic TE-rich region (chapter 3). Moreover, in the fourth chapter of this project, I

was able to identify a link between presence/absence polymorphisms of TE insertions in the 3'UTR of *AvrStb20q* and strain pathogenicity. Indeed, an absence of TE insertion in the 3'UTR was linked to higher strain pathogenicity while insertion seemed to have a deleterious effect of strains' success in infection. The TE haplotype was however not sufficient to explain all phenotypes, indeed some strains with lower pathogenicity had an absence of insertion haplotype. Another observation that I made is that longer insertions, over 3,300 bp, were only identified in the least pathogenic strains, these were also the only insert sizes which were found to carry class I transposable elements. The localization of these inserts in the 3'UTR is original compared with the examples presented above. However, as previously stated in chapter 4, 3'UTR TE-induced mutations and indeed 3'UTR length have been linked to gene transcription, translation regulation and mRNA stability and localization (Li et al., 2014; Mayr, 2019; Niu et al., 2019; Rodríguez-Romero et al., 2019; Shen et al., 2017; Xiong et al., 2018). The insertions in the 3'UTR of *AvrStb20q* could be directly influencing the expression or indeed encoded protein conformation thus directly influencing strain pathogenicity, although this remains to be demonstrated. An impact on protein structure would be particularly interesting bearing in mind the relatively new observation that despite most effectors being orphan genes with no sequence similarities with other genes or indeed with each other, some can be regrouped into families by structure. This was shown for MAX-effectors in *M. oryzae* and *Pyrenophora tritici-repentis* (Guillen et al., 2015), RALPH effectors of *Blumeria graminis* f. sp. *hordei* (Franceschetti et al., 2017) and LARS effectors in *Leptosphaeria maculans* (Lazar et al., 2022). Structural analyses of *AvrStb20q* could further elucidate the gene-for-gene mechanisms in which it is involved and could potentially lead to a clearer view of the effect of the 3'UTR TE insertions.

III. QUANTITATIVE AND QUALITATIVE NOTIONS OF DISEASE RESISTANCE ARE SHIFTING WITH NEW FINDINGS

Quantitative resistance has been previously qualified as “basal resistance” or “non-specific resistance” among other terms. Qualitative resistance on the other hand has been described as “race/strain-specific resistance” and as being associated with gene-for-gene interactions involving coupled *R* and *Avr* genes. Despite extensive examples described in the introduction, the definitions subsist as seen for example in article published as recently as 2020, which states: “Resistance to *Septoria blotch* can be either quantitative (horizontal) or isolate-specific (vertical)” (Toropova et al., 2020). With my experiments on Renan, I05 and I07, I have been able to formally demonstrate that gene-for-gene interactions and strain specificities are not the prerogative of qualitative

resistance and that qualitative and quantitative notions of disease resistance have overlaps. In the fourth chapter of this thesis I showed that *Stb20q* and *AvrStb20q* are involved in a gene for gene interaction. The mutant strain tests in chapter 3 showed that depending on the genetic background of fungal strains, the observed phenotype due to this interaction can lead to qualitative or quantitative phenotypes.

A. DIFFERENT FORMS OF QUANTITATIVE DISEASE RESISTANCE?

In literature, disease resistance QTL can be either strain/race specific with instances of gene-for-gene interactions or broad-resistance conferring. However, as pointed out by Ellingboe (1975), “broad resistance” could simply mean resistance which hasn’t yet been shown to be specific, a typical example is that of *Stb16q* (General introduction; this thesis). One might therefore wonder if there are genuine cases of broad spectrum quantitative resistance. *mlo* is a major resistance gene to powdery mildew in wheat. The resistance it confers is through a loss of susceptibility mechanism, which does not involve a gene-for-gene interaction, in a recessive allele which has proven to be broad spectrum and to withstand the test of time (Kusch and Panstruga, 2017). Additionally, a variant of the gene called *mlo-11 (cnv2)* was found to induce a reduction in disease rather than a full stop (Ge et al., 2016), demonstrating that quantitative resistance can indeed be broad spectrum. Another example of a loss of function-based resistance was identified in rice with the *pi21* gene leading to partial resistance (Zhang et al., 2016). *pi21* confers broad-spectrum resistance to rice blast (Angeles-Shim et al., 2020). Other broad resistance genes are found in the wheat-*P. triticina* pathosystem for example. *Lr34* is broad-resistance adult plant resistance gene from *T. aestivum*, which was found to also be effective at the seedling stage in durum wheat (Rinaldo et al., 2017). *Lr34* encodes an ATP-binding cassette transporter and has been found to be effective for partial resistance against not only leaf rust, but also stripe rust and powdery mildew (Krattinger et al., 2009; Li et al., 2020). Similarly, *Lr67* confers partial broad spectrum resistance to several diseases, it encodes a hexose transporter which is thought to modulate hexose transport, thus limiting colonization by biotrophic pathogens (Herrera-Foessel et al., 2014; Spielmeyer et al., 2013).

These examples show that quantitative resistance can be explained by a diversity of mechanisms and that it can be broad-spectrum just as it can be explained by gene-for-gene interactions.

B. RE-DEFINING TERMS

Following my experiments and all supporting bibliography, I propose that quantitative

and qualitative forms of disease resistance should only be differentiated by phenotypes, not by mechanisms, seeing as there are many overlaps where mechanisms are involved. Qualitative is a term that should only apply in the event of total resistance (no disease at all or HR) which is not due to non-host resistance. Following this, the only difference between quantitative and qualitative with regards to gene-for-gene interactions should be in terms of the effect on the phenotype of the “avirulent” allele. Avirulent is used here not to qualify the strain itself, but rather in reference to the allele of the avirulence gene under scrutiny, therefore encompassing the “less pathogenic” phenotypes that are observed with *Avr3D1* (Meile et al., 2018) or *AvrStb20q*. In a qualitative interaction, the recognition of the avirulent allele will lead to no disease symptoms at all or to HR depending on the pathosystem. In a quantitative interaction, the recognition of the avirulent allele will lead to a decrease in disease symptoms in comparison with phenotypes obtained for a strain with an equivalent genetic background carrying the virulent allele. If different alleles are identified, which lead to varying degrees in symptoms, some genes could even be considered as quantitative or qualitative resistance genes depending on the phenotypes observed with the different alleles. In this definition, the polygenic aspect is not taken into account, although, a multiplicity of resistance loci can of course have additive effects or indeed epistatic ones contributing to the overall phenotype.

C. NOMENCLATURE OF RESISTANCE LOCI

In the repertoire of *Stb* genes, two have a ‘q’ at the end of their name standing for ‘quantitative’. These are *Stb16q* and *Stb20q*. As previously stated, *Stb16q* was named this way as the locus was hypothesized as the QTL corresponding to locus could potentially have been explained by several underlying genes and was also hypothesized to confer broad resistance (Ghaffary et al., 2012). Had the definition proposed above been used, there would have been no question regarding the ‘q’ as the phenotypes were in line with what I describe as qualitative. The appellation has however endured despite the resistance conferred by *Stb16q* being overcome by several strains (Kildea et al., 2020; Orellana-Torrejon et al., 2021). *Stb20q* however does not lead to a qualitative phenotype, justifying the apposition of the ‘q’ (chapter 2). I propose that a ‘q’ should be apposed to the resistance locus only if the phenotype is what I have previously defined as quantitative. The ‘q’ for *Stb16q* should therefore be dropped.

Confusion derived from a potentially maladapted nomenclature of resistance genes is not unique to STB. As previously mentioned for resistance genes in apple to *V. inaequalis*, some of the resistance genes do not lead to a classic fully resistant

phenotype characterized by HR, but rather to chlorosis with little to no sporulation (Bowen et al., 2011; Khajuria et al., 2018), they are however named according to the same nomenclature as other apple scab resistance genes, which induce HR.

The nomenclature of rust resistance genes is particularly rich in sources of potential confusion. This is the case both for *Lr* genes, which confer resistance to *P. triticina*, and *Yr* genes, which confer resistance to *P. striiformis* f. sp. *tritici*. Discrepancies regarding the nature of the genetic resistance of wheat to both of these pathogens emerged before the first *Lr* genes were even named. Slow-rusting phenotypes did not fit into either the “horizontal” or “vertical” forms of resistance that had been previously described, prompting Johnson’s proposal to refer to “durable resistance” rather than “horizontal”, a denomination which was less loaded with assumptions allowing for both specific and non-specific forms of resistance (Johnson, 1979; Van Der Plank, 1966). The repertoire of *Lr* genes is about four times the size as that of *Stb* genes counting 80 genes (Kumar et al., 2021). Among these, the majority confer what is known as seedling-type resistance, in opposition to APR. Some race-specific APR genes, such as *Lr12*, *Lr13* for example are associated with HR, but are stage specific (Pinto da Silva et al., 2018), while others such as *Lr34* or *Lr74* are still considered to be race-non-specific (Pinto da Silva et al., 2018). The *Lr* denomination therefore encompasses what can be considered classic *R* genes leading to HR, race-specific genes that lead to HR but only at the adult stage, race-specific genes that lead to a partial resistance phenotype, race-specific genes that lead to a partial phenotype but only at the adult stage and race-non-specific genes that lead to partial phenotypes. The latter category at least, have demonstrated additivity with more resistant phenotypes being observed on cultivars carrying a combination of these resistance genes rather than one alone, as was the case for *Lr34* and *Lr68* (El-Orabey et al., 2019). Additionally, some are also temperature dependant, *Lr14a* for example is more effective for resistance at lower temperatures (Kolodziej et al., 2021). Resistance to stripe rust caused by *P. striiformis* f. sp. *tritici*, is equally as complicated, if not more, with at least 79 named resistance genes in wheat (*Yr1* to *Yr79*) also encompassing a mixture of seedling-type resistance, APR and temperature-dependant APR (Yang et al., 2019).

Possibly the main issue with these nomenclatures is that quantitative was resistance originally deemed to be only polygenic, and therefore a single gene underlying a resistance QTL was automatically added to the list of *R* genes associated with the disease regardless of its phenotype. Naming *Stb16q* with a ‘q’ was an idea to circumvent the issue, differentiating quantitative and qualitative loci, but dropping the QTL denomination. Though after all the ‘q’ cannot justifiably be applied to *Stb16*, the

solution is simple and easily understandable and is an at a glance way of differentiating quantitative and qualitative phenotype-associated genes in a given list, thus giving an indication in their name as to their potential or lack of, for durability.

D. DURABILITY OF QUANTITATIVE RESISTANCE IN LIGHT OF NEW FINDINGS

Durability of resistance as Johnson stated in the 1970s can only be evaluated in retrospect (Johnson, 1979), in other words, it is not conceivable to emit a definite statement on the durability of a resistance gene without its being implemented and put to the test over a number of years. In all probability, any form of resistance should be eventually overcome, even broad resistance, because of highly plastic and fast-evolving pathogen genomes subject to random mutations. For example, even the “universal weapon” to defeat powdery mildew disease *mlo* (Kusch and Panstruga, 2017) has been overcome in laboratory conditions following an experimental evolution protocol. The authors suggested that the absence of *mlo*-virulent strains in field conditions was due to a combination of variations in selective pressure induced by season-dependant resistant and susceptible cultivar rotations and a fitness cost of the adaptation (Kusch et al., 2021). The question of durability is then dependant on the time-frame. Even resistance that was effective for decades such as that conferred by *Rpg1* in barley to stem rust was eventually overcome by certain races (Solanki et al., 2019). Gene-for-gene interactions underlying quantitative phenotypes are indicative of potential break-down of the resistance, however the time-frame should be much larger than for those leading to qualitative phenotypes because of lower selective pressure. This can be illustrated in several ways. A first and simple explanation is that high selective pressure induced by strong resistance results in a reduction of the pathogen population, causing a bottleneck which promotes virulent strains (Iacono et al., 2013). Another explanation is given by the coevolutionary theory of hosts and parasites. This model predicts that in a gene-for-gene system, less effective effector triggered resistance genes, i.e. those conferring quantitative resistance, have higher frequencies in the long run, and that strong quantitative resistance selects a higher frequency of avirulence genes thus promoting durability (Cowger and Brown, 2019). Although data and models show that quantitative resistance is more durable than qualitative resistance, it is not everlasting. Evidence for quantitative resistance erosion and even breakdown has been observed as previously discussed in chapter 2. However, outside of the possibility that some of the breakdown is due to “weak *R* genes”, no other potential mechanisms plant-side are known (Cowger and Brown, 2019). On the pathogen-side, risk factors for durability are rapid reproduction, variety in reproductive modes and population size (Cowger and Brown, 2019; McDonald and Linde, 2002).

While durability can truly only be evaluated in retrospect, certain practices or deployment strategies have however been shown to promote durability of disease resistance these all have the reduction of selective pressure in common. These are crop rotations, cultivar mixtures, cultivar mosaics and gene pyramiding (Rimbaud et al., 2018). In gene-for-gene interactions, the pyramiding of genes with different specificities can dilute selective pressure (Pilet-Nayel et al., 2017; Luo et al., 2021), and the combination of broad-spectrum and narrow spectrum resistances is thought to minimize resistance erosion (Van et al., 2013). Taking into account the great many variables involved in resistance durability, some models have attempted to predict durability. One model showed that durability was dependant on the cropping ratio, meaning the proportion of land occupied by a resistant crop, taking fluctuations and variability in pathogen populations into account (Iacono et al., 2013), another model proposed that durability is a function of the fitness cost of adaptation (Vera Cruz et al., 2000). A more recent model, which takes rusts as an example, suggested that while quantitative resistance alone is more durable than qualitative resistance, the combination of two major resistance genes can be more durable than the combination of a major resistance gene and quantitative resistance. The level of durability was found to depend on the magnitude of the quantitative resistance. If it was too low, it did not slow adaptation of the pathogen to the major resistance gene (Rimbaud et al., 2018). This model did however not account for what the authors call "partially effective major genes", genes like *Stb20q*.

As a great many different components can affect resistance durability and that for the great majority of cases, the mechanisms underlying quantitative resistance remain unknown, it remains difficult to predict. The evidence that gene-for-gene interactions can underlie quantitative resistance does have an impact on durability as in all probability, specific resistance is more likely to be overcome than non-specific resistance (Cowger and Brown, 2019). However, efforts to dilute selective pressure by using a combination of specific resistance genes with different specificities and underlying mechanisms, among other measures, should contribute to increasing durability, although the contribution of too small effect loci may be put into question (Rimbaud et al., 2018).

Controlling STB epidemics remains a challenge for European and World agriculture, around 70% of the annual fungicide expenditure in Europe is dedicated to controlling this disease (Fones and Gurr, 2015). While STB has long been thought to be mostly broad spectrum and non-isolate specific, due to highly variable quantitative phenotypes, we now know that resistance to *Z. tritici* at least partly involves gene-for-

gene interactions. These interactions can underlie qualitative resistance, as for *Stb6* or *Stb16q*, but also quantitative resistance such as the QTL identified in this study. Understanding the genetic determinism and specificities of resistance genes is paramount to optimizing their deployment in the most durable manner. Selective pressure is one of the main drivers of adaptation to host resistance in pathogen populations. The highly polygenic and quantitative nature of resistance to STB in wheat is a strength that should be used in breeding to produce durably resistant wheat cultivars. The more we can learn and understand about the genes involved in plant-pathogen interactions, the more efficiently they can be put to use. One possibility is the stacking, or pyramiding of QTL. In this study, the three resistance QTL identified in 'Renan' seem to have been sufficient to confer durable resistance. Although some erosion has been observed, this cultivar is still used today in organic farming despite having been introduced at the end of the 1980s. The identification of *Stb20q* in particular illustrates that many resistance loci in wheat remain unknown, including relatively strong effect ones. This advocates maintaining phenotypic selection alongside marker assisted selection as these unknown loci do contribute to the overall resistance level of a given cultivar. With high diversity in fungal populations also, emphasis should be put on multilocal trials as these could contribute to the identification of highly specific gene-for-gene interactions which can hinder durability. Finally, selective pressure has also been shown to be one of the main drivers in adaptation to fungicides in this pathosystem, with a dilution of selective pressure by an increase of its heterogeneity limiting the emergence of fungicide-resistant strains (Ballu, 2021). Combining disease control methods could lead to a reduction of fungicide use, for example while genetic resistance could be favoured, its durability could be backed-up by fungicides to reduce the emergence of highly adapted fungal populations thus contributing to the durability of resistance to STB.

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QUANTITATIF VERSUS QUALITATIF DANS LE CADRE DES INTERACTIONS PLANTE-PATHOGENE, EST-CE UN FAUX DILEMME? LE CAS DE L'INTERACTION BLE-*ZYMOSEPTORIA TRITICI*

RESUME SUBSTANTIEL

Les notions de qualitatif et de quantitatif s'opposent en phytopathologie depuis plus de 60 ans. La résistance qualitative, également appelée résistance verticale, se rapporte à une distribution discrète des données phénotypiques en classes distinctes, résistante ou sensible. Cette distribution s'explique par les interactions gène-pour-gène entre les gènes de résistance *R* côté hôte et les gènes d'avirulence *Avr* côté agent pathogène. La résistance quantitative, en revanche, parfois appelée résistance basale ou horizontale, est caractérisée par une distribution continue des phénotypes expliquée par une combinaison de loci à effet quantitatif (QTL) à effet plus ou moins fort et dont les gènes et mécanismes sous-jacents sont généralement inconnus. La résistance quantitative est réputée plus durable que la résistance qualitative dû au contournement souvent rapide des gènes *R* impliqués dans des interactions gène-pour-gène avec les gènes *Avr*. Depuis une vingtaine d'années, de plus en plus d'exemples semblent contredire la théorie qui prône une stricte dichotomie fortement portée sur les mécanismes expliquant ces deux formes de résistance. L'objectif de cette thèse a été d'étudier les mécanismes sous-jacents aux interactions quantitatives avec comme hypothèse l'existence d'interactions QTL-pour-QTL similaires aux interactions gène-pour-gène décrites précédemment. La mise en évidence de telles interactions entre QTL de résistance et QTL de pathogénie soulève le besoin d'adapter les définitions conventionnelles aux dernières connaissances acquises. Nous avons choisi de travailler avec le pathosystème modèle *Zymoseptoria tritici*-blé tendre car les interactions entre l'hôte et l'agent pathogène sont considérées comme étant principalement quantitatives.

Le travail présenté dans cette thèse s'articule en cinq chapitres. Le premier, sous la forme d'une introduction générale, a eu pour objectif de présenter les grands principes liés aux notions de quantitatif et qualitatif en phytopathologie. Cette introduction présente d'abord de manière générale les méthodes employées pour contrôler les maladies des cultures et met l'accent sur le besoin de mieux comprendre les interactions entre les plantes hôtes et leurs agents pathogènes dans le but de mettre à profit les gènes de résistance aux maladies. La partie suivante décrit les mécanismes employés par les agents pathogènes filamenteux (champignons et oomycètes) pour

infecter leurs hôtes ainsi que sur le système immunitaire de ces hôtes qui est activé en réponse à une attaque. Ensuite, sont abordées les définitions des résistances quantitatives et qualitatives, et notamment les divergences à ces définitions qui sont observées dans nombre d'études. Cette partie met en évidence les discordances entre des définitions couramment utilisées et la réalité biologique. Une proposition est faite pour expliquer certaines de ces discordances, notamment celle de la possibilité d'interactions gène-pour-gène menant à des phénotypes quantitatifs. Cette hypothèse met en question la durabilité de la résistance quantitative. L'introduction générale se termine par une présentation du pathosystème étudié dans ce projet. La septoriose, dont l'agent causal est le champignon ascomycète *Z. tritici*, est une maladie qui engendre de fortes pertes de rendement chaque année dans les cultures de blé, une céréale qui est l'aliment de base pour un tiers de la population mondiale.

Les trois chapitres suivants sont présentés sous la forme d'articles scientifiques, dont le premier a été publié dans *Genes* le 31 décembre 2021.

Le deuxième chapitre a pour but de disséquer la résistance à la septoriose dans le cultivar de blé tendre 'Renan', principalement résistant à cette maladie. Pour ce faire, deux souches I05 et I07 ont été choisies préalablement pour leur pouvoir pathogène contrasté sur ce cultivar. Des données phénotypiques ont été obtenues après l'inoculation de I05 d'une part, et de I07 d'autre part, sur une population biparentale issue du croisement entre 'Renan' et 'Chinese Spring', un cultivar sensible. Une carte génétique ultra-dense a été construite pour cette population biparentale en utilisant des marqueurs SNP issus de deux puces différentes, la puce Breedwheat Affymetrix Axiom 410K et la puce Illumina Infinium iSelect Wheat 90K. Des analyses de liaison croisant les données phénotypiques et la carte génétique ont permis d'identifier trois QTL. Un QTL, identifié sur le chromosome 1D, confère de la résistance à la souche I07 et explique environ 10% de la variabilité phénotypique. Ce QTL colocalize avec un gène de résistance majeur *Stb19*. Un QTL, identifié sur le chromosome 5D, confère de la résistance à la souche I05 et explique environ 30% de la variabilité phénotypique. Le troisième QTL, identifié sur le chromosome 7B explique environ 35% de la variabilité phénotypique et confère de la résistance aux deux souches. De plus, ce QTL colocalize avec le gène de résistance majeur *Stb8*. Une liste de gènes candidats expliquant ces QTL a été proposée, avec comme cribles, une surexpression lors de l'infection par *Z. tritici* (données disponibles dans la littérature) et l'appartenance à des familles de gènes classiquement impliqués dans la détection des agents pathogènes par les plantes, à savoir les nucleotide-binding site leucine-rich repeat immunoreceptors (NLR), receptor-like kinases (RLKs) et autres protéines avec des domaines kinase. L'étude a

permis de démontrer que la résistance de 'Renan' à la septoriose est quantitative et polygénique. De plus, les QTL de résistance présentent des spécificités vis-à-vis des souches et deux des QTL détectés colocalisent avec des gènes de résistance majeurs. Ceci suggère que des interactions gène-pour-gène pourraient expliquer les phénotypes quantitatifs observés. Le QTL de résistance sur le chromosome 5D ne colocalisant pas avec une source de résistance connue a été désigné *Stb20q*, le 'q' indiquant l'effet quantitatif du locus.

Le troisième chapitre a pour objectif d'analyser l'architecture du pouvoir pathogène des souches I05 et I07 vis-à-vis du cultivar 'Renan', permettant ainsi, à la suite du deuxième chapitre, d'avoir une vue d'ensemble sur l'interaction Renan-I05/I07. Une population issue du croisement entre les souches I05 et I07 a permis d'une part la construction d'une carte génétique ultra-dense de *Z. tritici* et d'autre part l'acquisition de données phénotypiques suite à une inoculation des descendants sur 'Renan'. Par analyse de liaison, trois QTL de pathogénie ont été détectés dans ces souches. Pour deux de ces QTL, identifiés sur les chromosomes 1 et 6 de *Z. tritici*, l'allèle pathogène est porté par la souche I05, tandis que pour le troisième, sur le chromosome 13, c'est la souche I07 qui porte l'allèle pathogène. Deux gènes candidats *G_07189* et *Zt09_13_00364* ont été identifiés et clonés par 'Agrobacterium tumefaciens mediated transformation' (ATMT) dans les QTL des chromosomes 6 et 13 respectivement. *G_07189* n'était pas annoté avant ces travaux. Tandis que l'implication de *Zt09_13_00364* dans le pouvoir pathogène n'a pas pu être validé avec les tests des souches mutantes sur plante, celle de *G_07189* l'a bien été. Ce gène qui est surexprimé pendant l'infection, correspond à une petite protéine sécrétée riche en cystéines, classique des effecteurs fongiques. Une analyse de la diversité de ce gène dans un panel de souches diversifiées a montré que malgré la présence de signatures de sélection dans sa séquence protéique, il n'a pas une grande diversité de séquence, suggérant peu de sélection des génotypes virulents. Ceci pourrait indiquer que si les interactions gène-pour-gène peuvent expliquer des phénotypes quantitatifs, elles n'impliquent pas forcément une durabilité moindre des gènes de résistance impliqués.

Le quatrième chapitre de cette thèse vise à identifier des interactions QTL-pour-QTL expliquant des phénotypes quantitatifs. Nous avons tout d'abord travaillé avec la population issue du croisement entre 'Renan' et 'Chinese Spring' dans laquelle les trois QTL de résistance ont été précédemment identifiés (Chapitre 2). Cette population a été phénotypées avec les souches I05 et I07, trois souches F₁ sélectionnées pour les combinaisons d'allèles qu'elles portent au niveau des trois QTL du pouvoir pathogène précédemment identifiés (Chapitre 3), et la souche mutante I07_ΔG07189_cl.40

obtenue par 'knock-out' du gène *G_07189* dans un fond génétique I07 (I07 porte l'allèle non pathogène pour ce gène). Les analyses de liaison, réalisées avec les données phénotypiques obtenues pour ces six souches, ont permis de mettre en évidence les liens entre les QTL du pouvoir pathogène et les QTL de résistance. En effet, la détection d'un QTL de résistance est dépendante de la présence de l'allèle non-pathogène du QTL du pouvoir pathogène correspondant dans la souche inoculée. Ainsi, ont été démontrées des interactions QTL-pour-QTL entre *Qzt-I07-13* et *Qstb-Renan-1D* d'une part, et *Qzt-I05-6* et *Qstb-Renan-5D (Stb20q)* d'autre part. De plus, la détection de *Qstb-Renan-5D (Stb20q)* avec la souche sauvage I07 et l'absence de détection de ce QTL de résistance avec la souche mutante I07_ΔG07189_cl.40 démontre que *G_07189* est bien le gène causal de l'interaction côté champignon ; *G_07189* a donc été renommé *AvrStb20q*. Ce résultat a été confirmé par une étude d'association à l'échelle du génome (GWAS) réalisée à partir de l'évaluation d'un panel diversifié de 120 souches sur une lignée recombinante issue de la population Renan×Chinese Spring portant l'allèle résistant uniquement pour *Stb20q*. Une analyse plus fine de la région du génome de *Z. tritici* où se trouve *AvrStb20q* dans le panel de 120 souches a montré que cinq isoformes de la protéine correspondante sont décelables, et que les polymorphismes correspondants à ces isoformes n'expliquent pas seuls leur caractère pathogène ou non pathogène. En effet, une insertion d'éléments transposables (TE) dans la région 3'UTR (untranslated region) du gène semble être liée à la pathogénie, les isoformes les moins pathogènes étant préférentiellement associées à la présence d'une insertion de TE. Ce travail nous a donc permis d'identifier des interactions QTL-pour-QTL et également une interaction gène-pour-gène sous-jacente dans ce pathosystème, démontrant que, malgré les définitions usuelles, quantitatif n'est pas nécessairement synonyme de large spectre et aspécifique, mais peut également englober des interactions gène-pour-gène spécifiques impliquant des effecteurs. Ces résultats mettent en lumière la nécessité de travailler les définitions actuelles de ces notions et de discuter la durabilité présumée de la résistance quantitative.

Le dernier chapitre de cette thèse se présente sous la forme d'une discussion générale. Celle-ci est articulée en trois parties. La première traite des interactions gène-pour-gène menant à des phénotypes quantitatifs ainsi que de la possibilité que les gènes de résistance impliqués puissent être des gènes *R* à effet partiel. De telles interactions ne semblent pas être exclusives au pathosystème blé-*Z. tritici*, des exemples similaires existant dans d'autres pathosystèmes. Enfin, un commentaire est émis par rapport à la moindre place que prend la résistance quantitative dans les travaux scientifiques relativement à la résistance qualitative. Ce biais pourrait être expliqué en partie par la difficulté de travailler avec des phénotypes intermédiaires ou partiels par rapport à des

phénotypes clairement distincts. Une deuxième partie traite du rôle joué par les éléments transposables dans les interactions entre plante hôte et agent pathogène qui participent à la plasticité des génomes fongiques, mais pourraient aussi être impliqués plus directement dans le passage de pathogène à non-pathogène. La dernière partie traite des définitions des résistances quantitative et qualitative et de la nécessité d'adapter ces définitions au regard des résultats obtenus au cours de cette thèse. En effet, une définition en termes de mécanismes sous-jacents ne peut convenir dès lors que les mêmes mécanismes d'interactions gène-pour-gène ou de gènes à large spectre peuvent mener aussi bien à des phénotypes quantitatifs que qualitatifs. Ces notions sont reliées à la nomenclature des gènes de résistance qui ne traduit pas toujours bien la réalité biologique. La durabilité de la résistance aux maladies et ses différentes composantes sont discutées. Si la pression de sélection imposée par des gènes de résistance à effet quantitatif devrait être moindre par rapport à celle imposée par des gènes de résistance majeurs, toute résistance est contournable, la durabilité est alors une fonction du temps que prendra ce contournement. Des mesures de combinaison de différentes sources de résistance à l'échelle de la variété et la mise en place de mélanges variétaux, par exemple, à l'échelle du champ, pourraient être des clefs pour maximiser cette durabilité.

OUTCOMES

Articles

PUBLISHED

Langlands-Perry C, Cuenin M, Bergez C, Krifa SB, Gélisse S, Sourdille P, Valade R, Marcel TC. 2022. Resistance of the Wheat Cultivar 'Renan' to Septoria Leaf Blotch Explained by a Combination of Strain Specific and Strain Non-Specific QTL Mapped on an Ultra-Dense Genetic Map. *Genes* 13: 100.

TO BE SUBMITTED

Langlands-Perry C, Pitarch A, Lapalu N, Cuenin M, Bergez C, Noly A, Amezrou R, Gélisse S, Barrachina C, Parrinello H, Suffert F, Valade R, C. Marcel TC. 2022. Quantitative and qualitative plant-pathogen interactions call upon similar pathogenicity genes with a spectrum of effects.

Langlands-Perry C, Amezrou R, Lapalu N, Gélisse S, Gazeau G, Ute G, Valade R, Marcel TC. 2022. Gene-for-gene interactions between host and pathogen blur the lines between qualitative and quantitative definitions of resistance.

Talks

Langlands-Perry C. QTL-for-QTL interactions in the *Z. tritici/T. aestivum* pathosystem. Durabilité des résistances, Séminaire doctorants et post-doctorants, INRAE, visioconférence. 21-22 June 2021

Langlands-Perry C. QTL-for-QTL interactions in the *Zymoseptoria tritici - Triticum aestivum* pathosystem. First MoDip meeting, Montpellier, France. 2021 13-15 October 2021

Langlands-Perry C. QTL-for-QTL interactions in the *Z. tritici/T. aestivum* pathosystem. Fungal genetics IDF 2nd Symposium on Fungal Genetics Ile de France 2021, Paris, France. 23 November 2021

Langlands-Perry C. The interaction between *Stb20q* in wheat and *AvrStb20q* in *Zymoseptoria tritici* blurs the frontier between qualitative and quantitative resistances in this economically important pathosystem. International Symposium on Cereal Leaf Blights 2022, Tunis, Tunisia. 11-13 May 2022

Posters

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